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VICscience

UPDATED CONTENT

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biology

VCE UNITS
3 + 4

4E

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Fig. 2.2 p.39: Alamy Stock Photo/Science Photo Library The discoverers of the structure of DNA. James Watson (b. 1928) at left and Francis Crick (1916–2004), with their model of part of a DNA molecule in 1953

Fig. 6.1 p.203: PNAS March 30, 2004 101 (13) 4620-4624; https://doi.org/10.1073/pnas.0308001101 Human and avian influenza viruses target different cell types in cultures of human airway epithelium Mikhail N. Matrosovich, Tatyana Y. Matrosovich, Thomas Gray, Noel A. Roberts, and Hans-Dieter Klenk Figures 1&2 Copyright (2004) National Academy of Sciences, U.S.A.

Fig 6.34 p.243: National Archives UK. https://webarchive.nationalarchives.gov.uk/20060525120000/ http://www.bseinquiry.gov.uk/pdf/index.htm http://www.nationalarchives.gov.uk/doc/open-overnmentlicence/version/3/

Fig 9.20b p. 345: Markert, J.A., Champlin, D.M., Gutjahr-Gobell, R. et al. Population genetic diversity and fitness in multiple environments. BMC Evol Biol 10, 205 (2010). https://oi.org/10.1186/1471-2148-10-205 CC BY 2.0 https://creativecommons.org/licenses/by/2.0/

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VCE UNIT 4

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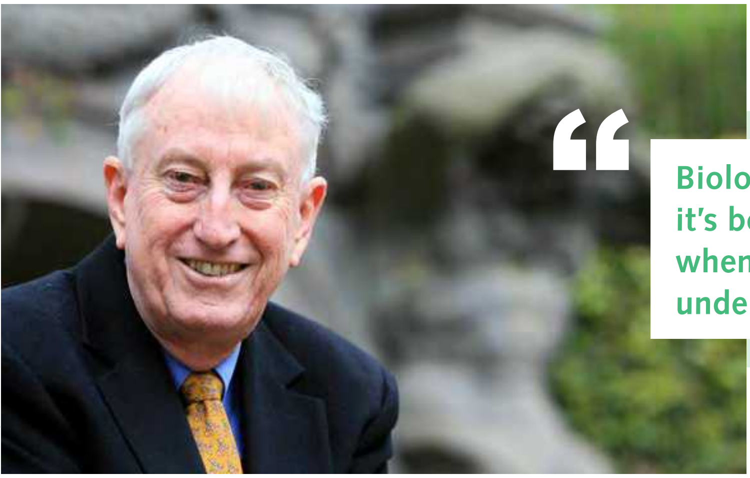
Foreword



Newspix/Richard Cisar-Wright

> “Biology is fantastic because it’s both complicated and, when you finally get to some understanding, beautiful!

Starting out as a veterinary scientist trying to understand how viruses kill, and how we might prevent that, I made a chance discovery with my Swiss colleague, Rolf Zinkernagel, that led to us sharing the 1996 Nobel Prize for Physiology or Medicine. Rolf trained as a medical doctor but, so far, I’m the only vet to win a Nobel. We found that the so-called transplant or *surveillance* molecules focus the *assassins* of immunity, the *killer* T cells, on cells that are damaged by infection or oncogenic (cancerous) changes. The following year I had the honour of being named the Australian of the Year. When asked to write this foreword my response was: are they thinking of Pete Doherty the musician? In a world where science communication has rapidly declined and fake news (or gossip over the back fence) is too often the norm, I felt it was important to add my opinion to the study of Science, specifically Biology, and say a little bit about how it can change thinking and lives.

I am passionate about promoting an evidence-based view of reality: my most recent book, *The Knowledge Wars*, describe the ‘warts and all’ view of science for non-scientists, even for people who don’t like science. That’s the great thing about biological science. It may be a surprise to some arty types, but science does teach you how to write clearly and concisely, though we’re no good at fiction! So far I’ve published a diverse range of general books about science and the scientific life: *A Light History of Hot Air, The Beginners Guide to Winning the Nobel Prize : a Life in Science, Sentinel Chickens: What Birds Tell Us About our Health and our World and Pandemics: What Everyone Needs to Know*, and *The Incidental Tourist*.

Biology is fantastic because it’s both complicated and, when you finally get some understanding, beautiful! Of course, it is important to pass exams if you want to get into medical or vet school, or become a researcher like me. Studying biology teaches all of us about ourselves, while strengthening important life skills such as critical thinking, problem solving, collaboration, scientific literacy and the importance of working together. And if you have a good basic grasp of biology, you’ll understand why this discovery or that is important. It will also help you to tell the difference between reality, hype and downright lies. The *VICscience Biology* series tackles some of the big ethical issues and teaches students how to think scientifically and question ideas.

Now, having been involved in infectious disease research (especially immunity) for more than 55 years, I’ve handed over my research lab (plus whatever grant money I bring in) to my younger colleagues. I’ve still got stuff to say, and my focus now is on writing more books. Part of the delight of being a senior researcher is to see those who’ve worked with you mature and become great scientists. And it starts for them, as it did for me, at one place: with learning the basics and being excited by biology. Apart from allowing me to live and work in different countries, and opening doors, in terms of social and economic mobility, being part of the unravelling of the story of life has been immensely gratifying. There is still an enormous amount to be discovered and even if you are not intending to be part of that, understanding the basics of biology can only serve to position you better for any future you might be contemplating.

Dr Peter Charles Doherty AC FRS FMedSci
Laureate Professor, The University of Melbourne.
5 December 2019

Author team

Dr Sarah Jones – consultant

Dr Sarah Jones is a medical research scientist who leads a team of scientists and clinicians in the School of Clinical Sciences at Monash University in Melbourne. The goal of Sarah's research is to develop a safe and effective new treatment for autoimmune and inflammatory conditions. Before this, Sarah held research positions at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Harvard Medical School, US, and Trinity College, Ireland. Sarah was a medal-winning member of the Australian International Biology Olympiad Team before becoming a tutor then acting director of the program, designing theoretical and practical learning material and exams for Australia's highly successful Olympiad teams. Sarah was lead author for the 2016 edition of *Nelson Biology VCE Units 3 & 4* and was scientific consultant and editor for *Nelson Biology VCE Units 1&2* (2016) and *Nelson Biology Units 3 & 4 for the Australian Curriculum* (2015).

Ann Cathcart

Ann Cathcart (MEdAdmin, BSc, DipEd) has extensive experience in the development of scientific curriculum content of a biological and scientific nature. She also has vast experience in the publication of materials for learning. Ann brings specific skills to an authoring role. She is a current and practising secondary school teacher who has taught Years 2–12 in many Australian school systems, predominantly in senior Biology and Chemistry, for more than 40 years. She has also managed school science departments in positions of Head of Science and Head of Biology. Ann has worked in science-related industries, such as medicine, agriculture and mining, and at the tertiary level in medical education. Ann understands secondary students. She has addressed stakeholders' needs in each part of her career. She has written educational materials and loves doing this. While applying attention to detail based on a strong technical background, she demonstrates a passion for enabling others, including her students, to achieve an outcome.

Taylah Bennett

Taylah Bennett attained a BSc (Hons) and is currently completing her PhD at the Biomedicine Discovery Institute at Monash University in Melbourne. Taylah received the Nairn Prize in 2017, which recognises the top Honours student in the Department of Immunology at Monash University. She has held positions as a teaching associate in the Department of Immunology and has previously worked at the Australian Regenerative Medicine Institute and School of Clinical Sciences. Taylah is a member of the Australian and New Zealand Society for Immunology, and the Immunology Group of Victoria and has been president of the Monash University Microbiology Postgraduate Society. Her research aims to understand how T cells are controlled to fight viruses and cancer but can also become pathogenic in situations of autoimmune disease.

Dr Tony Chiovitti

Tony Chiovitti attained a BSc (Hons) at the University of Melbourne in 1992. He completed his PhD at the School of Botany, University of Melbourne in 1997 investigating cell wall biochemistry of Australian red algae and algal evolution using gene sequences. He has eight years of postdoctoral research experience in Australia and overseas with biochemical studies of bacteria and microalgae, including collaboration in the first phytoplankton genomes to be sequenced. He obtained a Dip. Ed (2004) and joined the education team at the Gene Technology Access Centre (GTAC), Parkville, Victoria becoming Deputy Director in 2012. Tony has developed and delivered educational programs for students and professional learning programs for teachers on the themes of cell and molecular biology, bioinformatics, immunology, ecology and evolution. He has also led educational programs that enable secondary school students to contribute as citizen scientists to biological research projects.

Dr Amanda Clarke

Amanda Clarke's interest in biology started when she was a child. Initially, she wanted to be a veterinarian, but at university she became fascinated with genetics, microbiology and immunology. She was granted a PhD in Immunology from the University of Melbourne for her studies into house dust mite allergy. While studying, she also taught practical classes at several universities and thoroughly enjoyed opening her students, minds to the wonders of medical research. Amanda then decided to become a Biology and Chemistry teacher. She developed a special interest in the biomedical applications of nanoscience and nanotechnology. She was part of a team at St Helena Secondary College who won a Victorian Government Education Innovation Award for the development of a nanotechnology curriculum at the school. Amanda is still teaching Nanotechnology and Biology and thoroughly enjoys it. She is currently employed as a learning specialist at Balwyn High School.

Xenia Pappas

Xenia Pappas is a Biology teacher with more than 30 years' experience. She has taught across all sectors of the Victorian education system, including time with the Zoo Education Service and Museum Victoria. She has held leadership roles within the Department of Education's Gifted Education Unit as well as Head of Year and Head of Biology for many years in schools. Xenia has always worked to engage her students by offering alternative approaches that taps into a range of learning modalities. As a long-time author of Biology and General Science resources, Xenia has developed a well-rounded knowledge and understanding of the curriculum from Year 7 to VCE and works to deliver the curriculum in a manner that addresses the individual needs of students.

Adrianne Harrowfield

Adrianne studied Genetics and Microbiology at La Trobe University gaining her BSc and subsequently received an Honours degree in Genetics. She began her scientific career as a research assistant at the Walter and Eliza Hall Institute of Medical Research within the Genetics Department. After two years working in research, she completed her DipEd at the University of Melbourne and has been teaching VCE Biology for 20 years. She has been a VCE Biology examination assessor and is currently a passionate teacher of Biology.

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- Rebecca Famlonga, who reviewed the Aboriginal and Torres Strait Islander content. Rebecca is a proud Wadawurrung woman and Traditional Owner. She has taught and led in secondary schools for more than 20 years and is passionate about Aboriginal and Torres Strait Islander Education.

VICscience VCE Biology Learning Ecosystem

exam+

exam*plus* is a comprehensive and innovative assessment platform that simulates real exam practice. exam*plus* provides an extensive bank of filterable and difficulty-graded past VCAA and unseen exam questions, all in the one place.

Textbook

Students learn through stimulating, engaging and scaffolded content, activities and investigations. All content can be directly mapped to the VCAA *VCE Biology Study Design.*

A+ Biology Study Notes includes detailed summaries and revision questions, as well as study tips and information about the exam. ***A+ Biology Practice Exams*** contains 14 topic tests and two perforated practice exams.

Logbook

Students record all their investigation materials in one place for assessment and authentication purposes as required by the VCAA *VCE Biology Study Design.*

nelson net

Teachers expand their teaching and students reinforce their learning through the rich variety of extra resources provided on the NelsonNet student and teacher portals.

Workbook

Students develop, use and demonstrate key science skills through engaging activities; they practise exam skills by completing exam-style questions.

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To the student

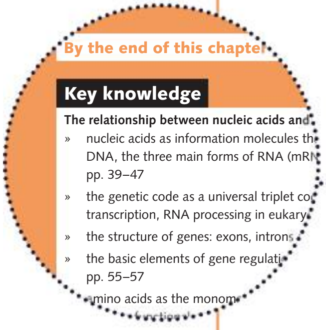
The VCE Biology course comprises both key knowledge and key science skills components, which will be assessed throughout your studies. We understand that undertaking VCE Units 3 & 4 can be an exciting but sometimes overwhelming time. You will learn a lot of content and develop scientific skills throughout a very busy year that will culminate in an external assessment. We have taken these stressors into account when designing the *VICscience Biology* suite of products. You will not need to go beyond these learning materials to study VCE Biology; they have been designed to work in unison so you can achieve at your best.

10 steps to study success

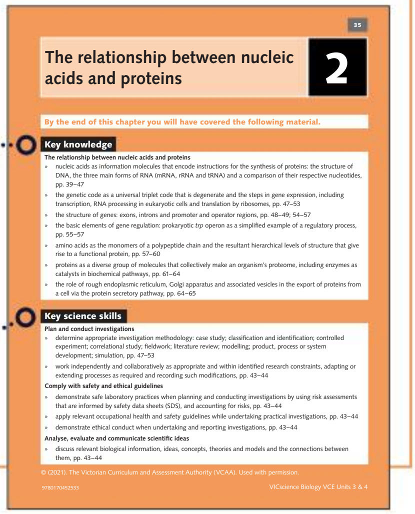
Ensure you take time to read the 10 ways we have organised your VCE biology study journey. You will see that at various stages in your studies, different aspects of this textbook will be more useful. Whether you are learning new concepts for the first time, reviewing what you have learnt or preparing for tests and exams, spending a little time now getting to know your textbook will help you reach your learning potential for VCE Biology.

1 Focus on the Study Design

Each chapter starts with a chapter opening page that will guide you through the **key knowledge** and **key science skills** that are covered in the chapter with page reference numbers to help find all the content that you need quickly.



By the end of this chapte
Key knowledge
The relationship between nucleic acids and
» nucleic acids as information molecules th
DNA, the three main forms of RNA (mRN
pp. 39–47
» the genetic code as a universal triplet co
transcription, RNA processing in eukary
» the structure of genes: exons, introns
» the basic elements of gene regulati
pp. 55–57
» amino acids as the monom



35

The relationship between nucleic acids and proteins **2**

By the end of this chapter you will have covered the following material.

Key knowledge

The relationship between nucleic acids and proteins

» nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides, pp. 39–47
» the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes, pp. 47–53
» the structure of genes: exons, introns and promoter and operator regions, pp. 48–49; 54–57
» the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process, pp. 55–57
» amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein, pp. 57–60
» proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways, pp. 61–64
» the role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway, pp. 64–65

Key science skills

Plan and conduct investigations

» determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation, pp. 47–53
» work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications, pp. 43–44

Comply with safety and ethical guidelines

» demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks, pp. 43–44
» apply relevant occupational health and safety guidelines while undertaking practical investigations, pp. 43–44
» demonstrate ethical conduct when undertaking and reporting investigations, pp. 43–44

Analyse, evaluate and communicate scientific ideas

» discuss relevant biological information, ideas, concepts, theories and models and the connections between them, pp. 43–44

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9780170452533 VICscience Biology VCE Units 3 & 4

of rough
a cell via the protein secreto
Key science skills
Plan and conduct investigations
» determine appropriate investigation met
experiment; correlational study; fieldwork
development; simulation, pp. 47–53
» work independently and collaboratively a
extending processes as required and recor
Comply with safety and ethical guidelines
» demonstrate safe laboratory practices
that are informed by safety data she
» apply relevant occupational heal
demonstrate ethical condu

2 Look for connections

Each chapter begins with a **chapter map** that:

- are easy-to-use
- use shorthand familiarisation
- is a navigational tool to guide you through each chapter
- offers a gentle entry into the more complex information.
- provides quick links to chapter content

36 UNIT 3 / AoS 1: What is the role of nucleic acids and proteins in maintaining life?

Online Chapter Map
Chapter 2 map

2 The relationship between nucleic acids and proteins

In Biology, using many small blocks to build larger and more complex structures is a concept that recurs again and again.

p. 39
2.1
Nucleic acids
The code for building proteins is contained in the DNA in the nuclei of your cells. Proteins are a major building component in your body. The DNA code is copied into messenger RNA, which is transported out of the nucleus to ribosomes in the cytosol.

p. 47
2.2
Gene expression
The mRNA code is read at the ribosome. Transfer RNA checks the cytosol to locate a specific amino acid to bring back to the ribosome to build the correct protein.

pp. 64
2.5
The protein secretory pathway
Proteins need to move about. From the endoplasmic reticulum within a cell, further processing occurs before proteins are moved to the Golgi apparatus for secretion out of the cell or for use in other areas of the body.

The structure of proteins is coded into our DNA. Scientists have ways to manipulate DNA.

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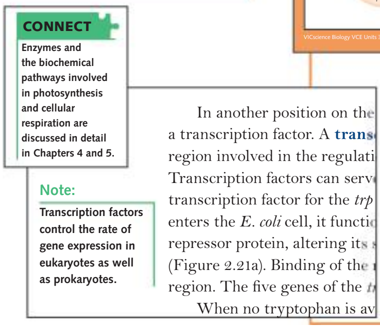
p. 47
2.2
Gene
expression
The mRNA code is read at the ribosome. Transfer RNA checks the cytosol to locate a specific amino acid to bring back to the ribosome to build the correct protein.



CONNECT
Enzymes and the biochemical pathways involved in photosynthesis and cellular respiration are discussed in detail in Chapters 4 and 5.

Note:
Transcription factors control the rate of gene expression in eukaryotes as well as prokaryotes.

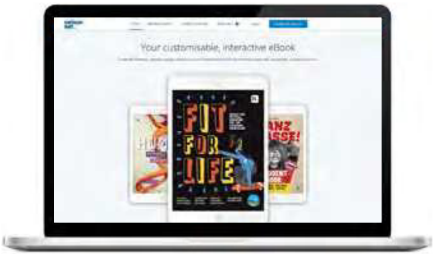
In another position on the
a transcription factor. A **trans**
region involved in the regulati
Transcription factors can serv
transcription factor for the *trp*
enters the *E. coli* cell, it functi
repressor protein, altering its
(Figure 2.21a). Binding of the
region. The five genes of the
When no tryptophan is av

We have made further connections throughout each chapter using margin **Notes**, which offer further explanation and **Connect** boxes, which link content to other relevant chapters where more information is provided.

3 Rehearse key terms

We have listed all the key terms at the beginning of each chapter.

You can use the **flashcards** study tool to learn and review key terms with their definitions, and assist with pronunciation and spelling of key vocabulary.



4 Test your memory

At the beginning of each chapter, use the **Remember** statements under the key terms list to bring previously learnt concepts to the front of your mind. Stronger foundations of knowledge make learning more difficult concepts easier.

The *VICscience Biology Skills Workbook* provides you with stepped questions to help you to engage your past learnings.

5 Develop your skills

Key science skills are examinable in the external assessment and therefore are a significant and important part of the course. To further develop and refine all the key science skills set out in the course, complete the activities in the accompanying *VICscience Biology Skills Workbook.* Signposts to workbook activities are found throughout the textbook. Look for the **Workbook icon**.

WB
REMEMBER
PAGE 22

6 Understand the concepts

Pictures tell a thousand words and are key in strengthening understanding, so ensure you look carefully at each **figure** and read the **labels** and **captions** so that you can understand what it is telling you.

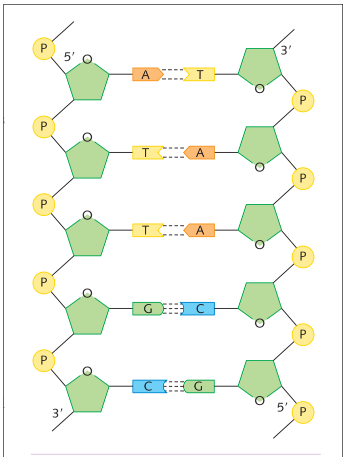
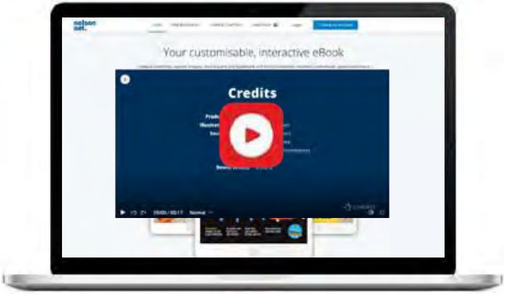
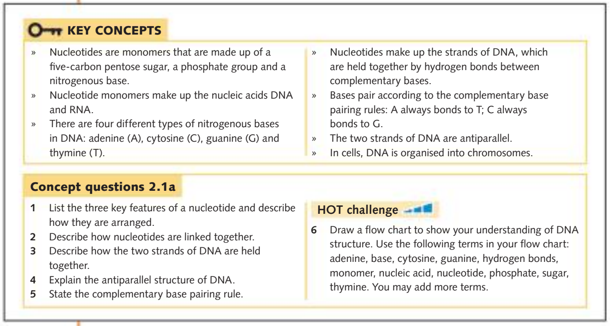


Figure 2.7 The antiparallel nature of DNA: the two strands of DNA run in opposite directions.

Sometimes words and pictures are not enough. Some of the key knowledge has been explained in videos. Look for the **video icons** throughout the chapters as these animated videos will help you to understand and make connections between content.



Important ideas, concepts and theories are summarised in **Key concept** boxes.



KEY CONCEPTS

- Nucleotides are monomers that are made up of a five-carbon pentose sugar, a phosphate group and a nitrogenous base.
- Nucleotide monomers make up the nucleic acids DNA and RNA.
- There are four different types of nitrogenous bases in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T).
- Nucleotides make up the strands of DNA, which are held together by hydrogen bonds between complementary bases.
- Bases pair according to the complementary base pairing rules: A always bonds to T; C always bonds to G.
- The two strands of DNA are antiparallel.
- In cells, DNA is organised into chromosomes.

Concept questions 2.1a

1 List the three key features of a nucleotide and describe how they are arranged.
2 Describe how nucleotides are linked together.
3 Describe how the two strands of DNA are held together.
4 Explain the antiparallel structure of DNA.
5 State the complementary base pairing rule.

HOT challenge

6 Draw a flow chart to show your understanding of DNA structure. Use the following terms in your flow chart: adenine, base, cytosine, guanine, hydrogen bonds, monomer, nucleic acid, nucleotide, phosphate, sugar, thymine. You may add more terms.

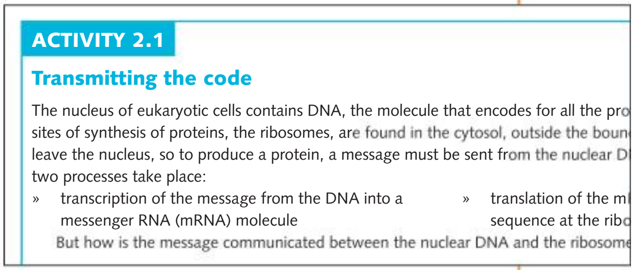
Concept questions follow each key concept box. These questions will help you to determine whether you have fully understood the content before you progress further in the chapter.

If you are feeling confident with the concepts you can give the **HOT Challenge** a go! This question is more difficult and may need further research. It will extend your understanding to a higher level.

7 Explore and learn

You will collaborate, explore and discover the living world through practical activities and investigations and also come to appreciate the collegial nature of Biology.

Complete short, hands-on tasks designed to clarify or reinforce a concept through the **Activity** boxes.



ACTIVITY 2.1

Transmitting the code

The nucleus of eukaryotic cells contains DNA, the molecule that encodes for all the pro
sites of synthesis of proteins, the ribosomes, are found in the cytosol, outside the boun
leave the nucleus, so to produce a protein, a message must be sent from the nuclear D
two processes take place:

- transcription of the message from the DNA into a messenger RNA (mRNA) molecule
- translation of the m
sequence at the ribo

But how is the message communicated between the nuclear DNA and the ribosome

Explore key knowledge and develop, use and demonstrate the key science skills through the **Investigations**. Investigations provide:

- guided instruction on the materials
- method
- collection
- analysis of results
- discussion.

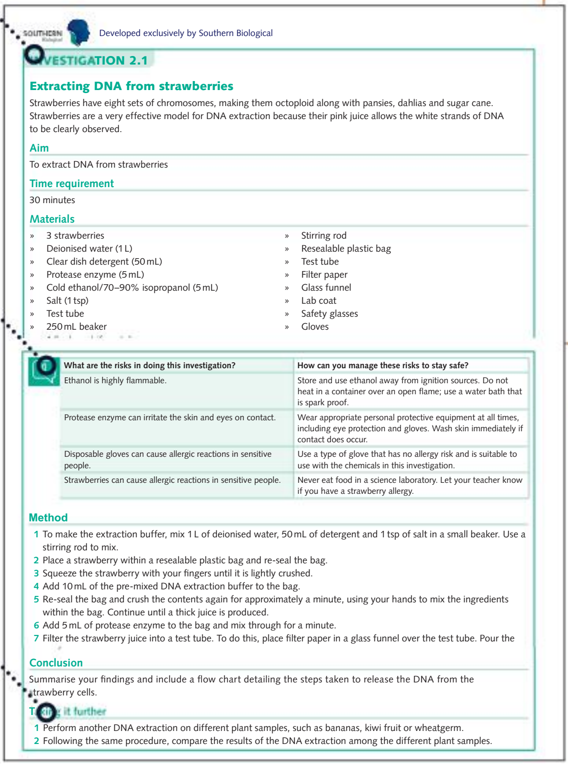
Investigations are not without risks and part of learning to work like a scientist is learning to work safely. **Risk assessment** tables highlight the risks of the investigation and provides suggestions on how you can minimise risks.

Taking it further questions found at the end of some investigations provide you with ideas on how you could extend or adapt the investigation for further study.

Remember that investigations work hand-in-hand with your logbook, which is where you record all your:

- investigation observations
- ideas
- data
- analysis
- discussion and conclusions.

Your logbook is an important assessment and authentication tool.



Developed exclusively by Southern Biological

INVESTIGATION 2.1

Extracting DNA from strawberries

Strawberries have eight sets of chromosomes, making them octoploid along with pansies, dahlias and sugar cane. Strawberries are a very effective model for DNA extraction because their pink juice allows the white strands of DNA to be clearly observed.

Aim

To extract DNA from strawberries

Time requirement

30 minutes

Materials

- 3 strawberries
- Deionised water (1 L)
- Clear dish detergent (50 mL)
- Protease enzyme (5 mL)
- Cold ethanol/70–90% isopropanol (5 mL)
- Salt (1 tsp)
- Test tube
- 250 mL beaker
- Stirring rod
- Resealable plastic bag
- Test tube
- Filter paper
- Glass funnel
- Lab coat
- Safety glasses
- Gloves

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Ethanol is highly flammable.	Store and use ethanol away from ignition sources. Do not heat in a container over an open flame; use a water bath that is spark proof.
Protease enzyme can irritate the skin and eyes on contact.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.
Strawberries can cause allergic reactions in sensitive people.	Never eat food in a science laboratory. Let your teacher know if you have a strawberry allergy.

Method

1 To make the extraction buffer, mix 1 L of deionised water, 50 mL of detergent and 1 tsp of salt in a small beaker. Use a stirring rod to mix.
2 Place a strawberry within a resealable plastic bag and re-seal the bag.
3 Squeeze the strawberry with your fingers until it is lightly crushed.
4 Add 10 mL of the pre-mixed DNA extraction buffer to the bag.
5 Re-seal the bag and crush the contents again for approximately a minute, using your hands to mix the ingredients within the bag. Continue until a thick juice is produced.
6 Add 5 mL of protease enzyme to the bag and mix through for a minute.
7 Filter the strawberry juice into a test tube. To do this, place filter paper in a glass funnel over the test tube. Pour the

Conclusion

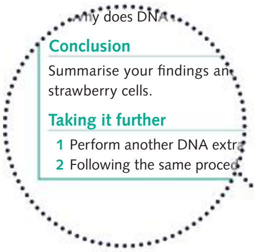
Summarise your findings and include a flow chart detailing the steps taken to release the DNA from the strawberry cells.

Taking it further

1 Perform another DNA extraction on different plant samples, such as bananas, kiwi fruit or wheatgerm.
2 Following the same procedure, compare the results of the DNA extraction among the different plant samples.


Developed e
INVESTIGATION 2.1
Extracting DNA from str
Strawberries have eight sets of chro
Strawberries are a very effective mo
to be clearly observed.
Aim
extract DNA from strawb


What are the risks in doing
Ethanol is highly flammable.
Protease enzyme can irritate the
Disposable gloves can cause
people.
Strawberries can cause al


y does DNA
Conclusion
Summarise your findings an
strawberry cells.
Taking it further
1 Perform another DNA extr
2 Following the same proce

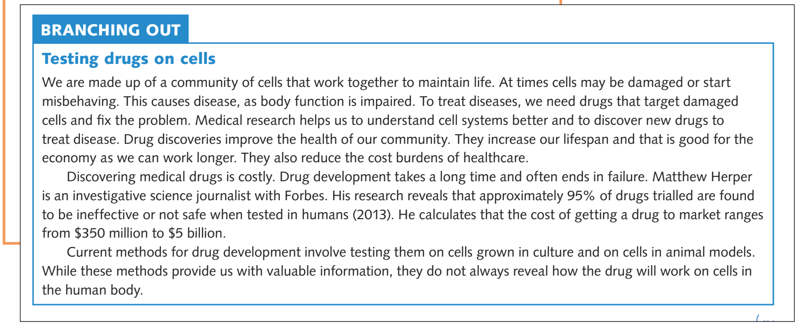
8 Prepare for tests and exams

The best way to prepare for exams is to use past exam questions. **Area of Study reviews** at the end of each Area of Study allow you to check your knowledge by completing difficulty-graded exam-style questions. The answers to these are at the back of the book. Look for **tips** in the margin that point out things to be aware of when answering exam questions.

EXAM TIP
Ensure you know the differences and similarities (compare) between DNA and RNA, their structure and functions.

9 Extend yourself

At the end of each chapter you will find a **Branching out** activity. This material is extension and non-examinable. It examines possible careers and future applications of what you have just learnt.



BRANCHING OUT

Testing drugs on cells

We are made up of a community of cells that work together to maintain life. At times cells may be damaged or start misbehaving. This causes disease, as body function is impaired. To treat diseases, we need drugs that target damaged cells and fix the problem. Medical research helps us to understand cell systems better and to discover new drugs to treat disease. Drug discoveries improve the health of our community. They increase our lifespan and that is good for the economy as we can work longer. They also reduce the cost burdens of healthcare.

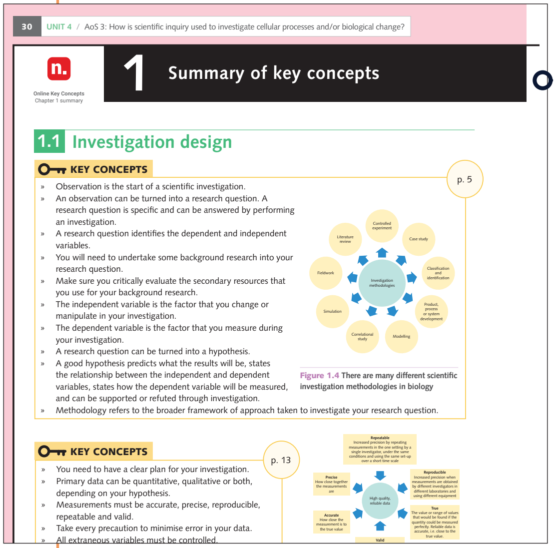
Discovering medical drugs is costly. Drug development takes a long time and often ends in failure. Matthew Herper is an investigative science journalist with Forbes. His research reveals that approximately 95% of drugs trialled are found to be ineffective or not safe when tested in humans (2013). He calculates that the cost of getting a drug to market ranges from $350 million to $5 billion.

Current methods for drug development involve testing them on cells grown in culture and on cells in animal models. While these methods provide us with valuable information, they do not always reveal how the drug will work on cells in the human body.

10 Consolidate your learning

At the end of every chapter you can consolidate your knowledge. Here you will find:

- **summary of key concepts** that you have met throughout the chapter. You can download a copy of the key concepts by accessing the worksheet icon on NelsonNet. Use this to assist you in revising and studying for internal and external assessments
- a **chapter glossary** of all the key terms for the chapter plus their definitions
- **chapter review** questions that will help you to recall, revise, understand and apply the concepts from the chapter. The questions are grouped under headings (**Remembering**, **Understanding**, **Applying**, **Analysing**, **Evaluating** and **Creating**) and reflect the level of thinking required to answer each question. These questions provide you with the practice needed to analyse and answer exam questions.



30 UNIT 4 / AoS 3: How is scientific inquiry used to investigate cellular processes and/or biological change?

Online Key Concepts
Chapter 1 summary

1 Summary of key concepts

1.1 Investigation design

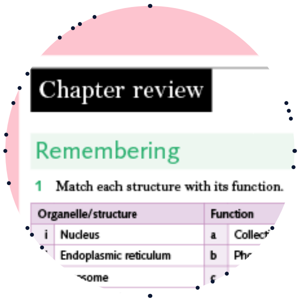
KEY CONCEPTS p. 5

» Observation is the start of a scientific investigation.
» An observation can be turned into a research question. A research question is specific and can be answered by performing an investigation.
» A research question identifies the dependent and independent variables.
» You will need to undertake some background research into your research question.
» Make sure you critically evaluate the secondary resources that you use for your background research.
» The independent variable is the factor that you change or manipulate in your investigation.
» The dependent variable is the factor that you measure during your investigation.
» A research question can be turned into a hypothesis.
» A good hypothesis predicts what the results will be, states the relationship between the independent and dependent variables, states how the dependent variable will be measured, and can be supported or refuted through investigation.
» Methodology refers to the broader framework of approach taken to investigate your research question.

Figure 1.4 There are many different scientific investigation methodologies in biology

KEY CONCEPTS p. 13

» You need to have a clear plan for your investigation.
» Primary data can be quantitative, qualitative or both, depending on your hypothesis.
» Measurements must be accurate, precise, reproducible, repeatable and valid.
» Take every precaution to minimise error in your data.
» All extraneous variables must be controlled.



Designing and conducting a scientific investigation 53

Chapter review

Remembering

1 Match each structure with its function.

Organelle/structure		Function	
i	Nucleus	a	Collecting and packaging centre of the cell
ii	Endoplasmic reticulum	b	Photosynthesis and storage
iii	Lysosome	c	Transport of substances around the cell
iv	Mitochondria	d	Control centre of the cell
v	Golgi apparatus	e	Aerobic respiration, which releases energy to the cell
vi	Chloroplast	f	Breakdown of materials

2 List two natural conditions that might cause plant cells to become plasmolysed.

Understanding

3 Explain what would happen, in terms of the diffusion of water molecules, if an animal cell was placed in a hypotonic solution.
4 A round cell with a diameter of 2 cm has a greater chance of survival than a round cell of 5 cm. Explain why this is so.
5 A student places living cells into a drop of liquid containing a 5% sugar solution. After 30 minutes, the student notices that the liquid contains less than 5% sugar. Explain what has happened.
6 Describe, by means of labelled diagrams, the processes of endocytosis and exocytosis.
7 Explain the importance of turgor to plants.
8 Explain why red blood cells become crenated in a hypertonic solution.

Applying

9 a Select three features that are common to all cells.
  b Describe features that are unique to:
    i prokaryotic cells
    ii eukaryotic cells

Online Resources

FOR THE STUDENT

The NelsonNet website provides you with material that will help you understand, explore, engage and organise the key knowledge and key science skills you have learnt about in your textbook. You will find it at **www.nelsonnet.com.au** and once your registration is complete, you can access digital resources for each chapter to further reinforce your learning. Look on the back cover of this book to find out how to complete your registration.

You will find:

- **NelsonNet ebook** with annotation capability
- **Weblinks** to more information, online interactives and videos
- **Worksheets** for some online videos
- **Downloadable versions** of each chapter map and summary of key concepts
- **Videos** explaining significant concepts
- **Play and say** to help you to pronounce and spell words in the key terms list
- **Flash cards** to assist with remembering and revising key terms and their definitions.

FOR THE TEACHER

Please note that complimentary access to NelsonNet and the NelsonNet ebook is only available to teachers who use the accompanying student textbook as a core educational resource in their classroom.

Contact your sales representative for information about access codes and conditions.

ALL THE EXAM PRACTICE YOU NEED, IN THE ONE PLACE

Research showed that VCE Biology teachers are spending significant time **sourcing quality practice exam questions.**

exam*plus* alleviates this stress with its an extensive bank of **filterable** and **difficulty-graded practice exam questions** and **answers** mapped to the VCE Biology study design for Years 11 & 12.

Choose from thousands of **quality exam style-questions** from past VCAA VCE Biology exams and written by trusted and experienced Nelson authors.

exam*plus* helps students **prepare** for and **succeed** at their **VCE Biology exam** by providing them with **exams/tests/worksheets** customised to their individual needs.

Contact your local sales representative for a demo or to register for a trial.

To the teacher

The VCE Biology course comprises both key knowledge and key science skills. The *VICscience Biology* suite of products provides you with the perfect resource to teach all the key knowledge and key science skills in an integrated way and to prepare your students thoroughly for the school-based and external assessments.

1 Stick to the Study Design

This textbook has been written so all content closely aligns with the VCAA *VCE Biology Study Design* (2022–2026). It has been authored and reviewed by experienced biology teachers, academics and researchers to ensure up-to-date scientific and accurate content for students.

3 Prepare for the exam

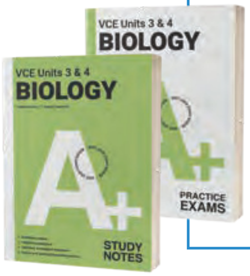
Students of VCE Biology are working toward external assessment at the end of Units 3 & 4. To fully prepare for this exam, students require access to a large number of quality exam-style questions with answers. *VICscience Biology* gives you the full complement including the following.

- **Area of study reviews** at the end of each Area of Study provide students with difficulty-graded multiple-choice and short-answer questions that have been adapted from VCAA exam questions with answers provided at the back of the book.
- You will also find a full practice **end-of-year exam with answers**.
- *VICscience Biology VCE Units 3 & 4 Skills Workbook* provides students with difficulty-graded multiple-choice and short-answer exam questions that have been adapted from VCAA exam questions.

2 Access differentiated material

Differentiation is built into each chapter to assist you in helping those students that may struggle with content or skill development and extending those students who want to achieve at a higher level.

- **Chapter maps** provide students with a gentle and visual introduction to each chapter enabling students to engage with the chapter content prior to entering the chapter.
- **Key terms** at the beginning of each chapter present all the bolded key terms throughout the chapter in one place. Students can use the **flashcards** study tool to learn and review key terms with their definitions, and assist with pronunciation and spelling of key vocabulary.
- **Remember** provides students the opportunity to recall concepts previously learnt that will be revisited during the chapter.
- **Concept questions** are pitched to be lower-order questions to assist with learning consolidation but end with **HOT Challenge** questions for those students who would benefit from answering higher-order questions.
- **Investigations** end with a **Taking it further** section, which provides ideas on how the investigation can be extended.
- **Weblinks** to external, vetted websites provide extra information; worksheets are provided for some weblinks.
- Each chapter finishes with a **Branching out** activity. This activity provides an extension activity for students who are looking for more information on a particular topic.



- **exam*plus*** simulates real exam-practice and comprises thousands of unseen exam-style and past VCAA exam questions with answers to use in your teaching. Simply select your questions for a quiz, topic test, or practice exam and **exam*plus*** generates a practice test or exam.
- Consider bundling ***A+ Study Notes*** and ***Practice Exams*** with your VICscience Biology booklist for the most economical solution for students' exam preparation and readiness.

4 Support for the teacher

There is a wealth of teacher support materials on the teacher NelsonNet site that accompanies this product. These include:

- **Cognero Assess** comprising auto-correcting multiple-choice questions and short-answer questions with answers to be shared with your students for every chapter
- **answers** to all textbook questions, investigations and Branching out activities
- sample SACs with suggested marking schemes
- **practice end-of-year exam with answers**
- **teaching plans** for every chapter showing how all the components of the *VICscience Biology* suite are integrated to provide your students with a thorough and complete learning experience designed to prepare them for internal and external assessment
- support for the investigations provided through **Southern Biological**.

DEVELOPED EXCLUSIVELY BY SOUTHERN BIOLOGICAL

ACCESS TO QUALITY INVESTIGATIONS

Practical work is a central component of the VCE Biology course and crucial in developing key science skills. The study design specifies the number of hours that students need to spend undertaking practical work. **Southern Biological and Cengage have partnered** to ensure that you and your students are provided with exciting and current practical investigations to introduce, reinforce and practise the key science skills listed in the *VCAA VCE Biology Study Design 2022–2026*, pages 7–8. Some of the investigations written by Southern Biological are exclusive to Cengage, and all investigations have been rigorously stress-tested by Southern Biological to ensure that they will work in your classroom.

Each investigation is accompanied by a risk assessment table that highlights risks to students or others posed by the materials or method. Teachers are expected to amend each table in the case of substitutions or in the case of any additional risks. This may mean obtaining and following Safety Data Sheets (SDS) for certain chemicals. All teachers are required to follow the safety guidelines of their specific school and associated government legislation when students are in their care.

Investigation support provided by Southern Biological include:

- **suggested answers** to investigation questions
- **videos to assist teachers** and laboratory technicians to prepare and deliver the investigations to students providing them with optimal hands-on experience
- **videos aimed at students** to assist with undertaking the investigations including suggested answers and hints
- **risk assessments** for investigations where applicable
- resourcing, safety and investigation **preparation sheets.**

Study design

Unit	Area of study	Chapters										
		1	2	3	4	5	6	7	8	9	10	11
3. How do cells maintain life?	**1: What is the role of nucleic acids and proteins in maintaining life?** Students explore the expression of the information encoded in a sequence of DNA to form a protein and outline the nature of the genetic code and the proteome. They apply their knowledge to the structure and function of the DNA molecule to examine how molecular tools and techniques can be used to manipulate the molecule for a particular purpose. Students compare gene technologies used to address human and agricultural issues and consider the ethical implications of their use. (p. 28 Study Design)		x	x								
	2: How are biochemical pathways regulated? Students focus on the structure and regulation of biochemical pathways. They examine how biochemical pathways, specifically photosynthesis and cellular respiration, involve many steps that are controlled by enzymes and assisted by coenzymes. Students investigate factors that affect the rate of cellular reactions and explore applications of biotechnology that focus on the regulation of biochemical pathways. (p. 30 Study Design)				x	x						
4. How does life change and respond to challenges?	**1: How do organisms respond to pathogens?** Students focus on the immune response of organisms to specific pathogens. Students examine unique molecules called antigens and how they elicit an immune response, the nature of immunity and the role of vaccinations in providing immunity. They explain how technological advances assist in managing immune system disorders and how immunotherapies can be applied to the treatment of other diseases. (p. 33 Study Design)						x	x	x			
	2: How are species related over time? Students focus on changes to genetic material over time and the evidence for biological evolution. They consider how the field of evolutionary biology is based upon the accumulation of evidence over time and develop an understanding of how interpretations of evidence can change in the light of new evidence as a result of technological advances, particularly in molecular biology. Students consider the biological consequences of changes in allele frequencies and how isolation and divergence are required elements for speciation. They consider the evidence for determining the relatedness between species and examine the evidence for major trends in hominin evolution, including the migration of modern human populations around the world. (p. 35 Study Design)									x	x	x
	3: How is scientific inquiry used to investigate cellular processes and/or biological change? Students undertake a student-designed scientific investigation in either Unit 3 or Unit 4, or across both Units 3 and 4. The investigation involves the generation of primary data relating to cellular processes and/or how life changes and responds to challenges. The investigation draws on knowledge and related key science skills developed across Units 3 and 4 and is undertaken by students in the laboratory and/or in the field. (p. 36 Study Design)	x										

Designing and conducting a scientific investigation

1

By the end of this chapter you will have covered the following material.

Key knowledge

Investigation design

- biological concepts specific to the selected scientific investigation and their significance, including definitions of key terms, pp. 4, 32–33
- characteristics of the selected scientific methodology and method, and appropriateness of the use of independent, dependent and controlled variables in the selected scientific investigation, pp. 8–11; 14–15; 23–24
- techniques of primary quantitative data generation relevant to the selected scientific investigation, pp. 12–13; 23–24
- the accuracy, precision, reproducibility, repeatability and validity of measurements, pp. 12–14
- the health, safety and ethical guidelines relevant to the selected scientific investigation, pp. 16–18; 23–24

Scientific evidence

- the nature of evidence that supports or refutes a hypothesis, model or theory, pp. 22; 23–24
- ways of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and uncertainty, pp. 19–21; 23–24
- authentication of generated primary data through the use of a logbook, pp. 18–19; 23–24
- assumptions and limitations of investigation methodology and/or data generation and/or analysis methods, pp. 9–14; 18–21

Science communication

- conventions of science communication: scientific terminology and representations, symbols, formulas, standard abbreviations and units of measurement, pp. 18–20; 23–24
- conventions of scientific poster presentation, including succinct communication of the selected scientific investigation and acknowledgements and references, pp. 25–29
- the key findings and implications of the selected scientific investigation, p. 28

Online Chapter Map
Chapter 1 map

1 Designing and conducting a scientific investigation

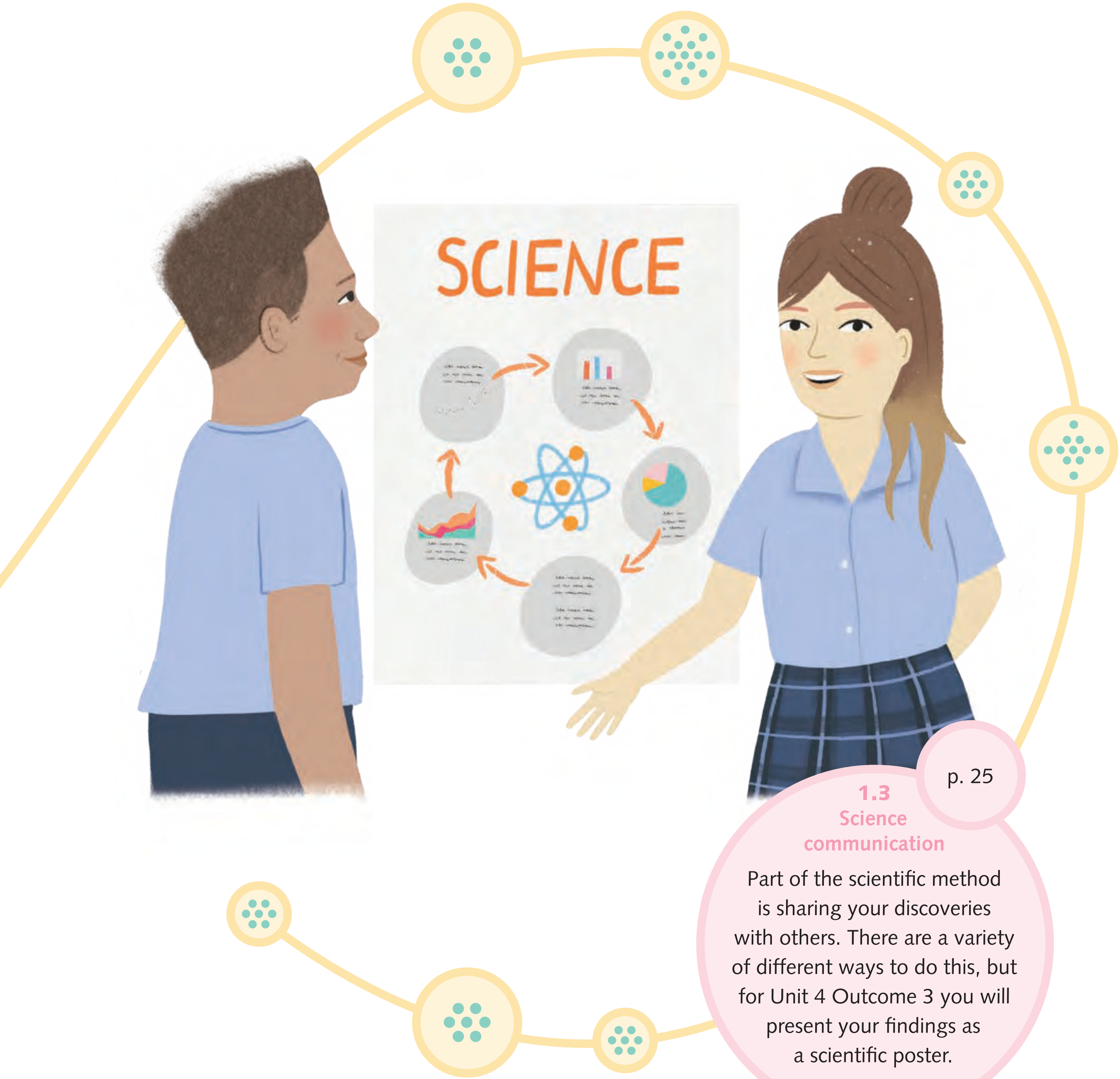
Scientists look curiously at the world around them and ask 'why?'. The answer to this question leads to other questions such as 'how?', 'what?' and 'when?'. Science involves investigation, asking questions and designing ways to find answers. For Unit 4 Outcome 3, you will be working in this way to find answers.

1.1 Investigation design p. 5

All scientific investigations begin with observations and questions, 'what if…?', 'why did that happen?' or 'how did it do that?'. The scientific method provides scientists with a well-tested process to follow. It ensures that the investigation is designed to answer the specific question asked.

1.2 Scientific evidence p. 18

In order to answer the question that you have asked, you need to collect data and, more importantly, find out what this data is telling you. This enables you to draw an evidence-based conclusion.



p. 25

1.3
Science communication

Part of the scientific method is sharing your discoveries with others. There are a variety of different ways to do this, but for Unit 4 Outcome 3 you will present your findings as a scientific poster.

Using the scientific method is integral not only in science but also in other aspects of your life. It provides you with a well-tested process to design investigations, collect data and draw evidence-based conclusions.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 1 map (p. 2)

Online Key Terms:
- Chapter 1 flashcards (p. 4)

Weblinks:
- Howard Florey (p. 5)
- APA style guide (p. 7)
- Harvard system (p. 7)
- CSIRO (p. 7)
- Australian Academy of Science (p. 7)
- CRAAP test (p. 7)
- Sterile technique (p. 17)

Online Key Concepts:
- Chapter 1: Summary of key concepts (p. 30)

Online Key Terms
Chapter 1 flashcards

Know your key terms

accurate
authentication
beneficence
bias
control group
controlled variable
dependent variable
ethics
extraneous variable
gradient
hypothesis
independent variable
integrity
justice
logbook
method
methodology
model
non-maleficence
observation
outlier
personal error
precise
primary data
primary source
qualitative data
quantitative data
random error
references
reliable
repeatable
replicates
reproducible
research question
respect
risk assessment
secondary data
secondary source
systematic error
theory
true value
uncertainty
valid
variable

Remember

REMEMBER
PAGE 2

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1. The scientific method is a procedure by which scientists can examine a theory and test a hypothesis.
2. Scientists perform investigations, take careful observations or use models and simulations to gain evidence to either support or refute their hypothesis.
3. When designing a scientific investigation, you should always consider safety and the ethical implications of your work.
4. A well-designed scientific investigation includes relevant controls and clearly defined variables.
5. Scientists keep carefully detailed records of their observations, their ideas and their data in a logbook.

VCE Biology Unit 4 Area of study 3 Outcome 3 requires students to use the key science skills to plan, design, undertake and communicate an investigation that investigates cellular processes and/or biological change. This investigation can be undertaken during studies of Unit 3 or Unit 4 or could be completed across both Units 3 and 4. To complete this outcome, you need to have a good understanding of the key science skills. You will find these listed in the *VCE Biology Study Design* (pp. 7–8).

This chapter will explain and illustrate these key science skills, and you will be able to use this knowledge to help you plan, design, undertake and communicate your investigation. You should always refer to the *VCE Biology Study Design* Unit 4 Area of study 3 (pp. 36–37) when planning your investigation to make sure you cover all the requirements set out in the Study Design.

1.1 Investigation design

Weblink
Howard Florey

The most important question you should ask when you begin this outcome is 'What am I going to investigate?' Remember that you can draw the idea for the investigation from Unit 3 or Unit 4 so be aware of this as you work through these units in class or at home. If you find something particularly interesting, then you might be able to develop it into an investigation that satisfies all the VCAA criteria.

As part of your assessment, you will need to maintain a **logbook**. (See p. 10 of the *VCE Biology Study Design*.) The logbook will be discussed in more detail on pages 18–19, but at this point it would be a good idea to set aside some pages in your logbook to devote to your investigation. Start with writing down ideas that could form part or all of your investigation.

WB
1.1.1
OBSERVATION
PAGE 3

Observation

Observation plays a critical part in any scientific investigation (Figure 1.1). It is usually the start of most investigations (Figure 1.2). You can observe through any of your senses – sight, smell, touch, hearing or taste.

If you observe something unusual or unexpected, you might wonder why and want to investigate it. This is exactly what Scottish scientist Alexander Fleming did in 1928. He was working at St Mary's Hospital in London, where he set up culture plates of *Staphylococcus* bacteria, which cause serious infections, particularly in people with weakened immune systems (such as hospital patients). He then went on a two-week holiday and, on his return, noticed that a mould had grown on the plates. Fleming observed that this mould was


Shutterstock.com/Likoper

Figure 1.1 Observation plays a critical part in any scientific investigation.

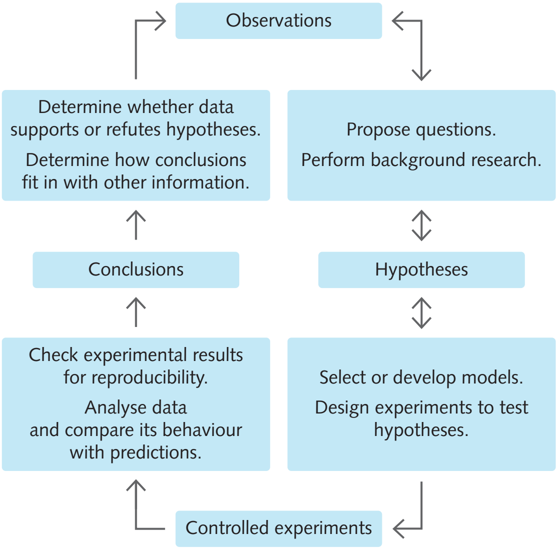
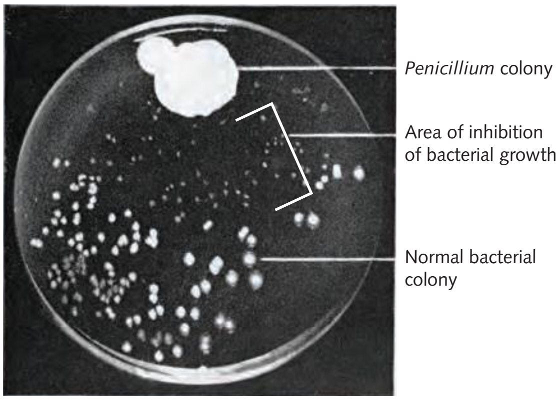


Figure 1.2 Steps in a scientific investigation



Alamy Stock Photo/Interfoto

Figure 1.3 Alexander Fleming observed that bacterial colonies did not grow near the *Penicillium* colony. He investigated further to find out why, which led to the discovery of the first antibiotic – penicillin.

inhibiting the growth of the *Staphylococcus* bacteria (Figure 1.3). This observation led to the development of the world's first antibiotic – penicillin. Imagine if he had just consigned those culture plates to the bin without observing them first. Millions of lives have been saved as a result of that one observation.

Another way to decide upon a topic is to pinpoint something that you find interesting in your course content. You could design a practical investigation to undertake further research on that topic.

Research question

Once you have decided on a topic for your investigation, or a list of topics that you can narrow down, you will need to come up with a research question.

An effective **research question** is specific and can be answered by performing your investigation and taking measurements with the resources and equipment that are available to you. A research question could be in the form 'What effect does a new fertiliser have on root growth?'. The aim of your research is to answer the question. It is important that you develop and frame the research question carefully.

Your research question needs to be specific enough that it guides the design of the investigation. Asking 'Does a new fertiliser increase root growth more than a standard fertiliser?' tells you what you will be varying (fertiliser) and what you will be measuring (root growth). It also gives a criterion for judging whether your results have answered the research question. If there is more root growth with the new fertiliser than with the standard fertiliser, then the results answer your research question positively. If there is not more root growth with the new fertiliser than with the standard fertiliser, then your results answer your research question negatively. This does not mean that your results are wrong. It just means the results showed that there was *not* more root growth with the new fertiliser.

Asking 'How can we optimise root growth?' is not a good question. This question does not say what will be varied, nor does it tell you when you have answered the question. The term 'optimise' is too vague and subjective.

Finally, a good research question should be feasible: you should be able to answer it with the time and equipment available.

A good research question identifies the independent and dependent **variables** that will be investigated. It asks what effect your proposed independent variable will have on your proposed dependent variable. Variables are factors or features that you vary on purpose or measure as part of your investigation and are discussed in more detail later in this chapter (p. 8).

Background research

Before you can turn your research question into a hypothesis, you need to undertake some background research on your topic and research question. This background research will inform you about what is already known about this topic and will probably lead you to change or refine your research question.

Primary sources

Primary sources of information contain original research; for example, findings within articles in scientific journals. The advantages of these sources are that they contain data from investigations for a specific purpose. They provide the background, method, results and discussion of the investigations. The disadvantage of these sources is that they tend to use a lot of highly technical language that can be difficult to understand.

Secondary sources

Secondary sources of information summarise, review or analyse primary sources. Secondary sources include reviews of the work of other scientists, and some periodicals and even textbooks. In general, secondary sources are written in a more accessible way. You may also be able to get an overview and understanding of a whole field of study through the one article.

Professional scientists consult both types of sources but prefer to read primary sources when planning their investigations. This helps them to develop a deep understanding of exactly what was done in the investigation, the **methodology** that was used and the method that was followed.

Table 1.1 lists the variety of sources of information that a scientist can use when undertaking background research.

Table 1.1 Types of information sources

Primary sources	Secondary sources
Scientific journals or periodicals Research reports Sessions presented at scientific conferences Patents Masters and PhD theses	Review journals or periodicals that summarise recent research Reliable websites, e.g. Nature.com Textbooks

Referencing source material

Keep a list in your logbook of all the primary and secondary source reference material that you use to research your research question. It is better to do this as you go than to have to go back later on and try to find out where you sourced the material.

There are several ways to reference material but the ones usually used in scientific research are the American Psychological Society (APA) style or the Harvard system. (See weblinks.) Remember that **references** refer to sources that you cite in the write-up of your scientific investigation. A bibliography is different because it collates a list of references that you may have consulted but not necessarily sources that you cited in the write-up. Make sure you check with your teacher about the preferred style of referencing.

Weblinks
APA style guide

Harvard system

CSIRO

Australian Academy of Science

CRAAP test

Evaluating source material

Be critical of what you read. Do not assume that everything you read online or even in books is true. Try to find **reliable** sources of information (Table 1.2, p. 8). Textbooks, websites from universities and government research agencies are usually very reliable. Publications and web pages from professional organisations such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Academy of Science (see weblinks), and equivalent international organisations are also good sources.

Websites containing student research such as science fair projects are not always reliable, although they can be useful for getting ideas. Online sources that try to sell you something or promote a particular point of view should be treated sceptically. If there is clear **bias** or a limited perspective contained in the information, then you should avoid using that information.

Talk to your teacher or librarian about sources of information. Your library may also have access to databases containing scientific journal articles. Your teacher or librarian will be able to help you to assess whether a website is reliable and suggest suitable sites. The CRAAP (Currency, Relevance, Authority, Accuracy and Purpose) test will provide you with a list of questions to help evaluate the resources that you are using. (See weblink.)

Table 1.2 Features of reliable and unreliable information sources

Reliable sources	Unreliable sources
Contain current information and seek to inform the reader Contain information that is relevant to your project or inquiry Are from a reputable source such as a university or scientific research institution Contain information that is likely to be accurate, e.g. a peer-reviewed journal article Avoid bias	Are not from reputable sources Present obvious bias Do not contain references for their claims, or they provide links to unscientific references Have not been updated regularly Contain unrelated advertising content Use vague terminology

WB
1.1.2
DESIGNING AN INVESTIGATION
PAGE 5

Variables

Once you have decided on your research question and done your background research, it is time to consider how to design an investigation to answer your research question. Before you do this, you need to understand variables. There are different types of variables.

Independent variable

The **independent variable** is the factor that you change or manipulate in your investigation. For example, if your research question was 'Does a new fertiliser increase root growth more than a standard fertiliser?', then your independent variable would be *type of fertiliser*. You would be using two different types of fertiliser and measuring their effect on root growth.

Dependent variable

The **dependent variable** is the factor that you measure during an investigation. For the above research question, the factor that you would be measuring is *root growth*, so this is your dependent variable.

Another example is a scientist testing the effect of water temperature on seed germination in *Acacia* seeds. The research question would be 'Do different temperatures of water affect the germination of *Acacia* seeds?'. The independent variable would be the temperature of the water poured onto the *Acacia* seeds and the dependent variable would be the number of *Acacia* seeds that germinate.

Usually your investigation will have one dependent variable and one independent variable. The investigator will change the independent variable and measure the influence on the dependent variable.

Hypothesis

If your research question requires a controlled experiment methodology, then your next step is to turn your research question into a hypothesis. A **hypothesis** is a tentative prediction, or a tentative explanation of an observation, based on an existing **model** or **theory**. A hypothesis should give you a prediction that you can test by performing an investigation. This means it should at least be falsifiable; that is, it should be able to be refuted (shown to be wrong). However, you will not generally be able to claim that you have proved your hypothesis. Rather, you may be able to say at the end of the study that your results support your hypothesis. Hence, an aim for an investigation should not start 'To prove …' because it is not possible to prove a hypothesis, only to refute it. If your investigations agree with predictions based on your hypothesis, then you can claim that they support your hypothesis. This increases your confidence in your model, but it does not prove that it is true.

A well-designed research question and hypothesis will guide your inquiry and help you to understand whether or not you have met the aims of your investigation (Table 1.3).

Table 1.3 Features of an effective research question and hypothesis

	Effective research question	Effective hypothesis
Phrasing	Framed as a question Specific and includes scientific terminology	Framed as a prediction based on your background research Specific and includes scientific terminology
Use of variables	Includes mention of the independent and dependent variables, e.g. 'How does the independent variable effect the dependent variable?'	Includes mention of the independent and dependent variables, e.g. 'If there is a change in independent variable, then this will result in a predicted trend in the dependent variable.'
Predicts results	No	Yes
Outcome	Can be answered by an investigation	Can be supported or refuted by an investigation

For example, using the research question 'Does a new fertiliser increase root growth more than a standard fertiliser?', the hypothesis could be: '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase in length.' You will note that this hypothesis meets all the requirements set out in Table 1.3. This hypothesis:

» predicts what the result will be (root growth will increase)
» is specific in that it states: if … *the independent variable is changed* … then … *something will happen to the dependent variable* …
» mentions the independent (fertiliser) and dependent (root growth) variables
» states how the dependent variable will be measured (root growth measured in length)
» can be supported or refuted by an investigation.

Methodologies

WB
1.1.3 METHODOLOGIES PAGE 6

Methodology refers to the broader framework of approach taken in the investigation to test your research question. If you wanted to test the effect of temperature on enzyme action, then you would choose a controlled experiment methodology. If you wanted to identify and name all the plant species in a particular area, then you would choose a classification and identification methodology. For the list of methodologies that are considered in VCE Biology, see pages 9–10 of the *VCE Biology Study Design*. These are summarised in Figure 1.4. Some of these methodologies can be carried out in the school science laboratory, others in the field (called fieldwork) and others in the computer lab using secondary sources or databases. The methodology that you choose depends on your research question. Table 1.4 gives you an idea of different methodologies that would be suitable for investigating different research ideas.

Figure 1.4 There are many different scientific investigation methodologies in biology.

Table 1.4 Types of methodologies and investigations (Not all methodologies mentioned in the *VCE Biology Study Design* have been included. Refer to the *VCE Biology Study Design* pages 9–10 for a full explanation of each methodology.)

Methodology	Where you would carry out your investigation		
	In the science laboratory	**In the field**	**In the computer lab**
Case study: research into a real or hypothetical situation that mirrors real life	Investigating the causes of recent environmental damage in an ecosystem		
Classification and identification: arranging and identifying individuals within a group	Sorting DNA from different species by gel electrophoresis Characterising DNA samples by restriction enzymes and gel electrophoresis	Surveying, classifying and identifying organisms in a particular habitat	Examining characteristics of fossils – e.g. hominin fossils from a museum database Comparing three-dimensional models (including ribbon diagrams) and/or amino acid sequences from protein databases
Controlled experiment: an experimental investigation that looks at the relationship between the independent and dependent variables, making sure all other extraneous variables are controlled	Enzymatic activity – e.g. examining the effects of pH, temperature and substrate concentrations on the rate of reaction; comparing commercial enzymes with those extracted from nature; comparing enzyme activity across different species (e.g. catalase in animal tissues and plant tissues) Cellular respiration – e.g. examining factors that affect the rate of cellular respiration (reactant concentration, enzyme activity, pH, age or subspecies/variety, such as comparing the rate of anaerobic respiration in baker's and brewer's yeast varieties) Photosynthesis – e.g. examining factors that affect the rate of photosynthesis (reactants, enzymes, pH); examining photosynthesis in different organisms; chromatography of different photosynthetic pigments		Determining evolutionary relationships between species by phylogenetic analysis of protein sequences Comparing genomes or chromosome maps from populations of organisms
Correlational study: a study to understand the relationship between two or more factors	Collecting leaves from different areas to discover if the number of chloroplasts in plant cells differs with environmental conditions		
Fieldwork: observing and recording observations beyond the classroom		Bioprospecting for useful antimicrobial substances (e.g. in native or indigenous flora) and their effectiveness against a range of bacteria	
Literature review: collation and analysis of **secondary data**			Surveying global distribution patterns of populations over time Analysing global health statistics – e.g. antibiotic resistance rates for particular microbial species and infection outcomes Conducting an epidemiological study of vaccination rates and infectious disease rates Systematically reviewing published studies – e.g. whether vitamin C cures the common cold

Methodology	Where you would carry out your investigation		
	In the science laboratory	**In the field**	**In the computer lab**
Modelling: constructing a physical, conceptual or mathematical model to represent a biological object, system or variables	Making a model to show how materials move through the plasma membrane		Making a computer model to show how proteins move through the protein secretory pathway Making a model to predict the impacts that an environmental policy has on an ecosystem
Simulation: using a model to study a biological system			Creating a simulation to show enzyme action in a number of different conditions, such as increased substrate or amount of enzyme

KEY CONCEPTS

- Observation is the start of a scientific investigation.
- An observation can be turned into a research question.
- A research question is specific and can be answered by performing an investigation.
- A research question identifies the dependent and independent variables.
- You will need to undertake some background research into your research question.
- Make sure you critically evaluate the secondary resources that you use for your background research.
- The independent variable is the factor that you change or manipulate in your investigation.
- The dependent variable is the factor that you measure during your investigation.
- A research question can be turned into a hypothesis.
- A good hypothesis predicts what the results will be, states the relationship between the independent and dependent variables, states how the dependent variable will be measured, and can be supported or refuted through investigation.
- Methodology refers to the broader framework of approach taken to investigate your research question.

Concept questions 1.1a

1 Define:
  - **a** research question
  - **b** independent variable
  - **c** dependent variable
  - **d** hypothesis.

2 What determines the type of methodology that you would use in your investigation?

HOT challenge

3 What are the three main features of a good hypothesis?

Designing your investigation to test your hypothesis

WB
1.1.4
DESIGNING YOUR INVESTIGATION TO TEST YOUR HYPOTHESIS
PAGE 7

Once you have decided on a specific research question, hypothesis and methodology, you can begin to design your investigation. This information should be recorded in your logbook. (See the information about maintaining a logbook on p. 18.) Having a plan ensures that you take the measurements that you need. The longer the investigation, the more important it is that you have a clear plan. There are several things to consider.

- What data will you need to collect?
- When and where will you collect the data?
- How will you analyse the data?
- What materials and equipment will you need?
- What are the independent and dependent variables?
- Have you identified all the variables that could influence your results (p. 8)?

Note:

Unit 4 Area of Study 3 Outcome 3 specifies that your scientific investigation must generate primary quantitative data.

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Figure 1.5 Plan exactly what you will measure to collect your data. Do you want to measure the length of roots or the mass of roots? Where do the roots end? Will you use fresh weight or dry weight?

Primary data

Primary data is data that you collect in an investigation. Generating primary data involves collecting quantitative and/or qualitative data.

Quantitative data is data that is a quantity and is recorded numerically. You measure the numerical value in the appropriate units. For example, you may measure root length in centimetres or mass of roots in grams.

Qualitative data is non-numerical and can be directly observed; it is a quality. For example, you may observe that when you add chemical X to chemical Y, a colour change of red to green occurs.

Sometimes you use a combination of qualitative and quantitative data. For example, you may describe the length of roots as reaching a maximum in centimetres (quantitative) but growing in a particular direction or pattern (qualitative).

As discussed earlier, your hypothesis should specify your dependent variable – what you are quantitatively measuring. In the case of the hypothesis '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase', you will be measuring root growth. You need to determine how you are going to measure root growth (Figure 1.5). Will it be by length or by mass? If it is by length, then which unit will you use (millimetres or centimetres), and where does the root start and where does it end? Are you measuring all the roots, or only the longest? If you are measuring by mass, you will also need to decide which unit you are going to use (milligrams or grams), where the root starts, and if you are going to use wet or dry mass (all the water removed). You need to make all these decisions before you carry out your investigation.

Keep a record of all of your planning in your logbook. This is useful information as your research project evolves and is needed for authentication purposes. Writing down what you plan to do, and why, will also help you to stay focused during the investigation.

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1.1.5
PRIMARY DATA
PAGE 9

Quality of primary data

Plan your investigation to generate the highest quality data. A well-designed investigation allows you to collect data that is accurate, precise, repeatable, reproducible and valid (Table 1.5 and Figure 1.6). This means you can rely on the data to draw conclusions, and be confident that a difference between one measurement and another reflects a real change in what is being measured.

Measurements are **valid** if they measure what is supposed to be measured. Validity of measurement is important because in a well-designed investigation, results are affected only by one single independent variable. Only investigations in which all extraneous variables have been controlled will produce valid results (p. 14). If the results are similar each time, then your results are more likely to be both valid and reliable.

A measurement is **accurate** if it is close to the **true value** of the parameter being measured. You will also aim to collect **precise** measurements; that is, repeated measurements that are close to each other. For example, you might measure the length of plant roots of five plants under the same conditions and record the following measurements: 15.2 cm, 16.1 cm, 127.9 cm, 14.9 cm and 16.7 cm. The 127.9 cm measurement stands out as not being close to the other measurements and you would have to ask whether the 127.9 cm measurement is precise.

If a result is not repeatable by you or reproducible by others, it is probably not a valid result. A result is **repeatable** if the same measurement (within the limits of experimental uncertainty) is made more than once by the same investigator using the same equipment under the same conditions. A result is **reproducible** if another investigator, following your method, obtains data that replicates the effect you observed and leads them to the same conclusion as yours, even if there are some small differences between your results and theirs (e.g. due to the different equipment used to take the measurements). If a result is not repeatable or reproducible, then a variable other than the one you are controlling is affecting its value. If this is the case, you need to identify the other variable and control it if possible.

Table 1.5 Quality of data

Valid	Does it measure what it is supposed to measure?
Precise	How closely do individual measurements agree with each other?
Accurate	Has the data been measured and recorded correctly?
Repeatable	Can the same investigator use the same investigation and equipment and get the same result?
Reproducible	Can another investigator use the same method and equipment and get a similar result?

Sometimes investigations simply do not work or cannot be done for some reason, such as equipment failure or unforeseen variables. For example, root growth will be affected if the plants contract a disease during the investigation. Try to think of all the things that could go wrong. If possible, come up with back-up plans. Allowing plenty of time helps as does starting your investigations as early as possible.

Think about how you can minimise **uncertainty**. Experimental uncertainty is the doubt associated with the value derived from measuring a variable, usually affected by the equipment used to take that measurement. For example, are your scales working properly, or has your tape measure stretched?

Make sure you have allowed time for analysis. Do as much analysis as you can while you collect results. If you plot graphs as you take measurements, then you will be able to identify outliers early. An **outlier** is a data point that does not fit the pattern of the rest of the data and may distort the data, acting as a source of error. If you identify an outlier while you still have access to equipment and space, then you can check the measurement and make sure that you did not make a mistake or that the investigation has not been compromised by an **extraneous variable** (p. 14).

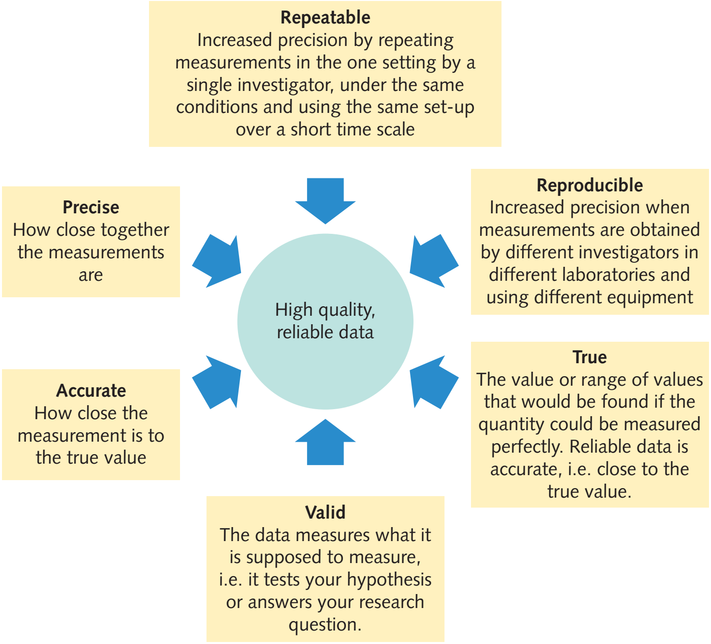

Figure 1.6 Features of data from a well-designed investigation

With these things in mind, you may need to consider the number of **replicates** to include in your investigations. These are independent samples that allow you to take multiple measurements, increasing the reliability of your data. In the root growth example, growing 10 plants in each experimental condition allows you to calculate an average value as well as the variation between values in your sample set. If the variation is small, it is likely that only one independent variable is acting in your investigation and your results are more likely to be reliable.

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1.1.6
MINIMISING
ERROR
PAGE 10

Minimising error

There are various causes of errors in investigations. For example, errors can be due to investigators' mistakes or desire to get particular results, equipment errors or if experimental subjects are not randomly assigned to experimental groups.

Personal errors arise as a result of investigators' mistakes or miscalculations.

Systematic errors are predictable errors that arise through imperfections in the equipment used to take the measurements. They cause measurements to differ from the true value by a consistent amount each time a measurement is made. To minimise systematic errors, you first need to make sure that all your equipment has been calibrated and tested. Calibration ensures that the equipment gives the correct readings with known standards. For example, scales can be calibrated by using known weights. The equipment should at least be calibrated at the top and the bottom of its range. Instructions for calibrating equipment correctly can be obtained from your teacher, laboratory technician or the user manual for the equipment.

Random assignment of subjects into experimental and control groups is an important part of the design of an investigation. For example, in a clinical trial of a new drug, patients should be randomly assigned to the **control group** or the drug (experimental) group with equal representation in both groups of age, gender, ethnicity and other variables. Clinical trials are also designed as double-blind studies in which neither the patient nor the nurse or doctor treating the patient knows which group they have been assigned to. These steps are essential for reducing bias.

Even in a well-planned investigation, **random errors** can occur. These are variations in the data and result in less precise measurements. The influence of random errors can be reduced by using multiple trials or samples (replicates) and ensuring that your investigation is repeatable.

When the person conducting the investigation and making the measurements has particular expectations about the results, this can introduce bias in the study. An example of bias in an investigation is an investigator choosing the tallest or healthiest plants to treat with the new fertiliser; these plants might give the biggest growth measurements at the end of the study. When an investigation is biased, the results are not valid and no conclusions can be made from the investigation.

For every step of your investigation, try to identify possible sources of error and come up with ways of eliminating systematic, random and personal errors, and incorporate these into your investigation design. If you make a mistake during your investigation, then you should repeat the investigation. For example, it is good planning to make sure that you have germinated enough seeds so that you have extra plants in case you need to repeat the investigation.

Science Photo Library/Cultura/Monty Rakusen

Figure 1.7 **Keeping conditions as consistent as possible helps to control the extraneous variables.**

Controlled variables

Consider the hypothesis: '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase in length'. You may already have realised that there are a few more things that can influence root growth other than type of fertiliser. For example, root growth could also be influenced by the species of plant, amount of water the plants receive, the amount of sunlight, the type of soil they are growing in and how much wind they are exposed to. These other things that could affect your results are extraneous variables and they need to be controlled or kept constant (Figure 1.7). By controlling all other variables, you are able to obtain baseline data that shows that only your independent variable is influencing the results, not any other extraneous variable (Figure 1.8).

An extraneous variable must become a **controlled variable** by identifying it and keeping it constant during the investigation. If this happens, then it does not affect the

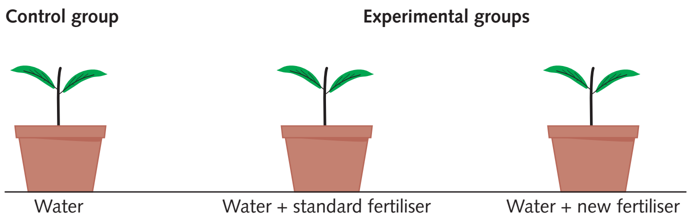


Figure 1.8 It is important to control all the extraneous variables so that only the independent variable influences the dependent variable. The following should be the same in the two groups: size of plants, potting mix, exposure to sunlight and other environmental factors (e.g. atmosphere, wind).

interpretation of the relationship between the dependent and independent variables. In the root growth investigation example, controlled variables would include plant type, plant health, growing medium, planting depth, light exposure, water available to plants, air circulation and the temperature of the growing medium. These are all potential confounding factors that would need to be controlled; that is, kept constant.

Table 1.6 summarises the different types of variables in an investigation.

Table 1.6 Types of variables

Type of variable	Definition
Independent variable	The variable that is changed or manipulated by the investigator. It is assumed to have an effect on the dependent variable.
Dependent variable	The variable that is measured. Its value depends on the independent variable, i.e. it responds to the independent variable.
Extraneous variable	A variable, other than the independent variable, that may affect the outcome of an investigation. These variables need to be controlled (kept constant).
Controlled variable	A variable that is kept constant during the investigation so that the investigator can determine the relationship between the independent and dependent variable.

Resources to carry out your investigation

When planning your investigation, you will need to consider several things (Table 1.7). As you are going to collect primary data for your investigation, you will need to make a list in your logbook of all the equipment that you will require. This will also help you later when you write your experimental **method**. An experimental method is the steps you take to carry out your investigation. You will need to consider how precise your measurements must be. If your hypothesis predicts a temperature change of 0.1°C but you can only measure to a precision of 0.5°C, then you will not be able to test your hypothesis. You may need to think carefully about how you measure some things. For example, in an investigation into root growth, you may need to measure the dry weight of the roots, which means finding a consistent way to dry them. Are you going to measure in grams or milligrams? If you measure in milligrams, you will need measuring scales that measure to that level of precision.

Table 1.7 Considerations when planning an investigation

Consideration	Questions to ask
Data and measurements	What kind of data needs to be collected, how often and by whom? Does data need to be collected outside class hours?
Materials and equipment	Can your equipment enable you to collect data that is precise enough? How can you best collect the data and minimise uncertainty?
Safety	Do you have access to all the required safety equipment, including a fume cupboard or specialised methods for disposal of waste?

The equipment you use in your investigation must be safe. Consider whether you will need personal protective equipment such as lab coats, safety glasses or gloves. (See Risk assessment below.) Include any safety equipment needed in your equipment list.

When you have made your list, discuss with your teacher the equipment that is available. You might need to modify your research question or hypothesis at this stage if the equipment that you require is not available. Consider where you will perform your investigations or observations. Can you use the normal classroom space, or do you need to be outside? If you are outside, how can you make sure that you can work without interference? Will you need to consider the convenience or safety of others? Talk to your teacher about what space is available.

Risk assessment

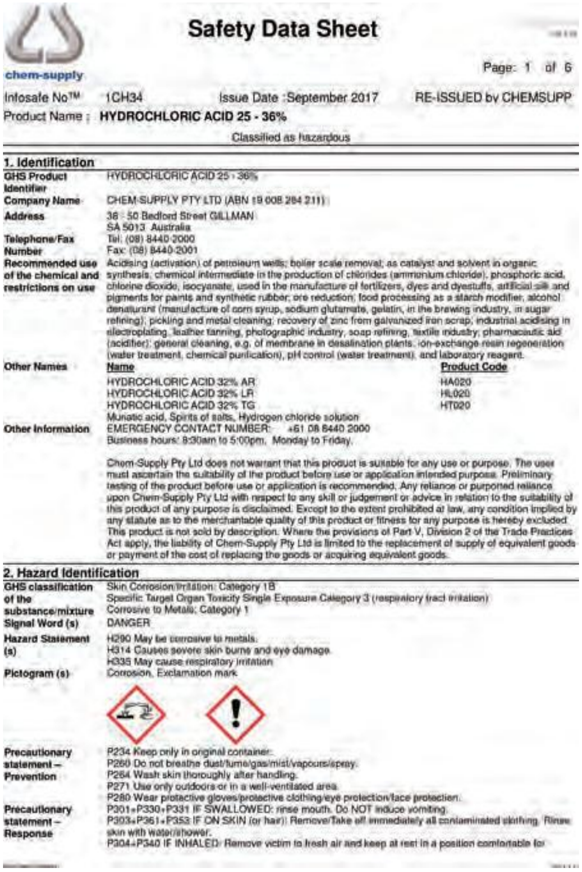
Risk assessment is the process of evaluating the potential risks of an investigation. Even if this is not a requirement for your own investigation, you should consider the following.

» What are the possible risks to you, other people, the environment or property?

» How likely is it that there will be an injury or damage?

» If there is an injury or damage to property or the environment, how serious are the consequences likely to be?

If you intend to use hazardous chemicals, you will need the relevant safety data sheet (SDS). This provides information on how the chemical affects health and safety (Figure 1.9). An SDS gives guidance on the safe handling, storage and disposal of the chemical, as well as emergency procedures for exposure. The SDS for a chemical can usually be found by an Internet search or by looking on the manufacturer's website. It is important to read the SDS when assessing the risk associated with using a chemical and the precautions you should take in your investigations. Table 1.8 shows a matrix for assessing the likelihoods and consequences of risks in investigations.



Safety Data Sheet

chem-supply

Page: 1 of 6

Infosafe No™ 1CH34 Issue Date :September 2017 RE-ISSUED by CHEMSUPP

Product Name : **HYDROCHLORIC ACID 25 - 36%**

Classified as hazardous

1. Identification

GHS Product Identifier HYDROCHLORIC ACID 25 - 36%

Company Name CHEM-SUPPLY PTY LTD (ABN 19 008 284 211)

Address 38 - 50 Bedford Street GILLMAN
SA 5013 Australia

Telephone/Fax Number Tel: (08) 8440 2000
Fax: (08) 8440 2001

Recommended use of the chemical and restrictions on use Acidising (activation) of petroleum wells; boiler scale removal; as catalyst and solvent in organic synthesis; chemical intermediate in the production of chlorides (ammonium chloride), phosphoric acid, chlorine dioxide, isocyanate, used in the manufacture of fertilizers, dyes and dyestuffs, artificial silk and pigments for paints and synthetic rubber; ore reduction; food processing as a starch modifier, alcohol denaturant (manufacture of corn syrup, sodium glutamate, gelatin, in the brewing industry, in sugar refining); pickling and metal cleaning; recovery of zinc from galvanized iron scrap; industrial acidising in electroplating, leather tanning, photographic industry, soap refining, textile industry; pharmaceutic aid (acidifier); general cleaning, e.g. of membrane in desalination plants, ion-exchange resin regeneration (water treatment, chemical purification), pH control (water treatment), and laboratory reagent.

Other Names

Name	Product Code
HYDROCHLORIC ACID 32% AR	HA020
HYDROCHLORIC ACID 32% LR	HL020
HYDROCHLORIC ACID 32% TG	HT020

Muriatic acid, Spirits of salts, Hydrogen chloride solution

Other Information EMERGENCY CONTACT NUMBER: +61 08 8440 2000
Business hours: 8:30am to 5:00pm, Monday to Friday.

Chem-Supply Pty Ltd does not warrant that this product is suitable for any use or purpose. The user must ascertain the suitability of the product before use or application intended purpose. Preliminary testing of the product before use or application is recommended. Any reliance or purported reliance upon Chem-Supply Pty Ltd with respect to any skill or judgement or advice in relation to the suitability of this product of any purpose is disclaimed. Except to the extent prohibited at law, any condition implied by any statute as to the merchantable quality of this product or fitness for any purpose is hereby excluded. This product is not sold by description. Where the provisions of Part V, Division 2 of the Trade Practices Act apply, the liability of Chem-Supply Pty Ltd is limited to the replacement of supply of equivalent goods or payment of the cost of replacing the goods or acquiring equivalent goods.

2. Hazard Identification

GHS classification of the substance/mixture Skin Corrosion/Irritation: Category 1B
Specific Target Organ Toxicity Single Exposure Category 3 (respiratory tract irritation)
Corrosive to Metals: Category 1

Signal Word (s) DANGER

Hazard Statement (s) H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation

Pictogram (s) Corrosion, Exclamation mark

Precautionary statement – Prevention P234 Keep only in original container.
P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P264 Wash skin thoroughly after handling.
P271 Use only outdoors or in a well-ventilated area.
P280 Wear protective gloves/protective clothing/eye protection/face protection.

Precautionary statement – Response P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for

Chem-Supply www.chemsupply.com.au/msds

Figure 1.9 A safety data sheet (SDS) for hydrochloric acid

Table 1.8 A matrix for assessing severity of risk

Likelihood	Consequences			
	Negligible	Marginal	Severe	Catastrophic
Rare	Low risk	Low risk	Moderate risk	High risk
Unlikely	Low risk	Low risk	High risk	Extreme risk
Possible	Low risk	Moderate risk	Extreme risk	Extreme risk
Likely	Moderate risk	High risk	Extreme risk	Extreme risk
Certain	Moderate risk	High risk	Extreme risk	Extreme risk

Once you have considered possible risks, you need to plan how to address them. What will you do to minimise them, and how will you deal with the consequences if something does happen? This may be as

Table 1.9 Risk assessment table

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
The fertiliser might be spilled on clothes or skin during application.	Wear a lab coat, gloves and safety glasses. Clean up spills immediately.

simple as using personal protective equipment such as a lab coat, gloves and safety glasses. Table 1.9 is an example of a risk assessment for an investigation.

Safe use and disposal of biological material

It is important to know how to safely handle and dispose of biological materials. For example, when growing known or unknown microbes on agar plates, you must use safe sterile techniques (see weblink) and wear lab coats, safety glasses, gloves and, if required, face masks. Treat all microbes on agar plates as potentially pathogenic and kill the bacteria by autoclaving (heating with pressurised steam) used plates before disposing of them. If you are uncertain about how to dispose of material used in your investigation, ask your teacher or laboratory technician.

Weblink
Sterile technique

Ethical guidelines

Ethics is a system of moral principles that considers what is good and bad for society. Put simply, it considers what is right and wrong. Bioethics is ethics in the context of biological research. You need to consider ethical guidelines relevant to your selected investigation during the planning stages of your investigation.

1.1.7 ETHICAL GUIDELINES
PAGE 11

The following approaches can help guide you through ethical considerations relating to your investigation.

- Consequences-based approaches aim to maximise the positive effects (benefits) and minimise the negative effect (harms) of a particular action. The *end results* are key in this approach.
- Duty- and/or rule-based approaches state that people have the duty to act in a certain way, and obey certain rules, regardless of the outcome. The *actions*, or *means*, are key in this approach.
- Virtues-based approaches consider the moral character or virtue of the person conducting the action: are they seeking to exhibit 'good' characteristics and behaviours? The *person conducting the action* is key in this approach.

There are several concepts relating to acting ethically in your research.

- **Integrity** is about being honest as a scientist. This means recording data accurately and not ignoring, hiding or changing any data that does not support your hypothesis. It means acknowledging and referencing sources of information, including books, websites, articles and people who have helped you. It means not using other people's ideas or data without their knowledge or permission. It also means allowing others to fully scrutinise your work to further public knowledge and understanding. Put simply, showing integrity is 'doing the right thing'. A good rule is that if you would not want someone to know what you were doing, you probably should not be doing it. It is no different from behaving ethically in any other area of your life.
- **Justice** is the moral obligation to consider competing claims, not place unfair burden on a particular group and fairly distribute or allow access to the benefits of an action.
- **Respect** means giving intrinsic or instrumental value to living things, and being considerate of their welfare, freedom, autonomy, beliefs, perceptions, customs and cultural heritage. It also means considering that living things can make their own decisions and empowering and protecting those who have diminished capacity to do so.

When planning your research, you should also be guided by the following ethical principles.

- **Beneficence** is a commitment to maximise the benefits and minimise the risks and harms involved in taking a particular course of action.
- **Non-maleficence** is the commitment to avoid causing harm and ensuring that any harm caused is proportional to the benefit gained from taking that course of action.

Refer to the *VCE Biology Study Design* for further information about the ethical conduct of scientific investigations (p. 5) and ethical approaches and concepts (pp. 15–16).

KEY CONCEPTS

- You need to have a clear plan for your investigation.
- Primary data can be quantitative, qualitative or both, depending on your hypothesis.
- Measurements must be accurate, precise, reproducible, repeatable and valid.
- Take every precaution to minimise error in your data.
- All extraneous variables must be controlled.
- The method details the steps that you will undertake to perform your investigation, including a risk assessment.
- Check whether you need to consider any bioethical issues in your investigation.

Concept questions 1.1b

1 Which data type records numerical measurements?

2 List five types of inputs you could use for qualitative data.

3 Define ‘validity’ in terms of scientific research.

4 Define ‘precision’ in terms of data collected.

5 Why is careful planning important when conducting an investigation?

6 What is a control and is it different from a controlled variable?

7 What is uncertainty and how can you take uncertainty into account when planning your investigation?

HOT challenge

8 Ethical considerations and risk assessment are two aspects of scientific methodology. How do these two important features intersect in experimental design? Is there potential scope for conflict between the two?

1.2 Scientific evidence

Scientific evidence is primary data that comes from scientific investigations. It is only valid if the investigation has been designed carefully, with errors minimised and bias eliminated, has been properly experimentally controlled, and has been found to be repeatable and reproducible.

Record keeping

Students studying VCE Biology are required to keep a record of their practical activities in a logbook. This is for recording, **authentication** and assessment purposes. You can keep your logbook in hard copy form or soft copy form (electronic), although hard copy is preferred.

Logbooks include details of investigations such as methods and results. They include comments and ideas, thoughts about the investigations, and analysis. They frequently include printouts of data, photocopies of relevant information, photos and other items (Figure 1.10).

Discuss with your teacher about the form of logbook records you are required keep and any specific formatting required for your logbook. Make an entry in the logbook every time you work on your investigation.

Each entry in your logbook needs to be dated. Write down what you do as you do it. It is easy to forget what you did if you do not write it down immediately. An accurate record is important if you need to repeat any measurements or if you get unexpected results. The more detail you include, the easier it will be to prepare your report or poster at the end of this study. Include large, clear diagrams of any experimental set-up and details of equipment used. You can also include photos of investigations.

Record the results of all measurements immediately and directly in your logbook. Never record data on bits of scrap paper. Use a pen, not a pencil, to record your results. Never use liquid paper or scribble over anything in your logbook. If you want to cross something out, put a line through it. It is also a good idea to make a note explaining why it was crossed out.

Collecting raw data

To determine a relationship between your variables, you need to have enough data points and the range of your data points should be as large as possible. A minimum of six data points (therefore six replicates) is generally considered adequate, but collect as many as you reasonably can in the available time.

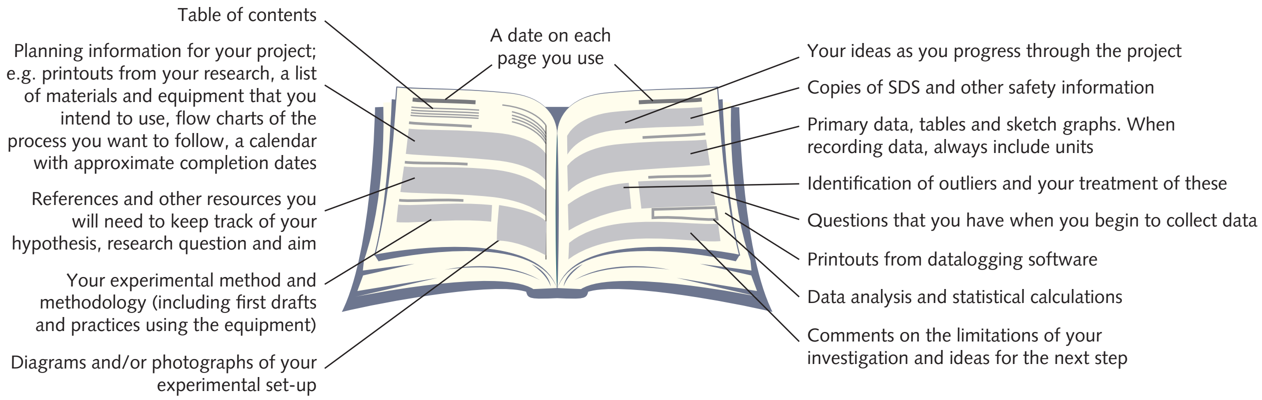


Figure 1.10 Features of an effective logbook

Always record the raw data directly in your logbook unless you are using a data logger connected to a computer to record the data. In this case, attach a printout of the data to your logbook and record the file name and location. Make sure that you measure and record everything you will need for your analysis. For example, if you were investigating root growth, you would record the amount of fertiliser used, the temperature and the starting length of the roots. It is much better to measure something that you discover later that you do not need than to start your analysis and realise that you did not measure something that you need.

Use appropriate units; for example, millimetres (mm) for length and grams (g) for mass. If you collect multiple data points, it is a good idea to record them in a table. Label the columns in the table with the name and units of the variables. Do not put the units in the table cells. The instruments that you use will often restrict the precision of your measurements. For example, a ruler may only have markings down to 0.1 cm. Make a note of these restrictions because they can also affect the accuracy of your final results, especially if the changes measured are small.

Analysing your data

Having gathered your data, there are usually a number of steps you need to take to analyse it. This allows you to draw meaningful conclusions from your investigation, leading you to either support or refute your hypothesis. Usually, you will use descriptive statistics to describe the data (such as calculating the mean), plot a graph of the data, and try to determine whether any trends or patterns emerge in the data.

Record all your analysis in your logbook. If you use a computer for your analysis, then record the file name and location and attach a printout of the analysis to your logbook. Many scientists have logbooks that are bulging with printouts of their analysis.

WB
1.2.1 ANALYSING YOUR DATA
PAGE 15

Diagrams

You may need to include scientific diagrams in your results. You can include drawings of your experimental equipment and how it is set up when collecting data, drawings of structures under the microscope, or drawings of organisms that you are studying in your investigation. Like other kinds of data, diagrams require a figure number and a clear title.

There are other important rules to follow when constructing scientific drawings (Figure 1.11).

- Use pencil.
- Create a large drawing that fills at least half a page.
- If using a microscope, include the magnification at which the image was viewed.

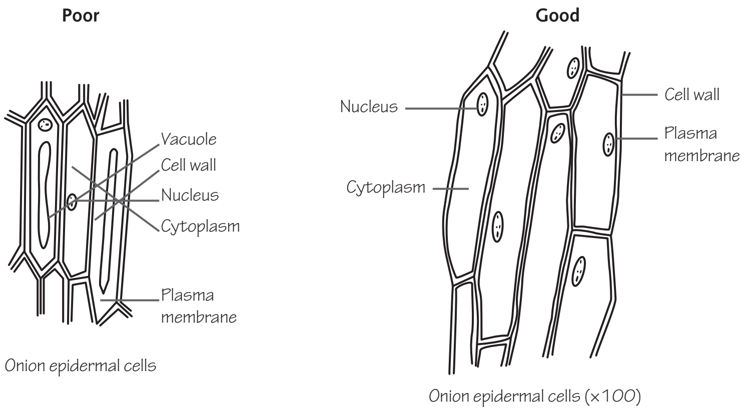


Figure 1.11 Effective scientific drawings: include the magnification (where appropriate) and make sure label lines do not cross.

- If the drawing is of an organism, include this information.
- If drawing your experiment, draw all equipment in cross-section.
- Label all the parts of your diagram and rule a line to each label. Ensure that the lines do not cross.

Tables

When recording data from your investigation, you will probably write it in a table. The advantage of this is that you can organise your data as you record it and begin to identify trends. A well-organised table saves you significant time later. Your table should have a clear title and be organised so that you can easily record and compare your independent and dependent variables. If you have completed any calculations on your data, you should record this information alongside your primary data. An example is shown in Figure 1.12.

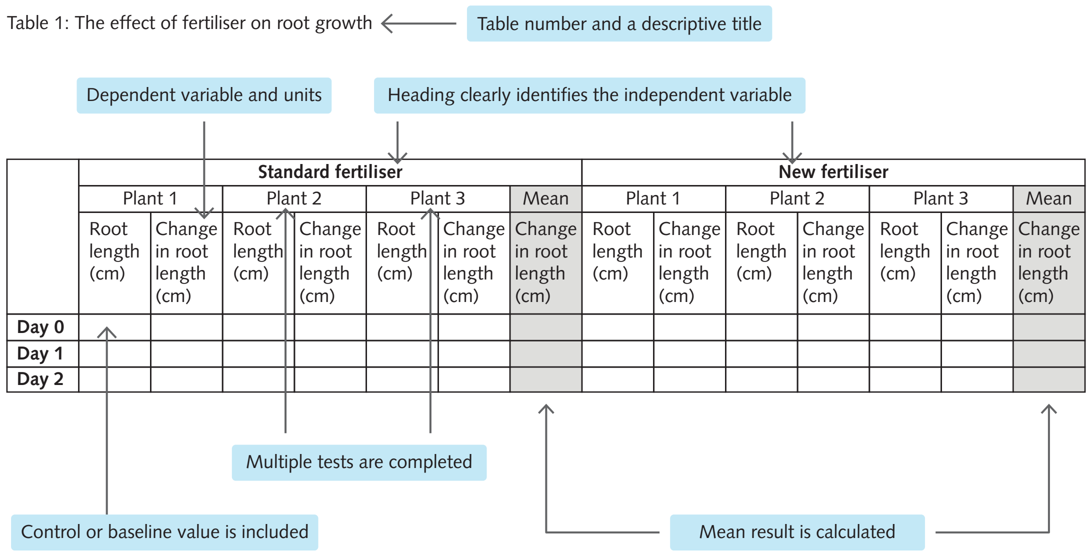


Table 1: The effect of fertiliser on root growth

	Standard fertiliser							New fertiliser						
	Plant 1		Plant 2		Plant 3		Mean	Plant 1		Plant 2		Plant 3		Mean
	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Change in root length (cm)
Day 0														
Day 1														
Day 2														

Figure 1.12 An effective table to record primary data and simple statistics

Note:

The acronym DRY MIX can help you remember how to plot variables on a graph.
D = dependent variable
R = responding variable
Y = *y*-axis
M = manipulated variable
I = independent variable
X = *x*-axis
Plot the dependent (responding) variable on the *y*-axis. Plot the independent (manipulated) variable on the *x*-axis.

Graphs

You may be able to see a pattern simply by looking at the list of numbers in your table. However, the easiest way to identify a pattern in data or a relationship between variables is to plot a graph.

A graph should be large and clear. The axes should be labelled with the names of the variables and their units (Figure 1.13). Put the variable that you are measuring (dependent) on the vertical axis (y) and the variable that you are altering (independent) on the horizontal axis (x). Choose a scale so that your data takes up most of the plot area. You don't always need to show the origin in a graph but, by including it, you will provide an honest representation of the data without any exaggeration.

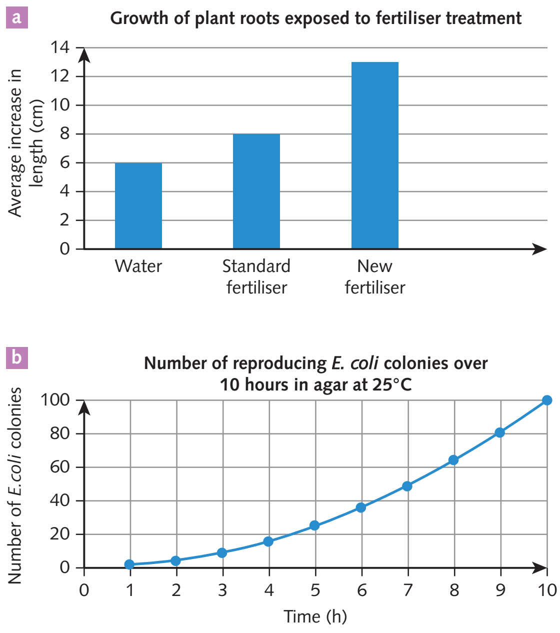


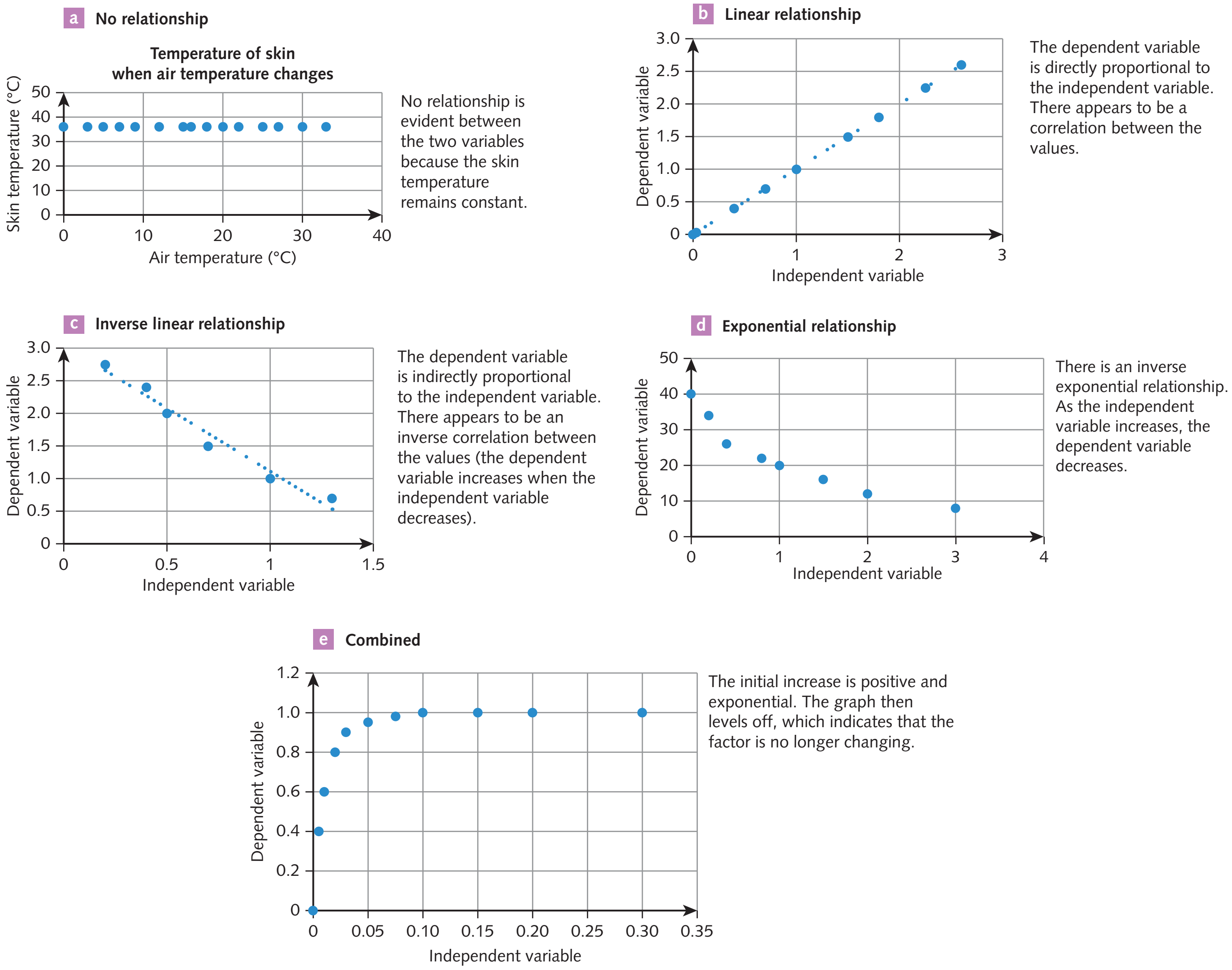
Figure 1.13 A correctly drawn **a** bar graph and **b** line graph

Interpreting your results

Once you have visualised your data by plotting it as a graph, you can begin to consider what trends, patterns or relationships the results are showing, and determine whether the trends are likely to reflect true relationships between the factors in your study. You can then relate your findings to your research question and hypothesis.

Determining relationships

When you have created a graph of your data, provided you have carefully considered the scale of your graph, and you have considered any outliers, you should be able to see any relationships. When writing your results section for your poster, you should describe any relationship that you notice between the independent and dependent variables, noting the **gradient** or slope of the graph. You should then try to interpret whether the relationship between the independent and dependent variables is a causative one (i.e. your data is a result of the treatments or test groups in your investigation). Your interpretation of the data, and what it means to the field of science that you are investigating, should be explained. Figure 1.14 shows the types of relationships that you may observe in your data.



a No relationship

Temperature of skin when air temperature changes

No relationship is evident between the two variables because the skin temperature remains constant.

b Linear relationship

The dependent variable is directly proportional to the independent variable. There appears to be a correlation between the values.

c Inverse linear relationship

The dependent variable is indirectly proportional to the independent variable. There appears to be an inverse correlation between the values (the dependent variable increases when the independent variable decreases).

d Exponential relationship

There is an inverse exponential relationship. As the independent variable increases, the dependent variable decreases.

e Combined

The initial increase is positive and exponential. The graph then levels off, which indicates that the factor is no longer changing.

Figure 1.14 Different types of relationships that may be evident in data. There may be **a** no mathematical relationship, **b & c** a clear linear relationship or **d & e** an exponential relationship.

Relating your results to your hypothesis

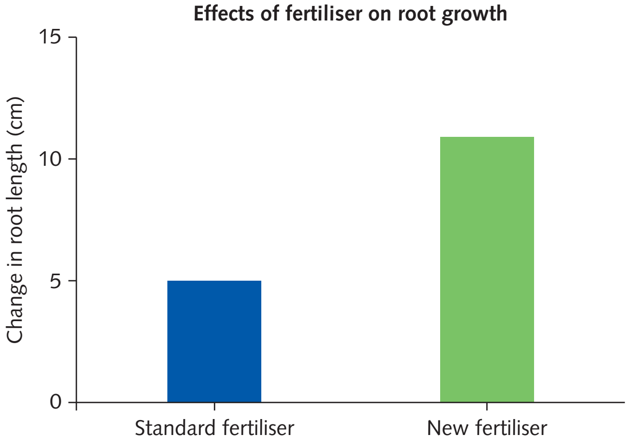


Figure 1.15 **This bar graph shows that there is a difference between the independent variables, which supports the hypothesis.**

Once you have analysed your results, you need to interpret them. This means being able to either answer your research question or state whether your results support your hypothesis. For example, if the new fertiliser induces greater root growth than the standard fertiliser, with all other variables being equal, this would support the hypothesis that 'The new fertiliser increases the rate of root growth in two weeks compared to standard fertiliser' (Figure 1.15). If there was no difference between the two, or if the new fertiliser induced significantly less root growth than the standard fertiliser, this would not support the hypothesis.

If your hypothesis is not supported

If there is not a significant difference in your data, your hypothesis may not be supported by the data. This may occur if the investigation could not show the effect posed by the hypothesis; for example, by not having enough replicates to reduce variability and produce a significant difference. Alternatively, the hypothesis may be wrong. However, it is not enough to simply say 'our hypothesis is wrong'. If the hypothesis is wrong, what is wrong with it?

Your method may have been too simple or may not have taken into account all of the other variables. For example, in the root growth investigations, the fertiliser may work best at a particular temperature, or over a longer time, or in conjunction with certain soil conditions. Or it may not work with the chosen type of plant. The investigation may have been too limited to fully test the hypothesis. You might conclude that further investigations are required to test these other variables.

However, before you decide that the method is at fault, check carefully that you have not made mistakes or ignored any variables. Think about factors that you did not consider, but which might have affected your investigation. Go through your method, results and analysis. Check that your equipment was correctly calibrated and that you were using it correctly. Check that you used the correct units for your data and that the units are correctly carried through all calculations during analysis. Check your analysis carefully. If you are working in a group, ask another person to repeat your calculations.

If you are certain your investigation results are real, and they still refute your hypothesis, do not be disappointed. The process of scientific enquiry is often propelled forward when old hypotheses are tested and refuted, and new observations pave the way to discoveries that change our understanding of biological systems.

SOUTHERN Biological Exclusively developed by Southern Biological

INVESTIGATION 1.1

The effect of temperature on trypsin activity

Casein is a common protein in mammalian milk. Casein is digested by trypsin, an enzyme that hydrolyses proteins into peptides, which other enzymes further break down to amino acids for use in the body. Trypsin works in the small intestine, after acid and pepsin in the stomach have started breaking down the proteins. Casein is relatively hydrophobic, making it poorly soluble in water; however, when trypsin is added to a dilute solution of milk powder, the casein is digested and the solution goes clear.

Aim

To determine the optimal temperature for trypsin activity

Time requirement

45 minutes

Materials

- 1% trypsin solution
- 3% solution of skim milk powder
- Buffer solution (pH 7)
- Water bath
- 6 test tubes and a test-tube rack
- Bungs or cork for test tubes
- Stopwatch
- Marker
- Plastic pipettes
- Thermometer
- Lab coats
- Safety glasses
- Disposal gloves

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Trypsin can cause allergic reactions in sensitive people.	Make sure your teacher is aware of any allergies.
Trypsin can irritate the skin and eyes on contact.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.
Hot water can scald.	Be careful when working with water hotter than 50°C. Do not touch the outside of the glass beaker.

Method

1 For the control test, set the water bath to 20°C.
2 Mark three test tubes with an ‘X’ halfway down each tube (Figure 1.16).
3 Using a pipette, add 10 mL of the milk powder solution to each of the three test tubes.
4 Collect another three test tubes and add 3 mL pH 7 buffer solution and 3 mL of trypsin solution.
5 Place all six test tubes in the water bath for 10 minutes. Ensure the six tubes are upright.
6 Pour the trypsin and buffer solution from one test tube into the milk powder solution in another test tube.
7 To mix thoroughly, place a cork in the test tube and invert 4–6 times.
8 Place the test tube in the test-tube rack with the X placed at the back; immediately begin the timer.
9 Record the time it takes for the milk solution to become clear by measuring the time it takes for the X to become visible through the solution.
10 Repeat steps 6–8 for the remaining test tubes and record the time for each of the three experiments in your logbook.

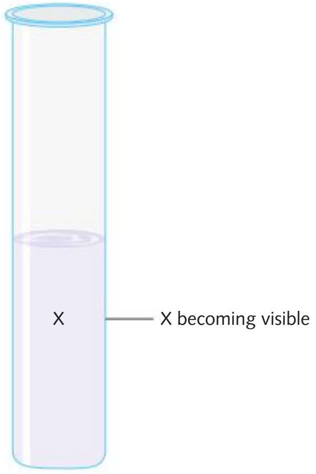


Figure 1.16 Measure the time it takes for the X to become visible.

11 Calculate the mean reaction time and record the result in your logbook.
12 Your teacher will now assign you one of four temperatures to test: 30°C, 40°C, 50°C or 60°C. As a class, you will test all four temperatures, pool your data and compare your results. Set the water bath to your assigned temperature. Once your water bath has reached the desired temperature, repeat steps 2–10 to test how the reaction time is affected by different temperatures.
13 Record your data in your logbook, calculate the mean reaction time and share your results with the class.
14 Record the class mean reaction time for each of the assigned temperatures in a combined results table. (See below.) If one of the temperature variables was tested more than once – i.e the 20°C control test – find the mean among them.
15 Draw a graph in your logbook using the data in the table. Be sure to clearly label both axes and use an appropriate scale.

Results

Sample class results for five temperatures

Temperature (°C)	Mean time for milk solution to clear (s)
20	
30	
40	
50	
60	

Discussion

1 What is your hypothesis?
2 What is the independent variable?
3 What is the range of the independent variable?
4 What is the dependent variable?
5 What are the extraneous variables and how were they controlled?
6 Why are all the test tubes left in the water baths for 10 minutes before the trypsin and milk are added together?
7 What are the advantages of calculating a mean of test samples as opposed to just one?
8 What type of distribution does the data show?
9 What is the optimal temperature for trypsin activity?
10 Is the hypothesis supported by the data?

Conclusion

Summarise the findings using the data provided. Relate the results to the hypothesis.

KEY CONCEPTS

- Maintain a logbook throughout your investigation for authentication and assessment.
- Raw data is analysed and summarised as diagrams, tables or graphs.
- Determine any relationships or trends in your data.
- Relate your results to your hypothesis, which is either supported or refuted.

Concept questions 1.2

1 How does keeping a logbook aid the scientist in their research?

2 List three reasons why a hypothesis may not be supported.

3 ‘Relationships and trends are inimical to scientific presentation of data.’ What does this mean?

4 List at least four features of a well-designed table.

HOT Challenge

5 There is a general rule about which axis of a graph the independent variable is applied to. State a general rule and itemise some exceptions.

1.3 Science communication

Once you have finished your investigation, you must be able to communicate what you have found to other people. This is important in science because often ideas from other people will spark new ideas and prompt further investigation. You are also adding to the body of scientific knowledge that you would have tapped into when you did your background research when you were planning your investigation.

There are a number of ways to present your findings, including journal articles and conference presentations. However, here you are required to present your findings as a scientific poster.

Presenting your work as a scientific poster

You need to format your scientific investigation into a poster of no more than 600 words. This word count is limiting, so you will need to think very carefully about what you put in each section of the poster (Table 1.10).

WB
1.3.1 PRESENTING YOUR WORK AS A SCIENTIFIC POSTER PAGE 18

Table 1.10 Sections in a scientific poster

Section	Description
Title	This should be the same as your research question.
Introduction	Make sure that you explain the reason for your research and link it to the underlying biological theory. You should also include an aim, hypothesis and/or prediction in this section.
Methodology and methods	Briefly outline your methodology here. Summarise the data generation and data analysis methods that you used.
Results	Your analysed data should go here. Your results may include graphs, perhaps with statistical analysis such as a line of best fit. You may also include tables of mean data. Remember that your raw data will be available in your logbook.
Discussion	In this section, you will need to show that you understand what your data means and be able to make links to the underlying scientific concepts that you were investigating. You need to analyse your data and explain the trends. You should also state whether you have answered your research question and/or whether the data supports your hypothesis or not. You may also have identified some limitations to your investigation and you may have ideas for further research.
Conclusion	This is a brief summary of your main findings. You should state whether or not your investigation answered the research question. Do not provide new information.
References and acknowledgements	You will need to include your references and acknowledge anyone who helped you or provided you with information. References and acknowledgements are not included in the poster word count.

Title

The title for your poster is the research question that you are investigating.

Introduction

The introduction to your poster could also be called the background research that you undertook in the planning stages of your investigation. The introduction outlines the existing knowledge about the research topic. This is where you summarise any existing theories, models, concept and similar studies, all of which should be correctly referenced (p. 28).

The introduction contains a clear aim and a stated hypothesis for the investigation with (or without) a prediction of whether you think the hypothesis will be supported or refuted.

Methodology and methods

The methodology is the framework of the approach that you have adopted to investigate your hypothesis.

You should briefly but clearly describe the method used in your investigation. In a poster, it is not necessary to have the complete sequence for someone else to follow. Instead, you can summarise it as a series of explained photographs or diagrams. You are not instructing anyone to do anything. You are telling people what you did. For example, you would write 'root length was measured' not 'measure the root length'.

If your study has potential safety issues or ethical considerations, identify them in this section and briefly describe the ways in which you handled them.

Results

The results section is a summary of your results together with schematic diagrams, flow charts, bar charts, tables or line graphs showing trends in the data. Do not interpret your results in this section. Make sure you label your graph axes, including units. Choose an appropriate scale so that the data takes up most of the plot area. Do not include tables of raw data in your poster.

When stating the findings of your study in the main text of the results section, refer immediately afterwards to the figure in which the finding is shown; for example, 'The vertical growth of *Arabidopsis* seedlings was significantly greater following two weeks of new fertiliser treatment than with a standard fertiliser or water alone (Figure 2).'

Figures

There are several types of figures that you may include in a scientific poster. The most informative figures are quantitative, such as graphs (Figures 1.17 and 1.18) or tables, although it is often useful to include qualitative data such as cross-sections or photographs. Regardless of the type of figure used, it should be chosen for its ability to best communicate the findings of the study. Sometimes a figure will have multiple panels (labelled A, B etc.). For example, a graph and a photograph showing the same pattern could be presented in two panels of the same figure.

Each diagram should have a figure number, and you should refer to it in the text of your poster. Position the diagram close to where it is referred to in the text. Figure captions are essential. They are usually below the figure and begin with the figure number followed by a brief description of the figure.

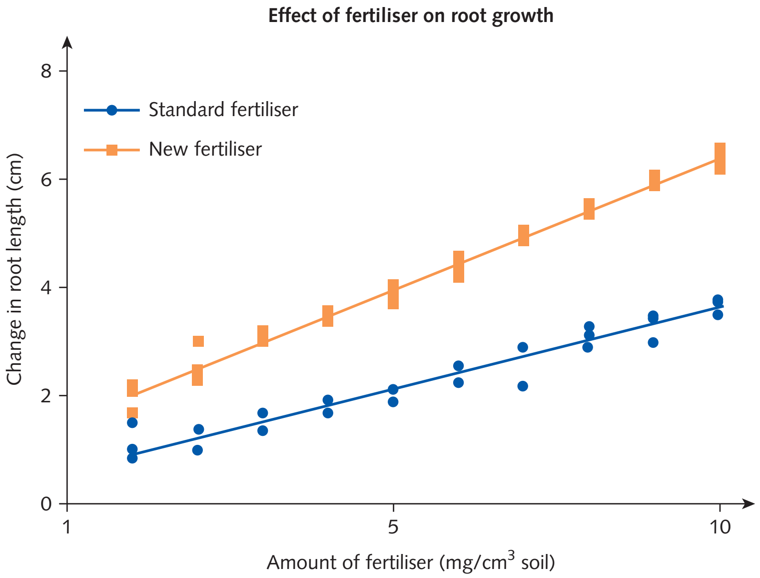


Figure 1.17 An example of a graph that demonstrates a mathematical relationship that could be used in the results section of a scientific poster

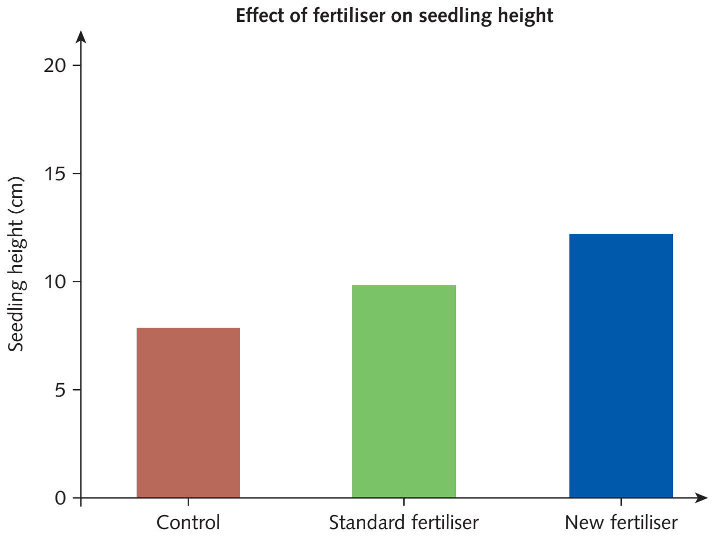


Figure 1.18 Seedlings were grown under standard conditions to a height of 2 cm, then separated and treated daily for two weeks with 50 mL of 2% new fertiliser (blue) or 2% standard fertiliser in tap water (green). The control group was given water alone (brown).

Discussion

In the discussion section, you need to use the evidence that you have produced from your investigation to construct a scientific argument about how well your investigation answered your research question and achieved the intended aim(s). This is probably the most difficult part of the report that you have to write.

Some questions you should consider when writing your discussion section are as follows.

- What relationships did you observe in your data?
- Did your results support or refute your hypothesis?
- How could you improve your investigation design to more accurately address your hypothesis?
- What do your results add to the current scientific knowledge of biological concepts?
- Do they agree with or contradict models or classification keys based on other published findings?
- What were the implications of your study, and how could you address these in future real or hypothetical studies?
- How might your findings affect the scientific community, industry, medical practice or the community at large?

It will be difficult to address all these questions in the limited space you have available. Start by writing down all the key points and then read through them several times, cutting down unnecessary words each time. Do not remove your own connections or ideas; this type of critical thinking is often a significant part of what is assessed in scientific writing. Remember that concise, coherent writing is an important scientific skill to practise.

Conclusion

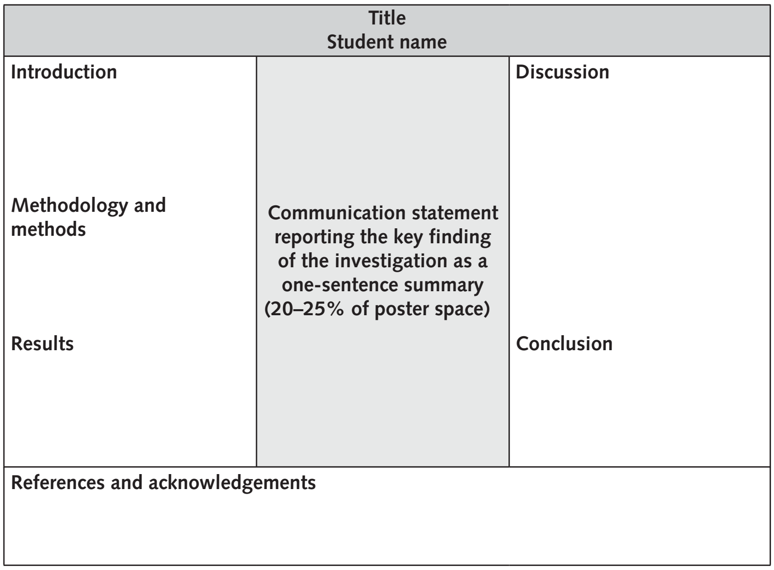
This short section allows you to draw conclusions from the evidence you have gathered during your study. It is a very brief summary of the results and their implications. It should provide a response to your research question and directly address the hypothesis you proposed in your introduction. The conclusion should also state the extent to which the analysis answered the research question, without introducing new information. A conclusion should only be a few sentences long.

References

A reference list details all the sources of information you used to write the text and figures for the poster. Whenever you use a piece of information or quotation in your poster, you must reference it at that point. This is typically done by placing a number in brackets [2], or the author and the year of publication (Smith 2019), depending on which referencing style you use. The complete reference list is provided in a single, alphabetical list at the bottom of the poster. You must reference in a consistent style (p. 7). Check with your teacher about the preferred style. References are not included in the poster word count.

Acknowledgements

You should thank anyone who helped you in your investigation. This includes people who supplied equipment or funding, as well as people who gave you good ideas or helped you with the analysis. Acknowledgements are not included in the poster word count.



Title
Student name

Introduction

Methodology and methods

Results

Communication statement reporting the key finding of the investigation as a one-sentence summary (20–25% of poster space)

Discussion

Conclusion

References and acknowledgements

Source: adapted from the *VCE Biology Study Design* (2022–2026) p. 11; © VCAA, by permission

Figure 1.19 Template for scientific poster

KEY CONCEPTS

- » Once you have finished your investigation, you have to communicate what you have found to other people.
- » A scientific poster includes a title, introduction, methodology and method, results, discussion, conclusion, references and acknowledgements.

Concept questions 1.3

1 Why is it important to include the appropriate units of measurement in quantitative data?

2 Discuss the specific requirements of the introduction section of a scientific poster.

3 Draw up a table that summarises the main features of tables and figures and include summarised examples to demonstrate your understanding.

4 Why does the discussion section of a report require you to consider the results in terms of the current theory?

5 Identify the three main pieces of information included in the conclusion of a report.

HOT Challenge

6 What is the specific manner in which you answer your research question?

Online Key Concepts
Chapter 1 summary

1 Summary of key concepts

1.1 Investigation design

KEY CONCEPTS

p. 5

- Observation is the start of a scientific investigation.
- An observation can be turned into a research question.
- A research question is specific and can be answered by performing an investigation.
- A research question identifies the dependent and independent variables.
- You will need to undertake some background research into your research question.
- Make sure you critically evaluate the secondary resources that you use for your background research.
- The independent variable is the factor that you change or manipulate in your investigation.
- The dependent variable is the factor that you measure during your investigation.
- A research question can be turned into a hypothesis.
- A good hypothesis predicts what the results will be, states the relationship between the independent and dependent variables, states how the dependent variable will be measured, and can be supported or refuted through investigation.
- Methodology refers to the broader framework of approach taken to investigate your research question.

Figure 1.4 There are many different scientific investigation methodologies in biology

KEY CONCEPTS

p. 11

- You need to have a clear plan for your investigation.
- Primary data can be quantitative, qualitative or both, depending on your hypothesis.
- Measurements must be accurate, precise, reproducible, repeatable and valid.
- Take every precaution to minimise error in your data.
- All extraneous variables must be controlled.
- The method details the steps that you will undertake to perform your investigation, including a risk assessment.
- Check whether you need to consider any bioethical issues in your investigation.

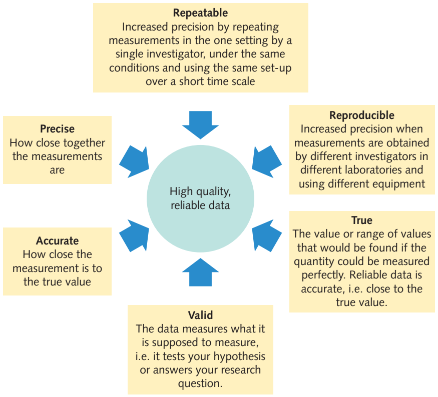


Figure 1.6 Features of data from a well-designed investigation

1.2 Scientific evidence

KEY CONCEPTS

p. 18

- Maintain a logbook throughout your investigation for authentication and assessment.
- Raw data is analysed and summarised as diagrams, tables or graphs.
- Determine any relationships or trends in your data.
- Relate your results to your hypothesis, which is either supported or refuted.

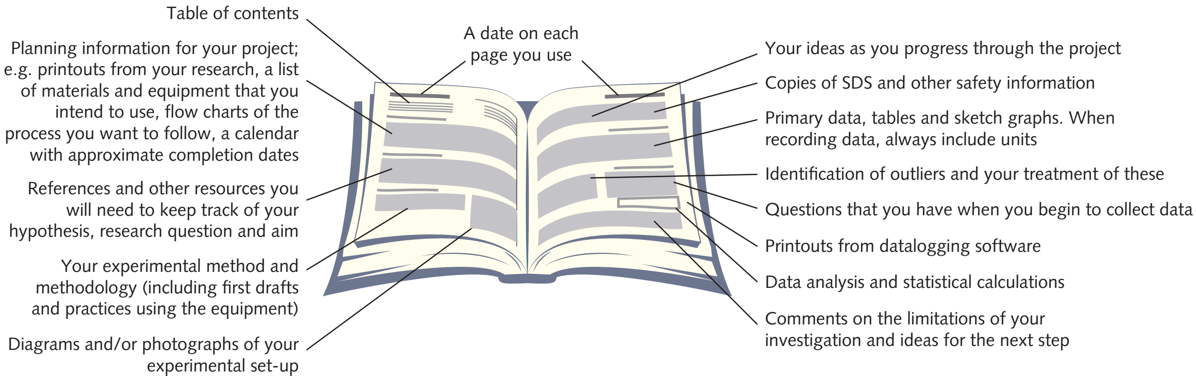


Figure 1.10 Features of an effective logbook

1.3 Science communication

KEY CONCEPTS

p. 25

- Once you have finished your investigation, you have to communicate what you have found to other people.
- A scientific poster includes a title, introduction, methodology and method, results, discussion, conclusion, references and acknowledgements.

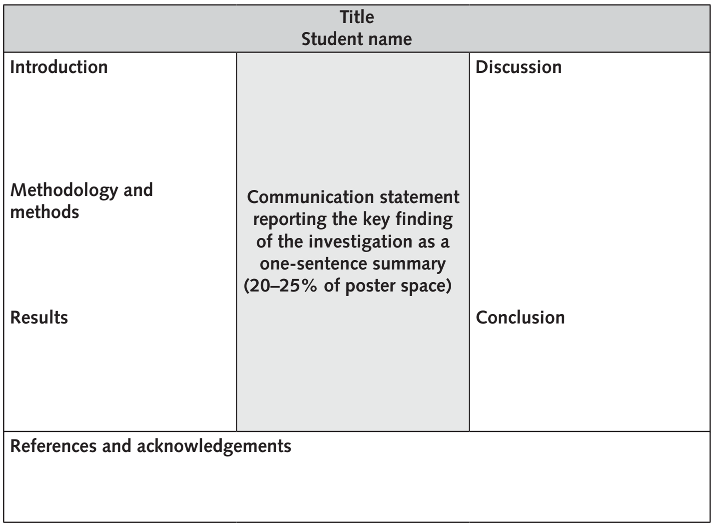


Figure 1.19 Template for a scientific poster

WB

1.4.1
KEY TERMS
PAGE 19

1.4.2
EXAM PRACTICE
PAGE 20

1 Chapter glossary

accurate without any mistakes

authentication confirming that the submitted assessment has been completed by the student

beneficence an ethical concept that involves taking positive action that maximises the benefit or 'good', and minimises the risks and potential harms

bias an error that occurs when an investigation is not randomised, particularly if the investigator is affected by their expectations of the outcome

control group a group in an investigation that receives no treatment (independent variable) so a baseline value can be established

controlled variable the variable that is kept constant during an investigation in order to determine the relationship between the independent and dependent variables

dependent variable the variable that is measured and whose value depends on the independent variable, i.e. it responds to the independent variable

ethics a system of moral principles that considers what is good and bad for society

extraneous variable a variable, other than the independent variable, that can influence the dependent variable

gradient the slope of a graph

hypothesis a tentative prediction, or explanation of an observation, based on an existing model or theory

independent variable the variable changed or manipulated by the scientist and assumed to have an effect on the dependent variable

integrity an ethical concept that means being honest about one's actions; in science it means fully reporting data (even if it doesn't fit your hypothesis) and acknowledging all sources of information

justice a moral obligation to give fair consideration to competing claims, not place unfair burden on a particular group, and ensure fair access and distribution of benefits of an action

logbook a record of experimental investigations kept by scientists performing the investigations; it is a legal record of the investigations and their results

method the steps taken to carry out a scientific investigation

methodology the broader framework of approach taken in the investigation to test your research question

model a representation of a system or phenomenon that explains the system or phenomenon; a model may be mathematical equations, a computer simulation, a physical object, words or some other form

non-maleficence an ethical concept that involves avoiding harm or ensuring that harm caused by action is proportionate to the benefit gained from the action

observation acquisition of information through your senses

outlier a data point that does not fit the pattern shown by the other measured data points

personal error a mistake or miscalculation due to human error

precise how closely together measurements are to one another

primary data data that you have measured or collected yourself

primary source an original source of information, created by the author and usually including primary data

qualitative data a measurement with descriptive or non-numerical results

quantitative data a measurement with numerical values

random error an unpredictable variation in measurement; can be improved by taking multiple measurements and calculating an average

references a list of all the sources that have been used in the write-up of a scientific investigation

reliable highly likely to be a trustworthy source of information or reproducible data

repeatable an investigation that can be conducted again by the same investigator under the same conditions to generate similar results

replicates independent samples that allow you to take multiple measurements, increasing the reliability of your data

reproducible giving the same result within uncertainty limits; when repeated measurements are made by a different investigator

research question a specific question that a particular investigation or investigator is attempting to answer

respect an ethical concept that considers the rights of an individual or a group, e.g. respect for animals considers their welfare

risk assessment a process of evaluating potential risks of an investigation

secondary data data that has been measured and collected by someone other than you

secondary source a source of information that has been obtained from another source and/or summarised, e.g. a popular science magazine

systematic error a predictable deviation in data, e.g. as a result of the equipment used

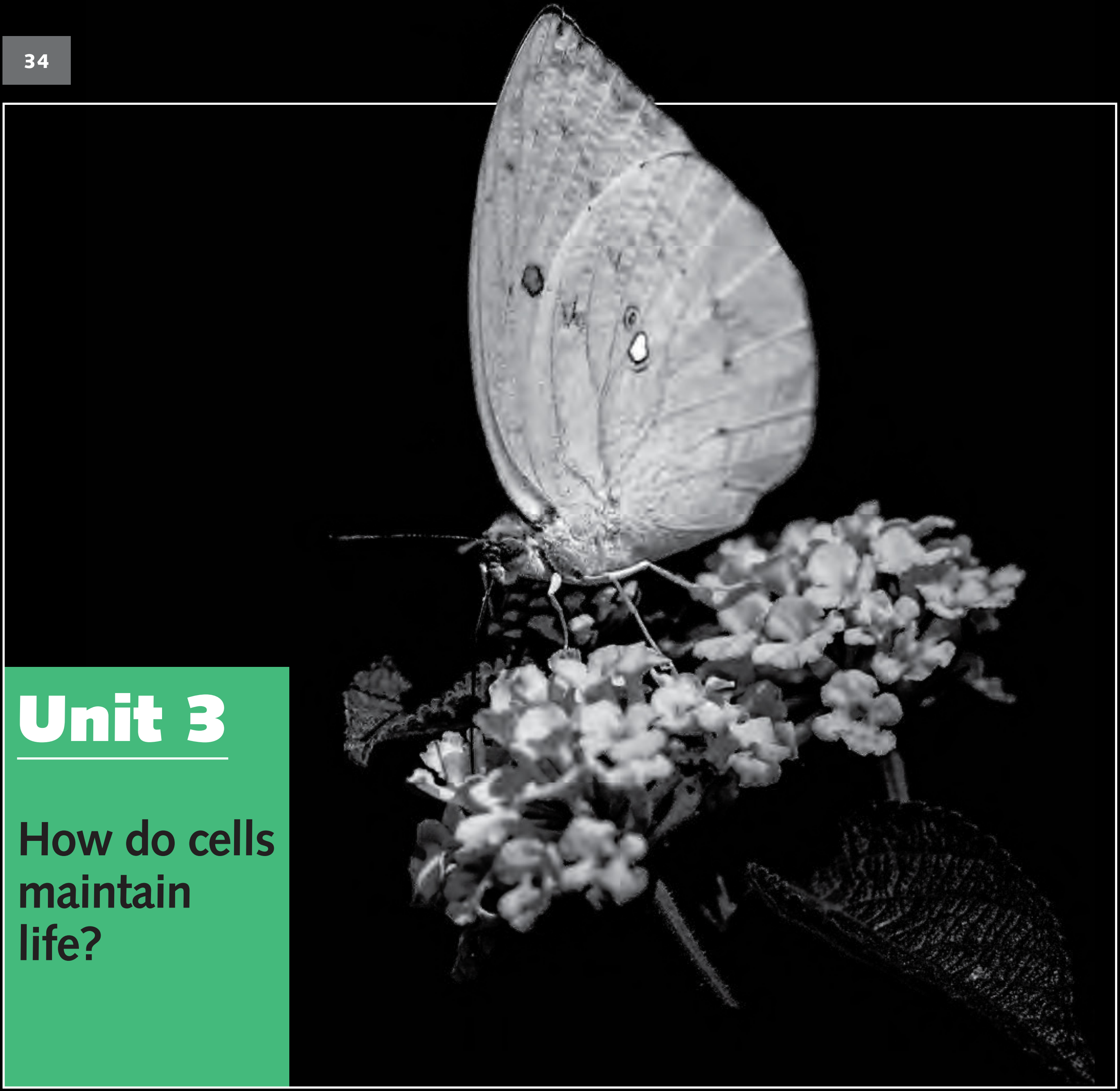
theory a collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

true value a value obtained in an ideal measurement

uncertainty a range of values that the true value falls within

valid describes results that are affected by only a single independent variable and hence are reproducible

variable something that can change or be changed, as distinct from a constant, which does not change



Unit 3

How do cells maintain life?

Getty Images/Eye Em/Jui Chieh Chang

Area of Study 1: What is the role of nucleic acids and proteins in maintaining life?

Area of Study 2: How are biochemical pathways regulated?

The relationship between nucleic acids and proteins

2

By the end of this chapter you will have covered the following material.

Key knowledge

The relationship between nucleic acids and proteins

- nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides, pp. 39–47
- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes, pp. 47–53
- the structure of genes: exons, introns and promoter and operator regions, pp. 48–49; 54–57
- the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process, pp. 54–57
- amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein, pp. 57–60
- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways, pp. 61–64
- the role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway, pp. 64–65

Key science skills

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation, pp. 43–44; 52–53
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications, pp. 43–44

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks, pp. 43–44
- apply relevant occupational health and safety guidelines while undertaking practical investigations, pp. 43–44
- demonstrate ethical conduct when undertaking and reporting investigations, pp. 43–44

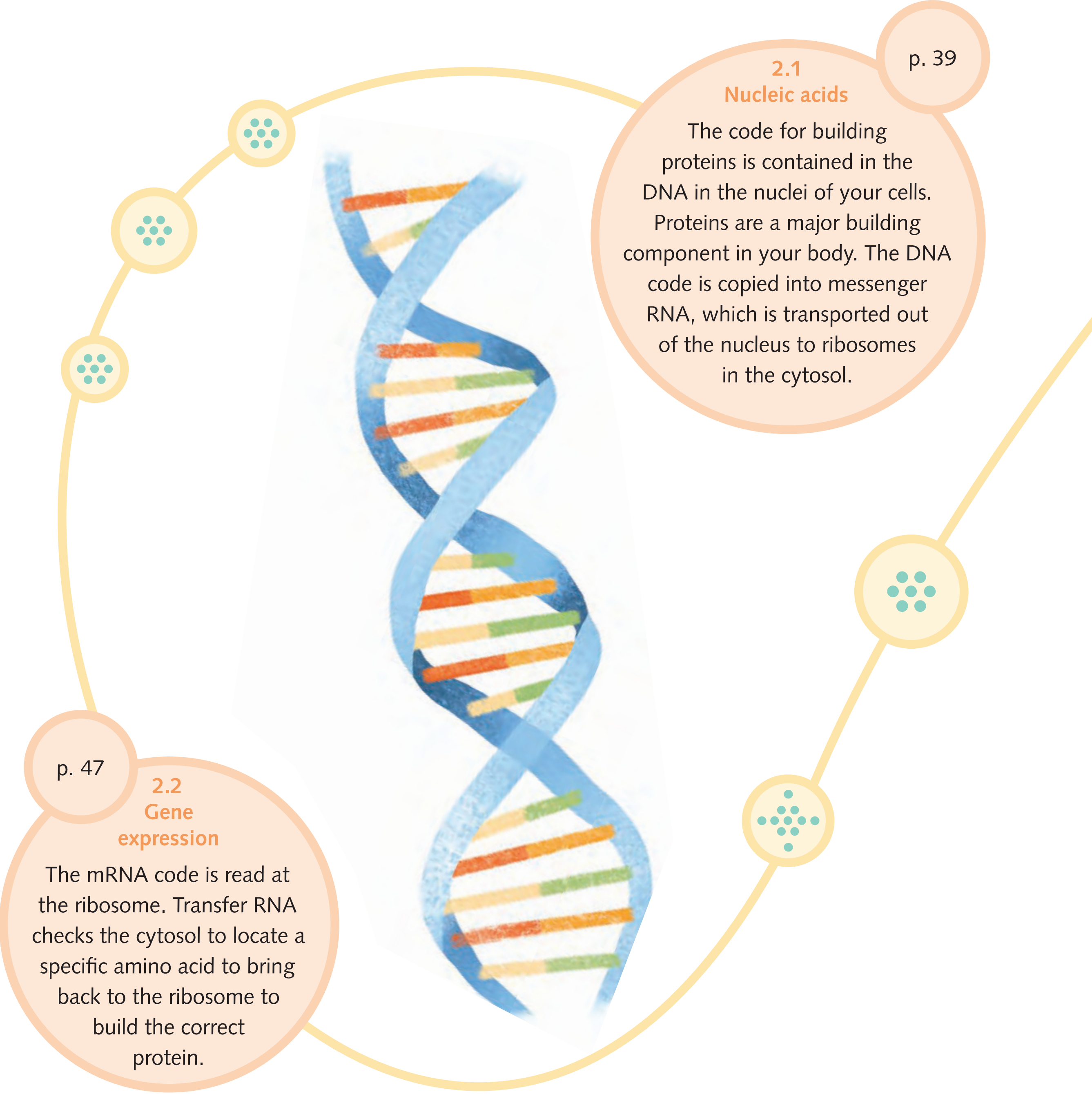
Analyse, evaluate and communicate scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them, pp. 43–44

Online Chapter Map
Chapter 2 map

2 The relationship between nucleic acids and proteins

In Biology, using many small blocks to build larger and more complex structures is a concept that recurs again and again.



p. 39

2.1
Nucleic acids

The code for building proteins is contained in the DNA in the nuclei of your cells. Proteins are a major building component in your body. The DNA code is copied into messenger RNA, which is transported out of the nucleus to ribosomes in the cytosol.

p. 47

2.2
Gene expression

The mRNA code is read at the ribosome. Transfer RNA checks the cytosol to locate a specific amino acid to bring back to the ribosome to build the correct protein.

2.3 Gene regulation

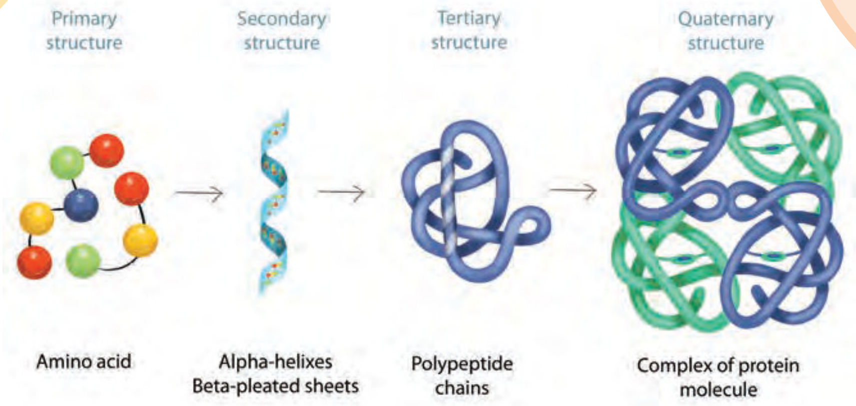
p. 54

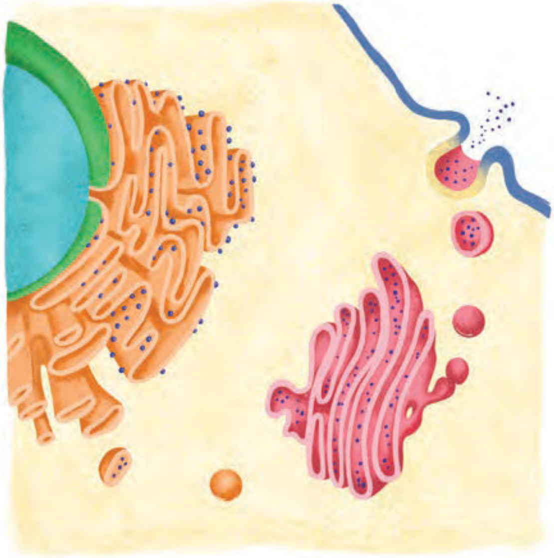
Not all proteins are built in every cell. Muscle cells and blood cells are made from different proteins. Regions of coding DNA can be switched on or off depending on the needs of the cell.

2.4 Proteins

p. 57

The sequences of amino acids determine the type of proteins produced. Chains of amino acids are folded, coiled and bonded to create more and more complex protein structures. Complexity determines the function of the protein.





2.5 The protein secretory pathway

pp. 64

Proteins need to move about. From the endoplasmic reticulum within a cell, further processing occurs before proteins are moved to the Golgi apparatus for secretion out of the cell or for use in other areas of the body.

The structure of proteins is coded in our DNA. Scientists have ways to manipulate DNA.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 2 map (p. 36)

Online Key Terms:
- Chapter 2 flashcards (p. 38)

Weblinks:
- Nucleic acids (p. 46)
- From DNA to protein (p. 51)

Online Worksheets:
- Nucleic acids (p. 46)
- From DNA to protein (p. 51)

Video:
- Gene expression (p. 47)
- The *trp* operon (p. 55)

Online Key Concepts:
- Chapter 2: Summary of key concepts (p. 68)

Know your key terms

Online Key Terms
Chapter 2 flashcards

α-helix
allele
alternative splicing
amino acid
anticodon
antiparallel
attenuation
β-pleated sheet
base pair
biological functionality
catalyse
chromosome
cisterna
codon
complementary base pairing
condensation polymerisation
conformation
degenerate
denature
deoxyribonucleic acid (DNA)
DNA polymerase
endoplasmic reticulum
enzyme
exon
functional proteomics
gene
gene expression
gene regulation
genetic code
genome
Golgi apparatus
histone
hydrogen bond
hydrophilic
hydrophobic
inducer
intron
leader region
messenger RNA (mRNA)
non-coding region
non-template strand
nucleic acid
nucleosome
nucleotide
operator
operon
peptide bond
phosphodiester bond
plasmid
poly-A tail
polypeptide
polyribosome
pre-mRNA
primary structure
product
promoter region
protein
protein secretory pathway
proteome
proteomics
quaternary structure
random coil
regulatory gene
repression
repressor protein
ribonucleic acid (RNA)
ribosomal RNA (rRNA)
ribosome
RNA polymerase
rough endoplasmic reticulum
secondary structure
semi-conservative replication
structural gene
substrate
subunit
template
template strand
tertiary structure
transcribe
transcription
transcription factor
transfer RNA (tRNA)
translation
transport vesicle
triplet

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 DNA contains the coded instructions to produce proteins, the 'workhorses' of a cell.
2 Organelles involved in protein synthesis and secretion include the rough endoplasmic reticulum, ribosomes and Golgi apparatus.
3 Proteins are building blocks for cellular structures and carry out many cellular processes.

WB
REMEMBER
PAGE 22

Origami is the ancient Japanese art of paper folding, where sheets of paper are transformed into sculptures through precise folding techniques. Several sheets of coloured paper can be used to produce complex sculptures such as those seen in Figure 2.1. Paper is the basic material of origami and complex folding of the paper produces the different sculptures.

Proteins are large complex molecules made from monomers called **amino acids**. Chains of amino acids form **polypeptide** chains, which can then be folded, in a similar way to folding paper in origami, to produce different types of proteins. Proteins are the building blocks for most of the structural and functional components of a cell. The complexity and diversity of the biological world around us can be explained by proteins.

This chapter will unfold the story of proteins from their beginning as a code stored in the **nucleotides** that make up the nucleic acids of the DNA molecule to how the cell deciphers that code to produce a complex protein molecule.

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iStock.com/CribbVisuals

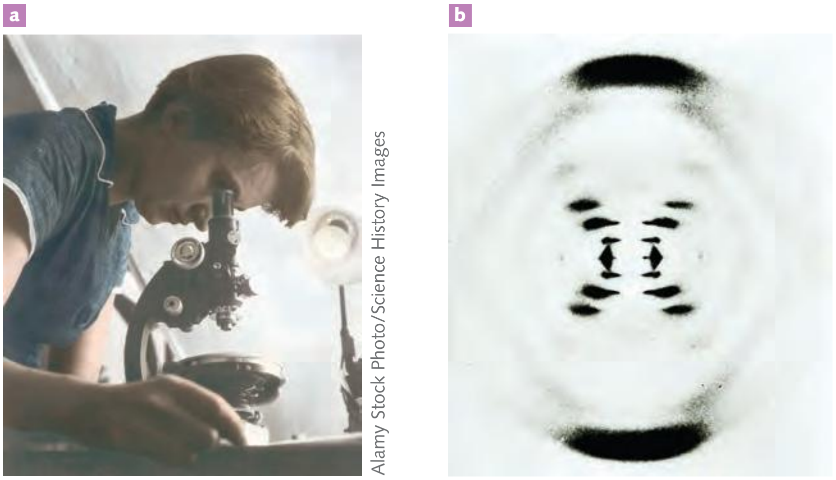
Alamy Stock Photo/Adonsky

Figure 2.1 Sheets of paper folded in different ways produce many different types of sculptures, just as chains of amino acids folded into different shapes can produce many different types of proteins.

2.1 Nucleic acids

Many years of work and investigation by scientists contributed to the body of knowledge that eventually led molecular biologists James Watson and Francis Crick to report the famous model of the double helix in 1953 (Figure 2.2). Watson and Crick used X-ray images of the DNA molecule that were made by X-ray

Figure 2.2 Watson and Crick with their DNA model



Alamy Stock Photo/Science History Images

Alamy Stock Photo/Science Photo Library

Figure 2.3 a Rosalind Franklin and **b** her X-ray diffraction picture of DNA, which was crucial for the discovery that DNA is a double helix.

crystallographer Rosalind Franklin, which were critical to determining its structure (Figure 2.3). DNA is an example of a nucleic acid. **Nucleic acids** store information in a chemical code that directs the cell to produce proteins. The genetic code of every living organism is contained in its DNA, the molecule of life. DNA is necessary for the survival of every living organism, and ultimately the evolution of life over time.

The nucleic acids **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)** are polymers that form when the monomers (nucleotides) bond together.

WB 2.1.1 STRUCTURE OF NUCLEIC ACIDS PAGE 23

Structure of nucleic acids

Nucleotides are the subunits, or monomers, which link together to form the polymers, called polynucleotide chains, of the nucleic acids. DNA and RNA differ slightly in the structure of their nucleotides. Each nucleotide has three distinct chemical components:

- a five-carbon pentose sugar (either ribose or deoxyribose)
- a negatively charged phosphate group
- an organic nitrogen-containing compound called a base (Figure 2.4).

EXAM TIP

Students should be able to draw and label the basic components of nucleotides and amino acids, however they are not expected to know or draw the chemical structure of these molecules.

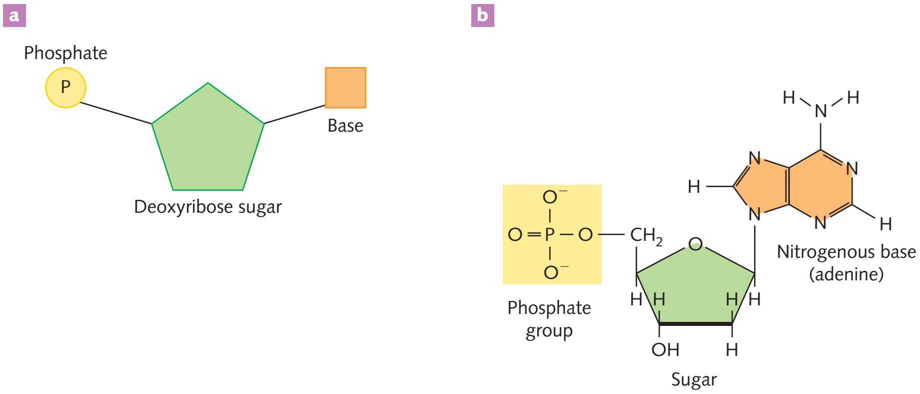


Figure 2.4 a A general representation of the structure of a DNA nucleotide. **b** A chemical representation of a nucleotide with the nitrogenous base adenine.

Structure of DNA

DNA is made up of nucleotides in which the pentose sugar has one fewer oxygen atom (hence: *de-oxy-ribose*) than the ribose sugar (p. 45). DNA is a stable molecule that carries genetic information of organisms through generations.

There are four kinds of nitrogenous (nitrogen-containing) bases in DNA:

- adenine (A)
- thymine (T)
- guanine (G)
- cytosine (C).

Thymine and cytosine are the smallest bases because they consist of one six-membered ring containing carbon and nitrogen. They are known as pyrimidines. Adenine and guanine are larger and they consist of two rings containing carbon and nitrogen. They are known as purines (Figure 2.5).

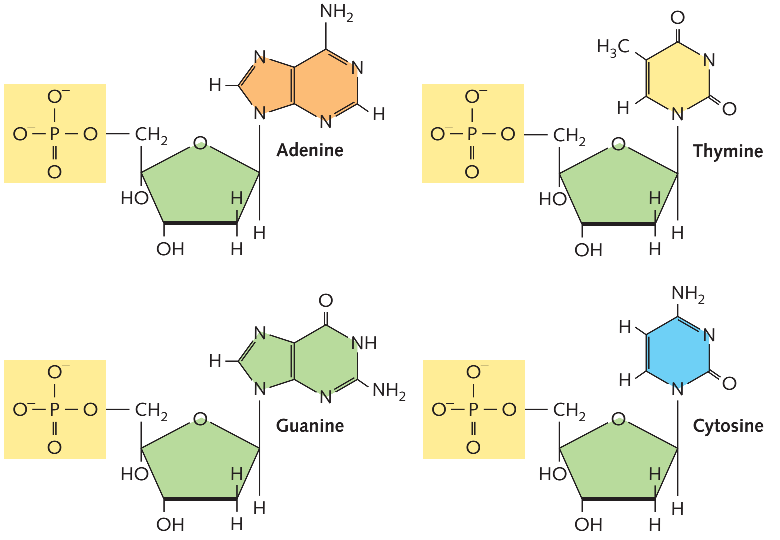


Figure 2.5 **The four nucleotides that make up DNA. Each nucleotide contains a different base: adenine, guanine, thymine or cytosine.**

DNA molecules are very long and consist of two strands of nucleotide polymers wound around each other to form a double helix. In each strand, the sugar–phosphate groups that make up the backbone of each strand are linked by **phosphodiester bonds**. The two strands are tightly bonded to each other. They are like a zip that can be unzipped when the genetic information is 'read'. **Hydrogen bonds** between the adjoining pairs of nitrogenous bases hold the two chains of the double helix together, much like the rungs of a ladder.

The nitrogen bases do not bond randomly. They bond according to strict **complementary base pairing** rules: adenine (A) always bonds with thymine (T), and guanine (G) always bonds with cytosine (C). A smaller base (T or C) is always bonded to a larger base (A or G), which ensures that the two strands remain a fixed distance from each other (Figure 2.6).

WB
2.1.2
STRUCTURE OF DNA, BUILDING A MODEL
PAGE 24

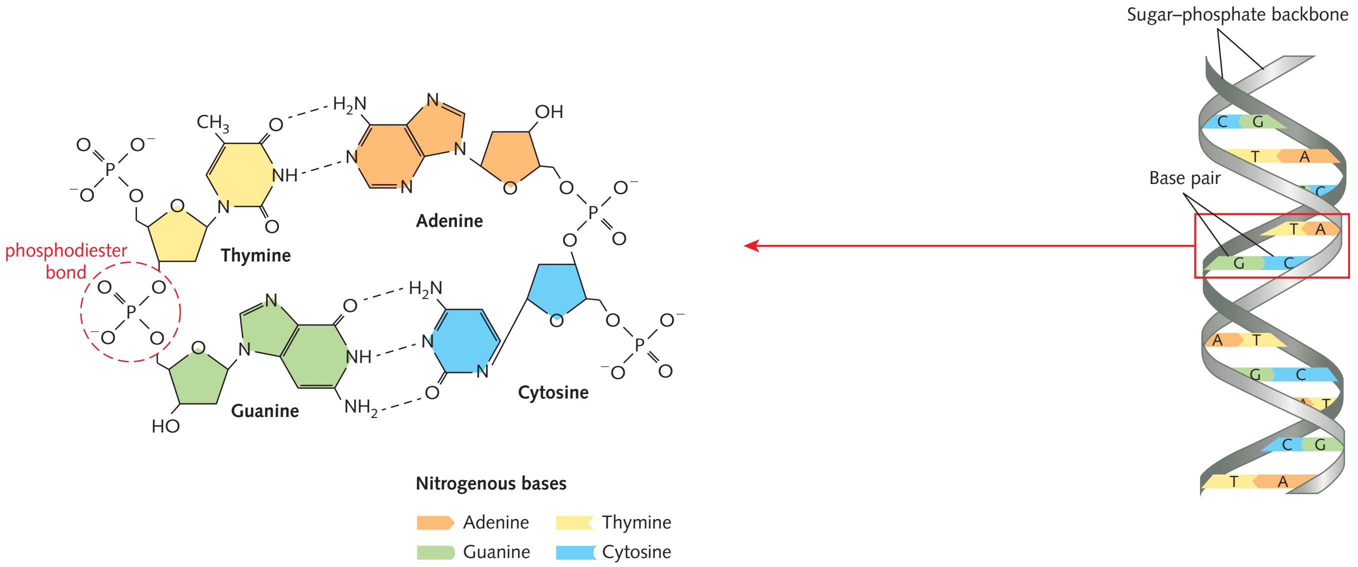


Figure 2.6 **The DNA helix is a double-stranded molecule. Two strands are held together by hydrogen bonding between complementary nitrogen bases. An A–T pair is held together by two hydrogen bonds, whereas a G–C pair is held together by three hydrogen bonds.**

Figure 2.7 shows that at the top left of the strand of DNA the 5′ (5 prime) carbon is facing upwards and this nucleotide (A) is bonded to a nucleotide (T) with the 3′ carbon facing upwards. This continues down the two strands of DNA so that they are running in opposite directions, with the top of one strand always laid against the bottom of the other strand. This is known as **antiparallel**.

In prokaryotic cells, the DNA is in the form of a single, circular **chromosome**. Some DNA is also found as small circular pieces of DNA called **plasmids**. The chromosome and plasmids are in the cytosol. In eukaryotic cells, the DNA is in the form of linear chromosomes contained within the nucleus. Proteins called **histones** are bound to the DNA in eukaryotic chromosomes to form **nucleosomes**. Like winding cotton around many reels, histones help pack the large DNA threads into the confined space of the nucleus (Figure 2.8). Nucleosomes are then coiled and condensed into a fibre, which are supercoiled to form chromosomes during cell division. Some DNA is also found in the form of circular chromosomes in the mitochondria and chloroplasts.

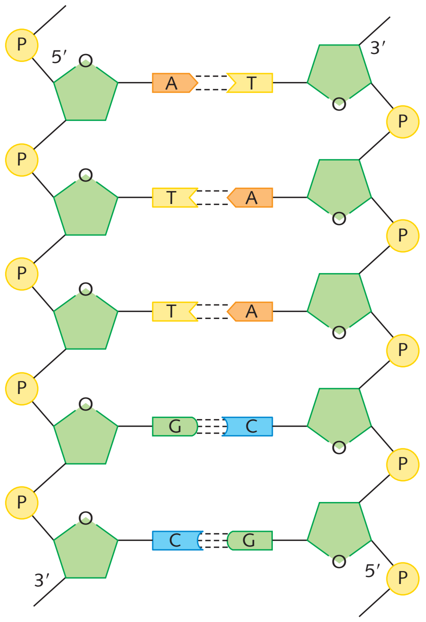


Figure 2.7 The antiparallel nature of DNA: the two strands of DNA run in opposite directions.

The complete base sequence of DNA in a single (haploid) set of an organism's chromosomes is called its **genome**. Segments of the DNA are called **genes** and certain sequences of nucleotides in the DNA of the gene code are responsible for making polypeptides. The sequence of the coding nucleotides in a segment of DNA ultimately determines the sequence of amino acids in the polypeptide. In turn, the sequence of amino acids determines which protein is formed.

Each cell of the human body contains more than a metre of DNA, twisted and coiled into 46 chromosomes that have more than three billion base pairs (bp). However, not all the DNA codes for polypeptides. Genes account for only about 1% of the human genome. Although the other 99% of the human genome is not protein coding, this does not mean that it is not important. In fact, **non-coding regions** of the genome contain many important regulatory regions that are involved in switching genes on or off. This is discussed in more detail on page 54.

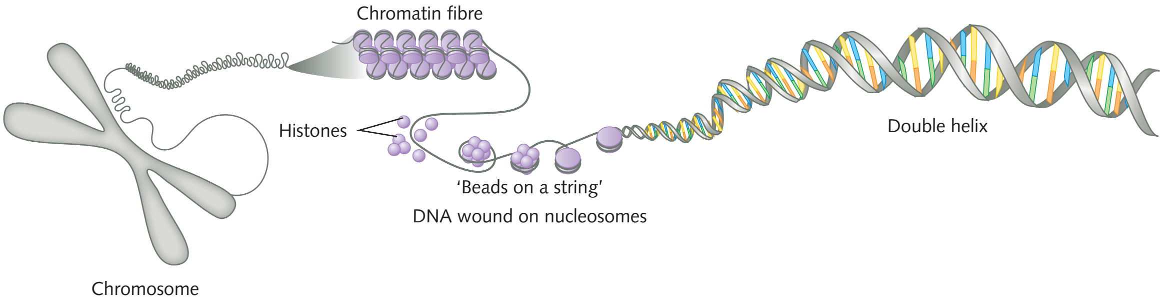


Figure 2.8 DNA is tightly wound around histones to package it inside the nucleus.

Most genes have small differences in the nucleotide sequences from one individual to another, except for identical twins. This means there may be differences in the polypeptides that are encoded by any given gene. These different versions of the same gene are called **alleles**. Alleles account for much of the variation between individuals in a population.

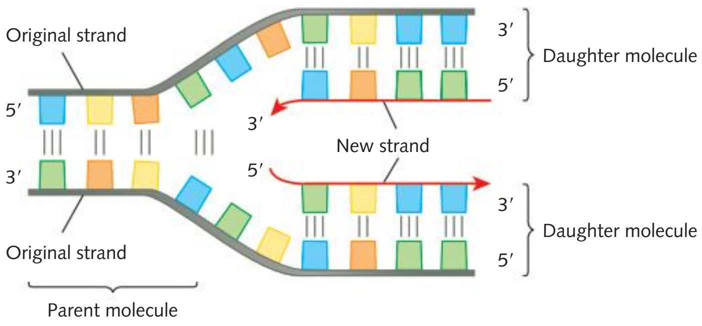


Figure 2.9 **A model for semi-conservative replication of DNA. Each daughter molecule contains one strand from the parent DNA molecule and one new strand.**

As already discussed, each DNA molecule consists of two complementary strands held together by hydrogen bonds. If the nucleotide sequence is known for one strand, it is possible to determine the sequence of the other strand because of the base-pairing rules (p. 41). When DNA is copied, the two strands are first separated by the action of **enzymes**. Each single strand serves as a **template** for the production of a new complementary strand (Figure 2.9). The new strand is built towards its 3′ end. The enzyme **DNA polymerase** moves along the template strand, adding complementary nucleotides. This is described as **semi-conservative replication** because each new daughter chromosome consists of one new strand and one conserved or original strand.

Double-stranded DNA is a persistent, long-lived molecule that carries the codes for protein synthesis, determining structure and functioning in cells. Because of its unique ability to replicate with high accuracy, the genetic information encoded in the nucleotides of DNA is passed from one generation of cells during cell division to the next and from one generation of organisms to the next through the process of reproduction. DNA is the master code that determines the very nature of cells and therefore of living things.

Developed exclusively by Southern Biological

INVESTIGATION 2.1

Extracting DNA from strawberries

Strawberries have eight sets of chromosomes, which means they are octoploid, as are pansies, dahlias and sugar cane. Strawberries are a very effective model for DNA extraction because their pink juice allows the white strands of DNA to be clearly observed.

Aim

To extract DNA from strawberries

Time requirement

30 minutes

Materials

- 3 Strawberries
- 10 mL DNA extraction buffer
- 5 mL Protease enzyme
- 5 mL ice-cold 95% ethanol
- 2 Plastic pipettes
- 1.5 mL centrifuge tube
- 1 Stirring rod
- 1 Inoculation loop
- 1 Resealable plastic bag
- 1 Test tube
- Filter paper
- 1 Glass funnel
- Lab coat
- Safety glasses
- Disposable gloves

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Ethanol is highly flammable.	Store and use ethanol away from ignition sources. Do not heat in a container over an open flame; use a water bath that is spark proof.
Protease enzyme can irritate the skin and eyes on contact.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.
Strawberries can cause allergic reactions in sensitive people.	Never eat food in a science laboratory. Let your teacher know if you have a strawberry allergy.

Method

1. Place three strawberries in a resealable plastic bag and re-seal.
2. Squeeze the strawberries in the bag with your fingers until lightly crushed.
3. Open bag and add 10 mL of the DNA extraction buffer.
4. Re-seal the bag and crush the contents again using your hands to mix the ingredients within the bag. Continue until a thick juice is produced.
5. Using a plastic pippete, add 5 mL of protease enzyme to the bag and mix for 1 minute.
6. Filter the strawberry juice into a test tube. To do this, place the filter paper in a glass funnel over the test tube. Pour the strawberries out of the bag and over the filter paper and a clear, pulp-free juice will filter into the test tube.
7. Remove the filtering apparatus and use a pipette to slowly add approximately 5 mL of cold ethanol into the test tube to cover the strawberry juice solution. Do *not* agitate the solution. The ethanol should sit separately on top of the strawberry solution.
8. White strands of strawberry DNA will become visible in the ethanol layer as the DNA is extracted from the nucleus as the cell walls break down. The strands will look like very fine spider webs.
9. Use the inoculation loop to ‘spool’ strands of DNA and observe them more closely. Alternatively, hold the test tube at eye level and use a pipette to draw up the DNA strands in the top layer fluid.
10. Transfer the DNA to a centrifuge tube for further examination.

Results

Describe what you see.

Discussion

1. DNA extraction buffer contains detergent and salt. What role did the detergent, protease enzyme, ethanol and salt have in the process of DNA extraction?
2. Many of the foods we consume contain DNA. Explain why ingesting DNA from other plants and organisms does not cause us harm or alter our DNA.
3. What is the function of DNA?
4. Where is DNA located within a cell?
5. Draw a diagram of DNA. Include five sets of nucleotide bases and label the hydrogen bonds between these bases.
6. Why is the ability to remove DNA from cells important to scientists?
7. Why does DNA rise towards the surface when the alcohol is added?

Conclusion

Summarise your findings and include a flow chart detailing the steps taken to release the DNA from the strawberry cells.

Taking it further

1. Perform another DNA extraction on different plant samples, such as bananas, kiwi fruit or wheatgerm.
2. Following the same procedure, compare the results of the DNA extraction among the different plant samples.

KEY CONCEPTS

- Nucleotides are monomers that are made up of a five-carbon pentose sugar, a phosphate group and a nitrogenous base.
- Nucleotide monomers make up the nucleic acids DNA and RNA.
- There are four different types of nitrogenous bases in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T).
- Nucleotides make up the strands of DNA, which are held together by hydrogen bonds between complementary bases.
- Bases pair according to the complementary base pairing rules: A always bonds to T; C always bonds to G.
- The two strands of DNA are antiparallel.
- In cells, DNA is organised into chromosomes.

Concept questions 2.1a

1 List the three key features of a nucleotide and describe how they are arranged.
2 Describe how nucleotides are linked together.
3 Describe how the two strands of DNA are held together.
4 Explain the antiparallel structure of DNA.
5 State the complementary base pairing rule.

HOT challenge

6 Draw a flow chart to show your understanding of DNA structure. Use the following terms in your flow chart: adenine, base, cytosine, guanine, hydrogen bonds, monomer, nucleic acid, nucleotide, phosphate, sugar, thymine. You may add more terms.

Structure of RNA

WB 2.1.3 STRUCTURE OF RNA PAGE 29

RNA is composed of nucleotides that are linked together by phosphodiester bonds. However, whereas DNA nucleotides contain a deoxyribose sugar, RNA nucleotides contain a ribose sugar, which contains one more oxygen atom (Figure 2.10).

RNA contains the base uracil (U) instead of thymine (T) found in DNA. The bases T and U are very similar, both being pyrimidines (Figure 2.11).

Another difference between RNA and DNA is that RNA is a single-stranded molecule and has a variety of folding patterns. DNA is a double-stranded molecule and coils into a double helix. RNA molecules are much shorter than DNA molecules. RNA ranges in size from a few dozen up to several thousand nucleotides, whereas DNA molecules range from tens of thousands up to hundreds of millions of **base pairs** in one molecule.

Table 2.1 summarises the differences between DNA and RNA.

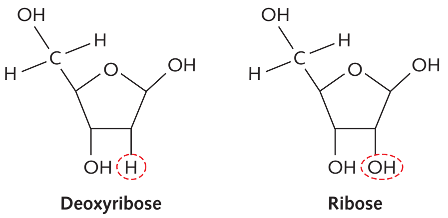


Figure 2.10 The pentose sugars of DNA (deoxyribose) and RNA (ribose)

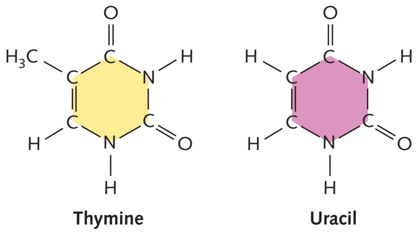


Figure 2.11 RNA contains the nitrogenous base uracil instead of thymine, which is found in DNA.

Table 2.1 Differences between DNA and RNA

	DNA	RNA
Nucleotide structure		
Sugar–phosphate backbone	Deoxyribose sugar	Ribose sugar
Nitrogen bases	Adenine, cytosine, guanine, thymine	Adenine, cytosine, guanine, uracil
Longevity	Highly stable; usually exists as a double helix that can form coils and supercoils	Unstable; exists as a single chain that often folds into secondary structures
Forms	Linear or circular chromosomes Plasmids	mRNA – linear shape tRNA – clover leaf shape rRNA – 2 subunits of ribosome (others that are beyond the scope of this course)
Enzyme responsible for synthesis	DNA polymerase	RNA polymerase
Location in eukaryotic cells	Nucleus, mitochondria and chloroplasts	Nucleus and cytosol

Function of RNA

The differences between the structure of DNA and RNA determine major differences in the longevity and functioning of the two types of molecules.

RNA is a single-stranded molecule that is relatively short-lived; it is made and degraded rapidly by cells. RNA is a versatile molecule with a variety of functions that facilitate and regulate protein production. The three main types of RNA are:

- **messenger RNA (mRNA)**
- **transfer RNA (tRNA)**
- **ribosomal RNA (rRNA)**.

The different forms of RNA are described in more detail in Table 2.2. Their roles in **gene expression** are discussed on page 47. Table 2.3 compares where DNA and RNA are found in cells.

Weblink
Nucleic acids
Watch the video on nucleic acids to revise the importance and structure of DNA and RNA.

Worksheet
Nucleic acids

Table 2.2 A comparison of the different forms of RNA in eukaryotic cells

	mRNA	tRNA	rRNA
Function	Carries the DNA code from the nucleus to the ribosome containing instructions for protein synthesis	Delivers amino acids to ribosomes for protein synthesis	A component of the ribosome: associates with proteins to form ribosomes
Location in which it functions	Nucleus and cytosol	Cytosol	Cytosol
Shape	Linear	Clover leaf	Three-dimensional fold

EXAM TIP
Ensure you know the differences and similarities (compare) between DNA and RNA, their structure and functions.

Table 2.3 Locations of DNA and RNA in prokaryotic and eukaryotic cells

Nucleic acid		Location in prokaryotic cell	Location in eukaryotic cell
DNA		A single circular chromosome in the cytosol Small circular plasmids in the cytosol	Linear chromosomes in the nucleus In mitochondria (circular DNA) In chloroplasts of plant cells (circular DNA)
RNA	mRNA	Cytosol	Nucleus and cytosol
	tRNA	Cytosol	Cytosol
	rRNA	Within ribosomes in the cytosol	Within ribosomes free in the cytosol, or attached to endoplasmic reticulum

KEY CONCEPTS

- RNA is single-stranded.
- RNA nucleotides contain ribose sugar.
- RNA contains the base uracil (U) instead of the DNA base thymine (T).
- RNA strands are shorter than DNA strands.
- RNA plays a major role in gene expression.
- There are three main types of RNA: mRNA, tRNA and rRNA.

Concept questions 2.1b

1 Identify three fundamental differences between the structures of DNA and RNA.

2 List the three major types of RNA and describe the function of each.

3 Explain the following statement. 'RNA plays a major role in gene expression.'

4 What are the main constituents of an RNA nucleotide?

5 Why are RNA strands shorter than DNA strands?

HOT challenge

6 In a table, construct diagrams that discriminate between the general structures of mRNA, tRNA and rRNA.

2.2 Gene expression

Gene expression is the transfer of the DNA code in a gene, by transcription, to **ribosomes** in the cytosol to produce a functional gene product, through translation (Figure 2.12).

Transcription

DNA is too large a molecule to leave the nucleus through a nuclear pore. The smaller molecule mRNA serves as the messenger that carries the information coded on the DNA out of the nucleus. The process of copying DNA to produce a complementary RNA molecule is called **transcription**. The DNA in the region of the gene first unwinds and then unzips with the aid of the enzyme helicase, exposing the nucleotide bases of both DNA strands. One strand of the DNA has the sequence that codes for the polypeptide and is called the **non-template strand**. The sequence of bases on the non-template strand is the same as the sequence of bases on the mRNA transcript, except thymine is replaced by uracil. The other strand is called the **template strand**. Due to the complementary base pairing of DNA, the template strand is read and the sequence is transcribed to produce the mRNA transcript.

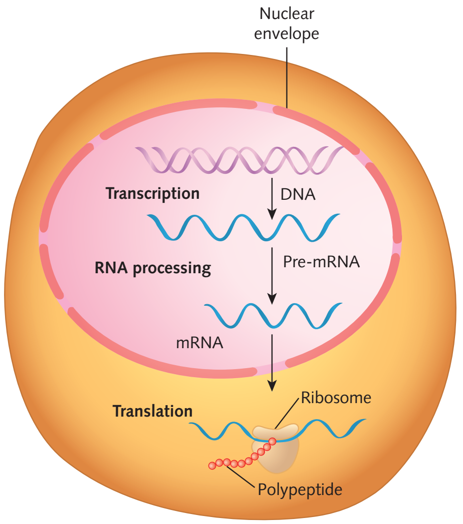


Figure 2.12 Gene expression relies on the processes of transcription and translation. During transcription, DNA is copied into pre-mRNA, which is processed into mature mRNA for export from the nucleus. During translation, the ribosome 'reads' the nucleotide sequence in the mRNA and forms the amino acid sequence of a polypeptide.

A sequence of DNA, called the **promoter region**, signals the start of the gene. The promoter region does not code for a gene but contains the information that determines where (in which cell type) and when (at what stage of development or activation) a gene is transcribed. Transcription begins when proteins position the enzyme **RNA polymerase** onto the DNA to bind with the promoter region. RNA polymerase then proceeds along the DNA, progressively building a strand of **pre-mRNA** from RNA nucleotides, that is complementary to the template strand (Figure 2.13).

Video
Gene expression

2.2.1 TRANSCRIPTION PAGE 30

Note:

The two strands of DNA have different names. In this resource they will be called the template strand and the non-template strand. In other resources you might also see them called the non-coding (template) and coding (non-template) strand or the anti-sense (template) and sense (non-template) strands.

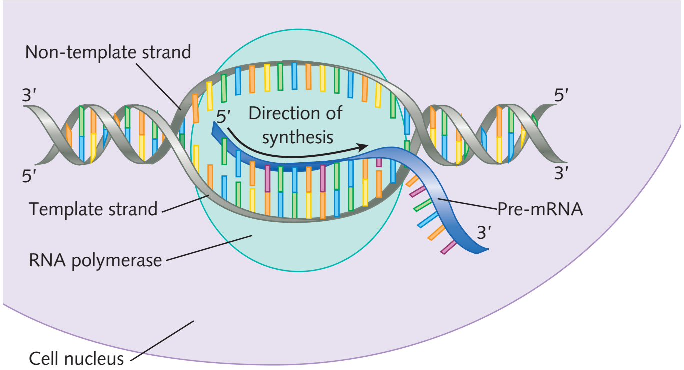


Figure 2.13 During transcription, the pre-mRNA strand is synthesised in the 5′ to 3′ direction from the template strand of DNA.

Although both DNA strands are exposed when the DNA separates in the region of the gene, the antiparallel structure of DNA determines which strand will be used as the template strand for transcription. Only the DNA template strand (from 3′ to 5′) will be **transcribed** into pre-mRNA (from 5′ to 3′). The nucleotide sequence of the mRNA is complementary to the non-template strand of DNA, with uracil added instead of thymine (Figure 2.13).

A sequence of nucleotides downstream of the gene serves as a signal to stop transcription. The RNA is released as a single strand of pre-mRNA. Once the pre-mRNA has peeled off, the DNA zips up and coils back into a double helix.

RNA processing

WB 2.2.2 RNA PROCESSING PAGE 35

Pre-mRNA is not yet in its mature mRNA form. It contains **introns**, which are non-coding regions, and **exons**, which contain the codes for the amino acids for polypeptide formation. Both the exons and introns are transcribed into pre-mRNA. The introns are then cut out, while exons rejoin by RNA splicing to form a shorter strand of mature mRNA that moves out of the nucleus to the cytosol.

As it is being transcribed, the pre-mRNA is modified by the addition of a methyl group (CH_3) at the 5′ end. Before it leaves the nucleus, the pre-mRNA is modified at the 3′ end by polyadenylation (the addition of 100–200 adenine bases) called the poly-A tail (Figure 2.14). The addition of these structures protects the RNA from degradation. When the methylated cap and **poly-A tail** are later removed, the RNA is rapidly digested and the nucleotides are recycled for further RNA synthesis.

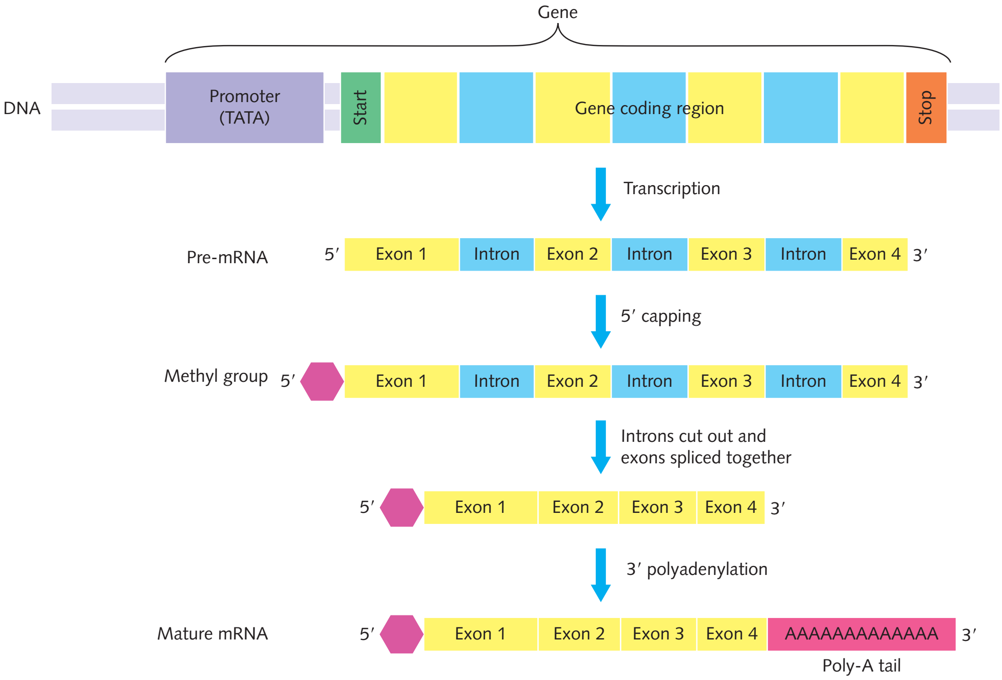


Figure 2.14 Transcription and RNA processing generates mature mRNA from pre-mRNA in the nucleus of a cell.

After processing, the mature mRNA is ready to leave the nucleus and move into the cytosol, where ribosomes translate the nuclear code into polypeptides. The average mRNA strand is 1000–2000 bases long, including the methylated cap and 100–200 adenine bases in the poly-A tail.

Alternative splicing

Before the human genome was sequenced, scientists predicted that it would contain more than 100000 genes, based on the estimated number of proteins in the human body. Surprisingly, after sequencing, scientists found that there are only 20000–25000 genes. This is because a single gene can code for several different polypeptides.

During pre-mRNA processing, different exons may be removed along with the introns to produce mRNA molecules of different length and sequence from the same pre-mRNA molecule (Figure 2.15). This process is referred to as **alternative splicing**. The polypeptides translated from the alternative mRNA molecules are of different sizes, have different sequences, and have their own unique functions.

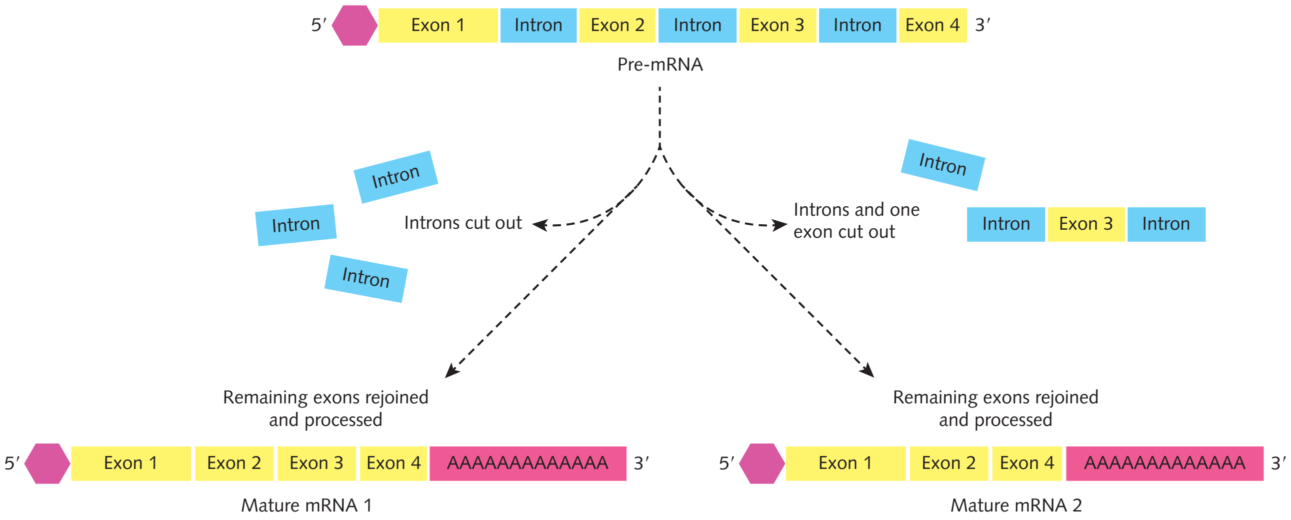


Figure 2.15 Alternative splicing allows the production of different mRNA molecules, and therefore different polypeptides, from the same gene. The more exons a gene has, the more options there are for alternatively spliced mRNA transcripts. The average number of exons per gene is nine, interspersed with eight introns.

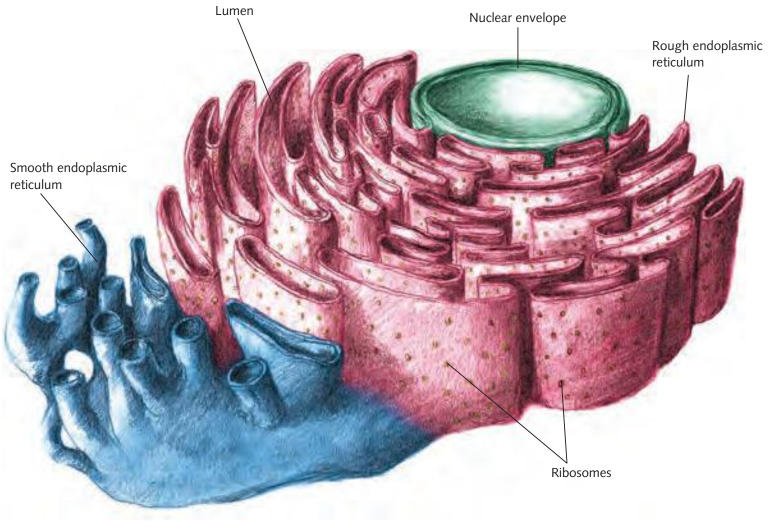
How a cell 'knows' which exons to keep and which to remove during alternative splicing is an area of intensive research. The fundamental mechanism involves interactions between specific mRNA sequences and nuclear proteins found in specific cell and tissue types. The same transcript expressed in two different tissues may be bound by different nuclear proteins present in each tissue. In one, the proteins may protect exons from removal. In the other, the proteins may loop introns together so that the exon in between is 'ignored' and cut out.

Ribosomes

In eukaryotic cells, ribosomal RNA (rRNA) combines with special proteins to form ribosomes in the nucleolus. Ribosomes are made up of two **subunits**: a smaller one called the 40 S subunit and a larger one called the 60 S subunit (S is a unit of size). The subunits move from the nucleolus in the nucleus into the cytosol. Both subunits contain many protein molecules together with rRNA. The subunits combine to form the functional units, the ribosomes, for translation.

Ribosomes are also found in prokaryotes, and in the mitochondria and chloroplasts of eukaryotes. These ribosomes are smaller than the ribosomes in the cytoplasm of eukaryotic cells, although they also consist of two subunits and are involved in protein synthesis. In eukaryotic cells, ribosomes are found free throughout the cytosol. Ribosomes are also bound to the **endoplasmic reticulum**, forming **rough endoplasmic reticulum**, an organelle consisting of a series of flattened sacs (Figure 2.16).

EXAM TIP
Make sure you write rough endoplasmic reticulum in full when answering exam questions. The abbreviation RER or rER is not an accepted abbreviation.



Science Source/Spencer Sutton

Figure 2.16 A ribosome attached to the endoplasmic reticulum is the site of synthesis of a polypeptide. The polypeptide is transferred to the lumen (inside) of the endoplasmic reticulum.

Generally, proteins that are to be used for structure and functioning in the cell are made on free ribosomes. In contrast, proteins that are secreted from the cell or expressed on the cell's surface are made on ribosomes bound to the rough endoplasmic reticulum.

Ribosomes can form chains, called **polyribosomes** or polysomes, which all bind to a single mRNA strand. The advantage of polyribosomes is that they allow many polypeptides to be made from a single mRNA strand in a comparatively short time. This greatly increases the rate of polypeptide synthesis. In bacterial cells, polypeptide synthesis happens even more rapidly. This is because prokaryotes lack a nucleus, they do not have introns, and protein synthesis can begin even before mRNA synthesis is complete.

Translation

WB
2.2.3
TRANSLATION
PAGE 36

When mRNA passes through a nuclear pore into the cytosol, it then moves to and binds with a ribosome. A small ribosome subunit loaded with an initiator Met-tRNA (one that can start translation) recognises the mRNA strand and binds to the methylated cap on the mRNA. It moves along it, 'scanning' for an AUG start **codon** (a set of three nucleotides). Once the start codon is found, a large ribosomal subunit joins with the small one. The ribosome then moves along the mRNA strand 'reading' the mRNA nucleotides in codons. This process is known as **translation**. The ribosome serves as the workbench for protein synthesis, while tRNA molecules provide the raw materials.

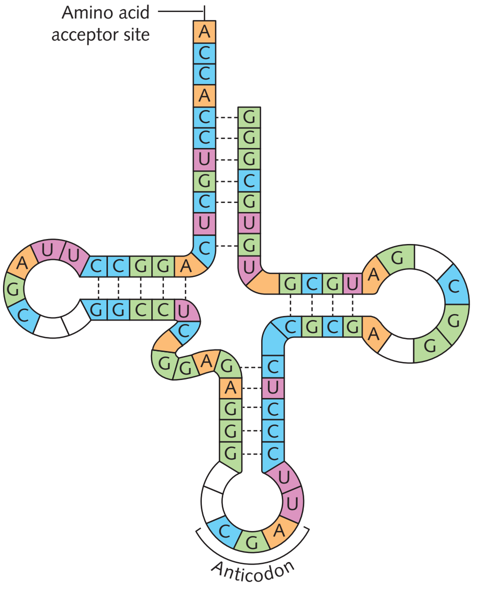
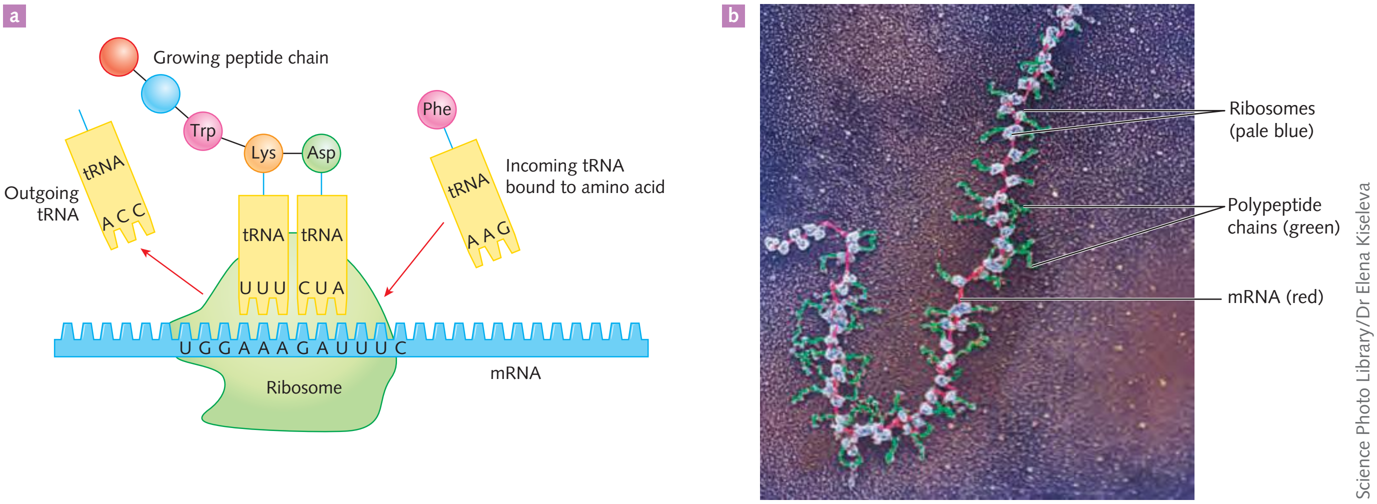


Figure 2.17 A tRNA molecule is a clover leaf shape, with three unpaired bases called the anticodon and a specific amino acid acceptor site.

Transfer RNA (tRNA) molecules each carry one specific amino acid of the 20 amino acids. They exist as free-floating molecules within the cytosol. Unlike mRNA, tRNA molecules are folded into loops with a distinctive clover shape (Figure 2.17). Three unpaired nucleotides in the central loop of the tRNA molecule are called the **anticodon** and will bind to a complementary mRNA codon, following the base-pairing rules. The amino acid is attached to an amino acid acceptor site on the stem of the tRNA molecule.

As the ribosome moves along the mRNA strand, a tRNA molecule moves into the correct position by binding its anticodon with a complementary mRNA codon and delivers a specific amino acid. As the ribosome moves on to the next codon of the mRNA strand, another tRNA molecule with a complementary anticodon binds to the next codon and another amino acid is placed into position, and so on. (How the specific amino acid is determined from the mRNA code is explained below.) As the ribosome moves further along the mRNA strand, more and more amino acids are delivered and joined by peptide bonds to produce the growing polypeptide chain. In this way, the amino acids are linked in an order corresponding to the sequence of codons in the mRNA. As this is determined by the sequence of base triplets in the original DNA, it follows that the base sequence in the DNA determines the order in which the amino acids link up. The process is shown in Figure 2.18. Several



Science Photo Library/Dr Elena Kiseleva

Figure 2.18 a The mRNA sequence of codons determines the order in which amino acids link up to form the polypeptide chain. The three-base anticodon of each tRNA molecule is complementary to the three-base codon in the mRNA. b An electron micrograph of ribosomes and growing amino acid chains of the proteins being made.

ribosomes can move along one mRNA strand simultaneously carrying out translation, each synthesising a polypeptide chain as they go. On reaching a stop codon, the ribosome releases the mRNA strand and the newly synthesised polypeptide chain.

A protein molecule is made up of one or more polypeptide chains joined to make a three-dimensional structure. The diversity of proteins can be explained by how their building blocks, the 20 amino acids, are arranged in various combinations. It is rather like arranging 20 kinds of beads in unique ways to make different necklaces of different lengths. The necklace chains can then be arranged variously in loops and folds to give each its characteristic features.

The genetic code

DNA is the molecule of life – it is essential in all living organisms to code for the proteins produced for the structure and functioning of the cells. It functions primarily as an information molecule by determining the sequence of the 20 different types of amino acids joined in a polypeptide.

A three-letter DNA code (A, C, G or T) can specify for each of 20 different amino acids in polypeptides. The nucleotides are read in groups of three, called **triplets** in DNA and codons in mRNA. If one of four nucleotides can occur in each of the three positions in a codon, then there are $4 \times 4 \times 4 = 64$ possible combinations. One codon in mRNA (AUG) codes for start, three codons (UAA, UAG and UGA) code for stop (no amino acid will be added and polypeptide synthesis is terminated), and the other 60 codons code for the 20 amino acids.

Eighteen amino acids are coded for by several different codons. Amino acids such as serine (Ser) and arginine (Arg) are specified by six codons, while threonine (Thr) and leucine (Leu) are specified by two codons (Figure 2.19). These observations show that there is a level of redundancy within the genetic code. Therefore, the genetic code can be described as **degenerate** because most amino acids can be encoded by two or more codons. This means that if a mutation occurs in the DNA, there is less chance that it will result in an amino acid change when the codons are read, and this means that the resulting protein can still be functional.

When using the genetic code table (Figure 2.19), it is important to remember that it is usually presented as a table of mRNA codons. (If the information given is for DNA, the template strand of the DNA must first be transcribed into mRNA, and then the genetic code table can be used to determine the amino acid sequence.) To use the table, read the first letter of the mRNA codon in the left-hand column of the table, then read the second letter of the codon in the row across the top of the table, and finally read the third letter in the column on the right side of the table. The point of intersection of these three will be the name of the amino acid coded for by the mRNA codon. For example, if the mRNA codon is GCU,

EXAM TIP

Could you create a flow diagram to explain the steps involved in gene expression and could you show each step with a labelled diagram? Make sure you understand how the expression of a single gene can lead to the production of several different proteins.

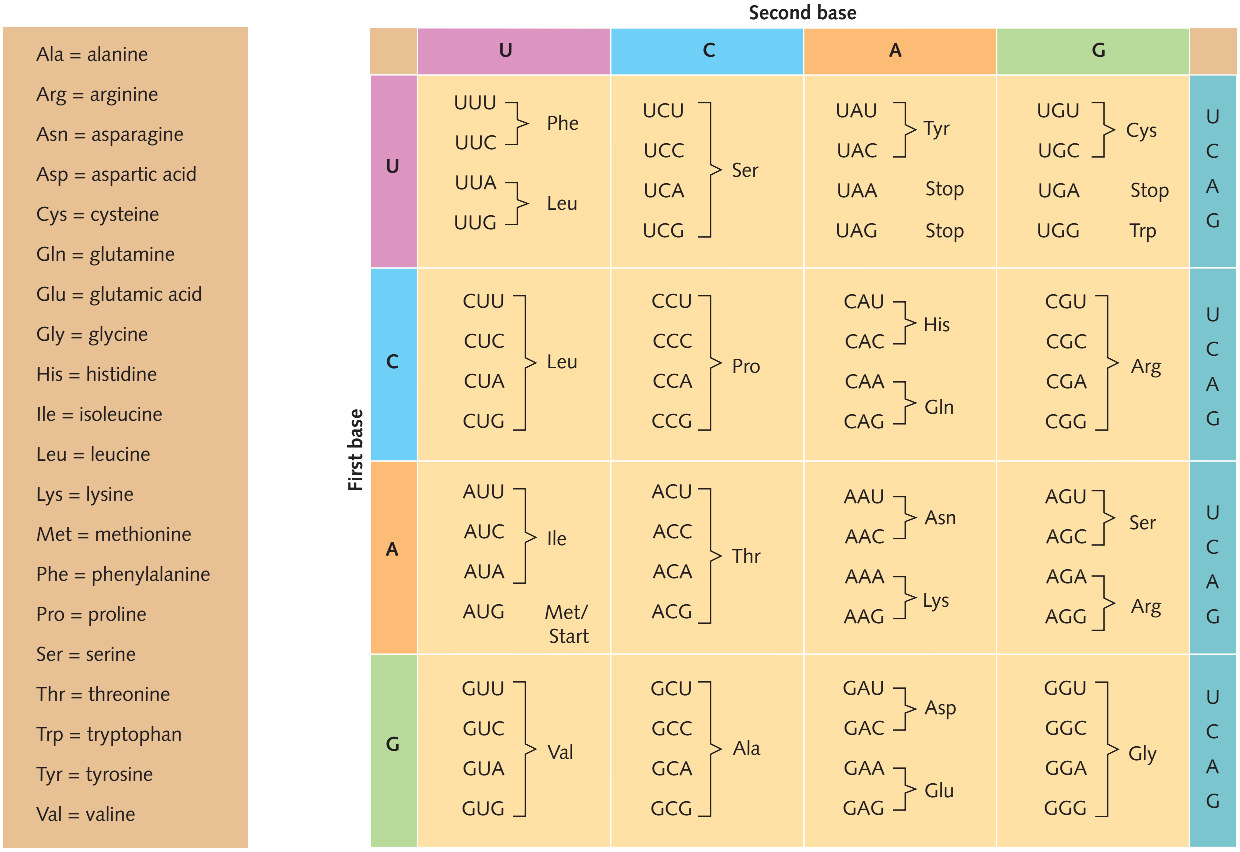
WB
2.2.4 THE GENETIC CODE
PAGE 37

n.

Weblink
From DNA to protein

Online Worksheet
From DNA to protein

WB
2.2.5
SCIENTIFIC LITERACY
PAGE 43



Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Cys = cysteine
Gln = glutamine
Glu = glutamic acid
Gly = glycine
His = histidine
Ile = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = tyrosine
Val = valine

Second base

First base	U	C	A	G	Third base
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met/Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Figure 2.19 The genetic code. Sixty of the mRNA codons correspond to the 20 amino acids; three codons act as stop codons; and the codon AUG initiates protein synthesis.

according to the genetic code, the GCU codon specifies the amino acid alanine (Ala). Similarly, an mRNA codon ACU specifies the amino acid threonine (Thr). Figure 2.19 shows the mRNA codon sequences for each of the 20 amino acids needed to produce all the proteins required by cells.

As already stated, the order of the codons in the mRNA specifies the order of the amino acids in the polypeptide. The fundamental rules of base-pairing are the foundation of the **genetic code**. With very few exceptions, the genetic code is universal to all organisms because they all have the same 20 amino acids specified by the same codons.

ACTIVITY 2.1

Transmitting the code

The nucleus of eukaryotic cells contains DNA, the molecule that encodes for all the proteins produced by the cell. The sites of synthesis of proteins, the ribosomes, are found in the cytosol, outside the boundary of the nucleus. DNA cannot leave the nucleus so, to produce a protein, a message must be sent from the nuclear DNA to the ribosome. To do this, two processes take place:

- transcription of the message from the DNA into a messenger RNA (mRNA) molecule
- translation of the mRNA into a specific amino acid sequence at the ribosome.

But how is the message communicated between the nuclear DNA and the ribosome in the cytoplasm of a cell?

Aim

To simulate how the genetic code is transcribed from DNA and translated into a polypeptide

You will need

Each pair of students requires:

- paper
- scissors
- coloured pencils

What to do

The following sequence of nucleotides is from the *lol* gene of a fungus.

DNA 5′ A T G G A A A C T T G T A T A T A A 3′
DNA 3′ T A C C T T T G A A C A T A T A T T 5′

1 On two separate strips of paper, write each sequence of nucleotides and label each strand ‘DNA’. Ensure the base pairs align when the two strips are brought together.

2 On a sheet of paper, draw a cell with a nucleus. Ensure the nucleus and cytoplasm are both large enough so the strips with the DNA sequences will fit inside them. Place the strips with the DNA sequences inside the nucleus.

Transcription follows base pairing rules, except that thymine in DNA is replaced with uracil in RNA. The complementary sequences in RNA are adenine–uracil and guanine–cytosine.

3 Label a third strip of paper with ‘mRNA’ at one end. Separate the two nucleotide sequences and position the third strip of paper in between them. Using the template strand of DNA write the mRNA sequence on this strip, going from the 5′ end to the 3′ end.

Translation of mRNA occurs at ribosomes in the cytoplasm. The sequence of nitrogen bases in mRNA is read in codons – groups of three. Transfer RNA (tRNA) molecules contain an anticodon, which is complementary to the codon of mRNA, and each tRNA carries a specific amino acid. The tRNA molecules bring these amino acids to be bonded together and form a long chain of amino acids in a specific sequence.

4 Move the mRNA transcript to a ribosome in the cytosol of your model cell. Place a fourth strip of paper alongside it and label it ‘Polypeptide’. Use the genetic code table in Figure 2.19 to translate the mRNA codons into amino acids.

5 Complete Table 2.4 to show the sequences.

Table 2.4 Transcription and translation of the *lol* gene

DNA template sequence						
mRNA sequence						
mRNA codons						
tRNA anticodons						
Amino acid sequence						

What did you discover?

1 a Identify from the model which DNA strand was the non-template strand, and which was the template strand for transcription.
  b Explain how you decided which strand to use as the template strand.
  c Describe the relationship of the nucleotide sequence in the mRNA with the nucleotide sequences of the non-template and template strands.

2 Explain how you decided from which end of the mRNA to start translating.

3 What is the first amino acid in the polypeptide sequence? Is it possible for any other amino acid to appear first in any polypeptide? Explain.

4 a How many nucleotides were in the mRNA sequence?
  b How many amino acids were coded for?
  c What relationship is there between the number of nucleotides in the mRNA transcript and the number of amino acids in the translated polypeptide?

5 Has this model of transcription and translation made the processes easier to understand?

6 Identify any improvements you could make to this model to make the processes easier to understand.

KEY CONCEPTS

- Information in DNA is coded into groups of three nucleotides, or triplets, and each triplet corresponds to a specific amino acid.
- During transcription, mRNA is copied from template DNA.
- Pre-mRNA is processed by cutting, splicing and capping to become mature mRNA before it leaves the nucleus.
- Pre-mRNA can be alternatively spliced to generate different mRNA transcripts.
- mRNA is translated into a polypeptide by ribosomes in the cytosol or ribosomes bound to the endoplasmic reticulum.
- Translation is accomplished with the assistance of tRNA molecules that bring amino acids into position for incorporation into the polypeptide.

Concept questions 2.2

1 Define two key processes in gene expression. Explain how they are different.

2 Explain why only one of the DNA strands can serve as the template strand during transcription.

3 Define 'exons' and 'introns' and explain how they relate to the processing of the pre-mRNA.

4 Describe your understanding of the relationship between codons, anticodons and the different forms of RNA.

5 Explain what is meant by a *degenerate* code.

6 Translate the following mRNA into a polypeptide using the genetic code shown in Table 2.19.
AUGUCCUACCGGGCCUAG

7 A particular mRNA contains 102 nucleotides, excluding the 5′ and 3′ caps.
  a How many codons does this mRNA have?
  b How many amino acids will be translated from this mRNA?

8 Is a triplet different from a codon?

9 What is a STOP codon? Use Figure 2.19 to identify two STOP codons.

HOT challenge

10 The following statement describes current theoretical understandings. 'Splice sites in pre-mRNA are marked by two universally conserved sequences contained at the ends of the introns.' Unpack this statement by explaining what it means. You may use annotated diagrams.

2.3 Gene regulation

The information encoded in DNA determines which proteins are made in particular cells or tissues, and under what environmental conditions gene transcription occurs. Proteins and enzymes (expressed in particular cells at particular times) can bind to these regulatory regions of DNA to turn gene expression on or off. The process of switching gene expression on and off is referred to as **gene regulation**. Genes are regulated during cell differentiation, development, or in response to physiological or environmental cues. Consider how a brain cell differs from a skin cell. These cell types carry out vastly different functions, yet they are packaged with the same DNA. A gene critically important for the functioning of a brain cell might never be expressed in a skin cell.

Cells use several mechanisms to regulate gene expression. For example, the promoter region of DNA where RNA polymerase binds to begin transcription of the gene determines when and in what tissue a gene is transcribed. As scientists sequence and study more genomes, and compare them with the proteomes of the same cells, more methods of gene regulation may be discovered.

Regulatory genes and structural genes

One way of broadly classifying genes is based on whether or not they control the expression of other genes. **Regulatory genes** are involved in controlling the expression of one or more other genes. The products of these genes may be functional pieces of RNA or proteins. The proteins may be enzymes, signalling molecules, receptor molecules or DNA-binding proteins. The key feature of a regulatory gene is that its product alters the expression of other genes.

Structural genes are any genes that are not regulatory genes. Structural genes are those that code for proteins that form the cytoskeleton, keratin in hair and nails, tRNAs and rRNAs, enzymes and signalling proteins that control cellular processes other than the expression of genes.

Structural genes are regulated by regulatory genes. This is shown in the example of the *trp* operon in the bacterium *Escherichia coli*, in which the genes were first described.

The *trp* operon: an example of gene regulation

WB 2.3.1 THE *TRP* OPERON: AN EXAMPLE OF GENE REGULATION PART A PAGE 45

The bacterium *E. coli* inhabits the mammalian intestine, living on sugars and other nutrients. As in all living organisms, *E. coli* requires amino acids to build proteins. One of the amino acids needed by *E. coli* is tryptophan, which *E. coli* takes up and uses from the environment. However, if tryptophan is unavailable, *E. coli* living in a mammalian gut produces the necessary protein enzymes for tryptophan synthesis. When tryptophan is present in the environment, this protein is not produced because tryptophan is energy - expensive and it would be a waste of the bacterium's resources and energy to do so.

The enzymatic proteins required to produce tryptophan are encoded by five structural genes: *trpE*, *trpD*, *trpC*, *trpB* and *trpA*. The coding regions for these five genes appear next to each other on the *E. coli* chromosome and are transcribed as a single mRNA strand. They are under the control of a promoter region, an operator region, and a **leader region**; a repressor *trpR* regulatory gene is located in a different location upstream on the circular chromosome. A group of structural genes together with the promoter, the operator, and the leader segment is known as an **operon**. The *trp* operon is shown in Figure 2.20.

Video
The *trp* operon

Note:
Transcription factors control the rate of gene expression in eukaryotes as well as prokaryotes.

There are two mechanisms that regulate the expression of the structural genes of the *trp* operon when the level of tryptophan is high: first, **repression**, in which the *trp* repressor protein blocks the initiation of transcription; and second, **attenuation**, which allows the cell to terminate transcription of the *trp* operon without binding of the repressor protein. In the first mechanism, repression, the regulatory gene *trpR*, codes for a transcription factor. A **transcription factor** is any protein that binds to DNA, at a promoter or other region involved in the regulation of gene expression, to control the rate of transcription from a gene. Transcription factors can serve to initiate or enhance transcription, or they may act to prevent it. The transcription factor for the *trp* operon is a **repressor protein**. When tryptophan from the environment enters the *E. coli* cell, it functions as an **inducer**, a kind of signalling molecule. Tryptophan binds to the repressor protein, altering its shape so that it can bind to a region of non-coding DNA, called an **operator** (Figure 2.21a). Binding of the repressor protein blocks RNA polymerase from attaching to the promoter region. The five genes of the *trp* operon cannot be transcribed and gene expression is switched off. As the level of tryptophan in the cell is high and the cell does not need to synthesize more tryptophan, this repression mechanism will save the cell energy and resources.

WB 2.3.2 THE *TRP* OPERON: AN EXAMPLE OF GENE REGULATION PART B PAGE 46

When no tryptophan is available to bind to the repressor, the repressor is inactive and cannot bind to the operator. The promoter is now exposed for RNA polymerase binding, and the five structural genes are transcribed into pre-mRNA. Gene expression is switched on (Figure 2.21b).

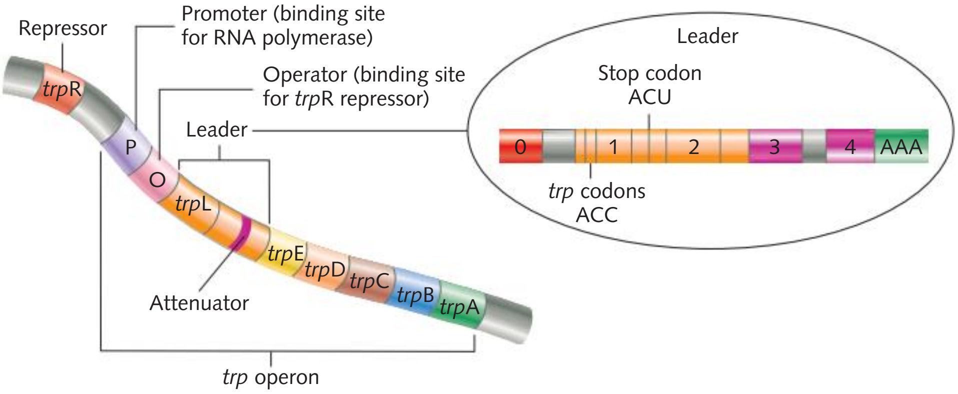


Figure 2.20 The structure of the *trp* operon, including the operator (the binding site for the repressor protein), the promoter (the binding site for RNA polymrase), the leader segment (which is involved in attenuation), and the five structural genes that code for proteins involved in tryptophan synthesis. The regulator gene *trp*R encodes the repressor protein and is located at another position on the chromosome of *E.coli.*

The binding of the tryptophan and the repressor

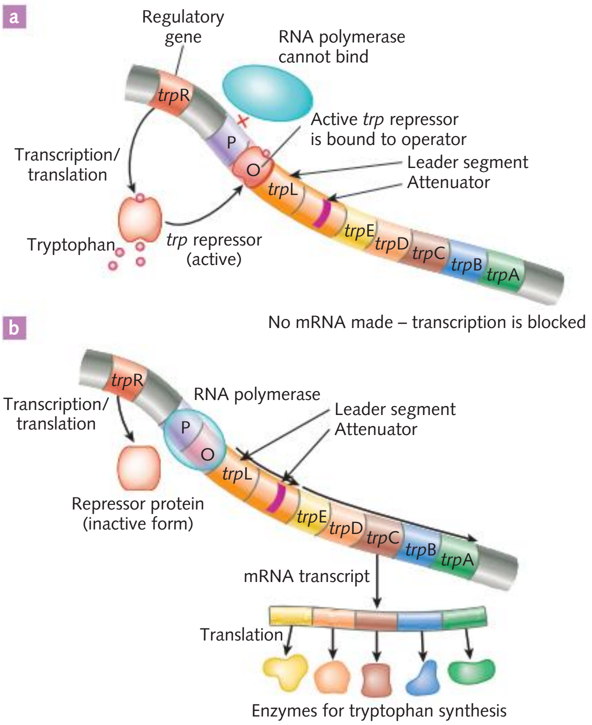


Figure 2.21 **a** When tryptophan is present, it binds to the repressor protein and alters its shape, so that the repressor can bind to the operator. The repressor then covers part of the promoter, preventing RNA polymerase from binding to the promoter, and blocking transcription of the structural genes in the operon. **b** When tryptophan is absent, the repressor protein is inactive, and it cannot bind to the operator. RNA polymerase can bind to the promoter and transcribe the five genes into a single mRNA transcript.

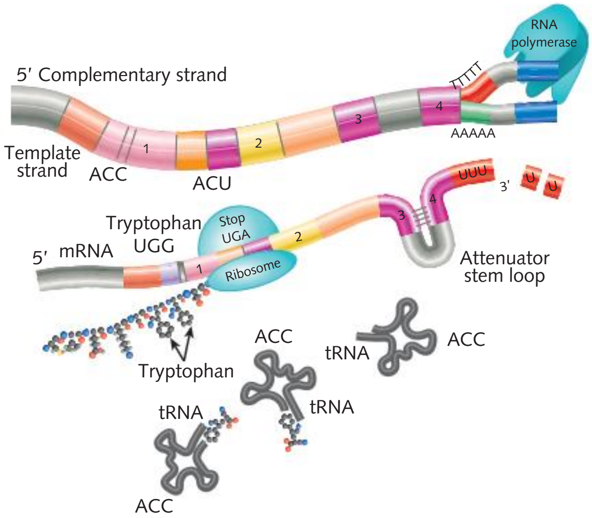


Figure 2.22 Attenuator stem loop has formed between sections 3 and 4 causing a pulling force which tears the weakly bonded adenine and uracil bases apart, thereby stopping transcription.

is reversible. When tryptophan concentrations are sufficiently high, tryptophan binds to the repressor and keeps the *trp* operon repressed. When tryptophan levels decrease, the repressor no longer binds to the operator. RNA polymerase can now bind to the promoter and the five structural genes can be transcribed.

In reality, there is a low rate of transcription of the *trp* operon because the *trp* repressor can fall off and then reattach to the operator. This means that tryptophan production is periodically turned on and off.

The second mechanism, attenuation, regulates gene expression without the repressor protein binding to the operator region. Attentuation occurs because of the structure of the leader region of the *trp* operon. The leader region contains 4 sections (1, 2, 3 & 4). Section 1 contains two adjacent *trp* codons (ACC) and in between sections 1 and 2 there is a STOP codon (ACU). Due to the bases that occur in sections 1, 2, 3 and 4, the sections can stick together due to complementary base pairing. Section 1 and 2 can stick together and form a hairpin loop, as can sections 2 and 3, and 3 and 4. Sections 3 and 4 are called the attenuator because their presence reduces (or attenuates) the transcription of mRNA in the presence of high levels of tryptophan. At the end of these 4 sections is a string of adenine bases (Figure 2.20).

As transcription of the DNA by RNA polymerase producing mRNA is occurring the ribosome can immediately start translation. This is because a prokaryotic cell does not have a nucleus and the mRNA and ribosomes occupy the same space. As the ribosome moves along the newly transcribed mRNA it passes by the two *trp* codons (UGG) in section 1. If there is a large or only a small amount of tryptophan in the cell, this will be added to the growing polypeptide chain that is being produced. The ribosome continues on and arrives at the UGA or STOP codon. The ribosome stops overlapping both sections 1 and 2. This means sections 1 and 2; and 2 and 3 are prevented from forming hairpin loops, but sections 3 and 4 stick together and form an attenuator stem loop.

Remember that transcription is still occurring. Also remember the string of adenine bases at the end of the leader section. As the attenuator stem loop is formed between sections 3 and 4 it creates a pulling force on the mRNA. As adenine and uracil are only weakly bonded by two hydrogen bonds the uracil in the mRNA pulls away from the adenine on the DNA and a shortened piece of attenuated mRNA falls away and the ribosome detaches. Transcription has stopped before the RNA polymerase reaches the structural genes (Figure 2.22).

If there is no tryptophan present in the cell the ribosome pauses at the *trp* codons in section 1 and waits for a delivery of tryptophan by a tRNA. This enables sections 2 and 3 to form an anti-attenuator stem loop. This loop formation does not provide enough force to pull the mRNA away from the DNA so transcription of the structural genes continues. Another ribosome will come along and attach to the transcribed mRNA to translate the five structural genes and more tryptophan will be produced.

KEY CONCEPTS

- Gene regulation is the process of switching gene expression on or off. The *trp* operon is an example of gene regulation in prokaryotes.
- In the *trp* operon, a regulatory gene codes for a repressor protein that blocks RNA polymerase from transcribing the *trp* operon.
- Tryptophan acts as an inducer that activates the repressor and consequently switches off expression of the *trp* operon genes.
- If there are low levels of tryptophan in the cell, the repressor will not bind to the operator, so the RNA polymerase will bind to the promoter and the five structural genes will be expressed.
- Alternatively when cell tryptophan is high, without repressor binding, attenuation can terminate transcription when the leader segment causes a hairpin loop to form. This acts as a transcription terminal signal and transcription ends prematurely.

Concept questions 2.3

1. What is the promoter of a gene?
2. What is meant by an operon?
3. What is the difference between a regulatory gene and a structural gene?
4. What might happen if genes are not regulated?
5. Tryptophan is coded for by five structural genes found in the *trp* operon. List these.
6. Name the two mechanisms that regulate the expression of the structural genes of the *trp* operon when the level of tryptophan in the cell is high. Explain how these two mechanisms are different.

HOT challenge

7. Gene regulation enables gene expression to be controlled. The genes can be switched on or off according to the prevailing environmental conditions or cell signalling. Order by number the steps involved in regulating the production of tryptophan in an *E. coli* cell. Include two pathways that demonstrate what happens when there are high and low levels of tryptophan using the repressor mechanism.

2.4 Proteins

Virtually everything a cell is or does depends on the **proteins** it contains. Proteins are large complex molecules that are the building blocks for many different structures and are produced by the activities of cells. For example, keratin is a strong fibrous protein found in your hair, the feathers of birds, the scales of snakes and the spines of echidnas. Haemoglobin is a protein in the blood that carries oxygen from the lungs to the rest of the body (Figure 2.23).

Proteins are large molecules, consisting of polymers called polypeptides. The monomers making up the polymers are amino acids.

NASA

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Figure 2.23 Proteins have a diverse range of functions. **a** Spindle fibres attach to chromosomes in cell division. **b** A spider web is composed of fibroin. **c** Haemoglobin carries oxygen to the cells in the body. **d** The castor oil plant produces the deadly toxin ricin.

Structure of amino acids

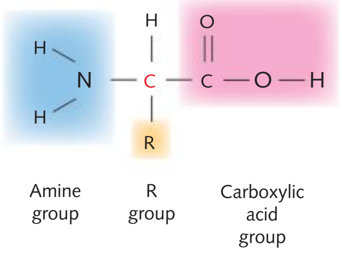

Figure 2.24 A generalised structural formula for an amino acid. Each amino acid has a different R group, which affects the structure and properties of the amino acid.

Amino acids are small molecules that have the same fuctional groups in their basic structure. Each of the 20 amino acids has a central carbon atom that has attached a hydrogen atom, an amine group (NH_2) and a carboxyl group (COOH) (Figure 2.24). Also attached is an R group, which is a different group in each amino acid. This is the group that varies in composition between amino acids and is called the variable group. The amine and acid groups react with water to become charged NH_3^+ and COO^- groups, respectively. The different R groups distinguish one amino acid from another and give the amino acids their particular chemical properties.

Some R groups make regions of the protein molecule polar (have a positive end and a negative end). Polar amino acids are **hydrophilic** ('water loving'). These R groups tend to be on the surface of proteins because of their affinity for the polar water molecules in their environment (Figure 2.25). There are 10 polar amino acids, including glutamine, tyrosine and serine. Other R groups make regions of the protein non-polar. Non-polar **hydrophobic** ('water hating') regions are generally tucked within the protein molecule, away from the water molecules in the aqueous environment. Hydrophobic amino acids can also be on the outside of sections of a protein that are embedded inside the hydrophobic centre of the plasma membrane. Non-polar amino acids include lycine, alanine and proline.

Plants synthesise all of their amino acids, but animals only synthesise some and must obtain the rest from their diet. More than 100 kinds of amino acids can be found in cells but only 20 are used to make up proteins.

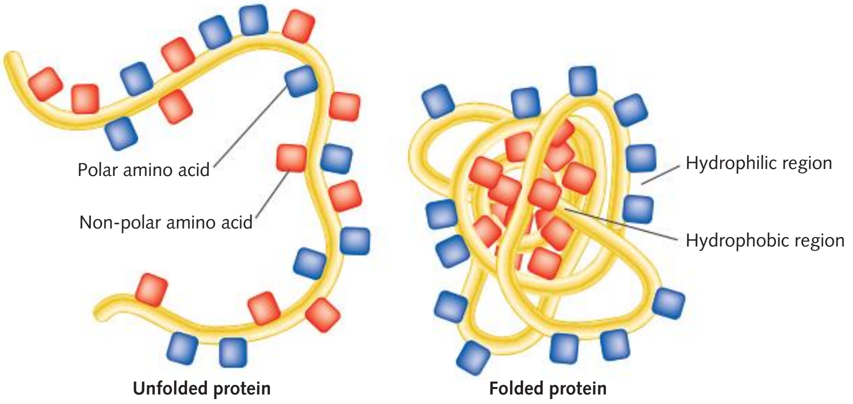

Figure 2.25 In an aqueous environment, hydrophobic amino acids (red) associate in the centre of the protein and the hydrophilic (blue) amino acids tend to interact with the surrounding environment.

WB

2.4.1
PROTEINS:
BUILDING
A MODEL
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Note:

Details of chemical structures, the specific names of bonds, and details of the condensation reaction or polymerisation, are not required as knowledge by VCAA in Biology.

Hierarchy of protein structure

The shape of a protein is very important to its function. To understand how a protein gets its final shape or conformation, it is necessary to understand the hierarchical classification of the structure of proteins. There are four different levels that give rise to the final structure.

Primary structure

The genetic code stored in the form of DNA determines the linear sequence of amino acids in the polypeptide. This is the **primary structure** of the protein. Amino acids bond together in the process of

condensation polymerisation, releasing a water molecule. The bond between two adjacent amino acids is called a **peptide bond** (Figure 2.26). A dipeptide is formed when two adjacent amino acids are linked together with a peptide bond. When many amino acids are joined in a linear sequence with all adjacent amino acids linked to each other by peptide bonds, a polypeptide chain is formed.

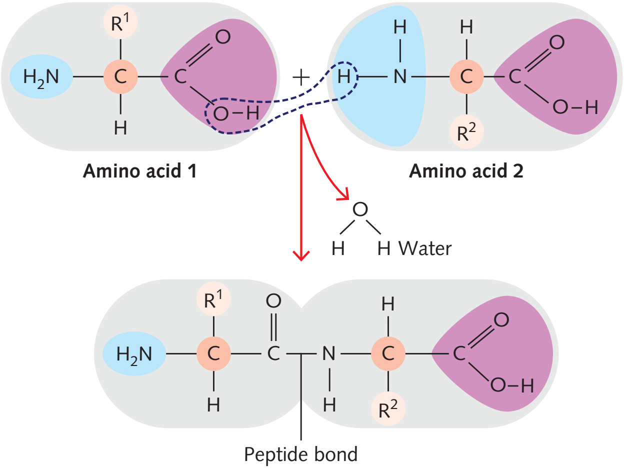

Figure 2.26 A condensation polymerisation reaction forms the peptide bond between two amino acids. Water is released during the reaction.

Secondary structure

Once the polypeptide chain is formed, various parts undergo coiling and folding due to hydrogen bonding between the peptide bonds of neighbouring amino acids. Hydrogen bonds are weak chemical bonds that form between a partially charged hydrogen atom on one amino acid and a partially charged oxygen or nitrogen atom on another amino acid. These coiled and folded portions of the polypeptide chain form the **secondary structure** of the protein (Figure 2.27). Tight coils are called alpha-helices (α-helices; singular, **α-helix**) and flattened folding forms are called beta-pleated sheets (**β-pleated sheets**). Other parts of the polypeptide chain do not fold into defined arrangements and are called **random coils**. This nomenclature (naming system) is perhaps misleading because the random loops are usually highly functional.

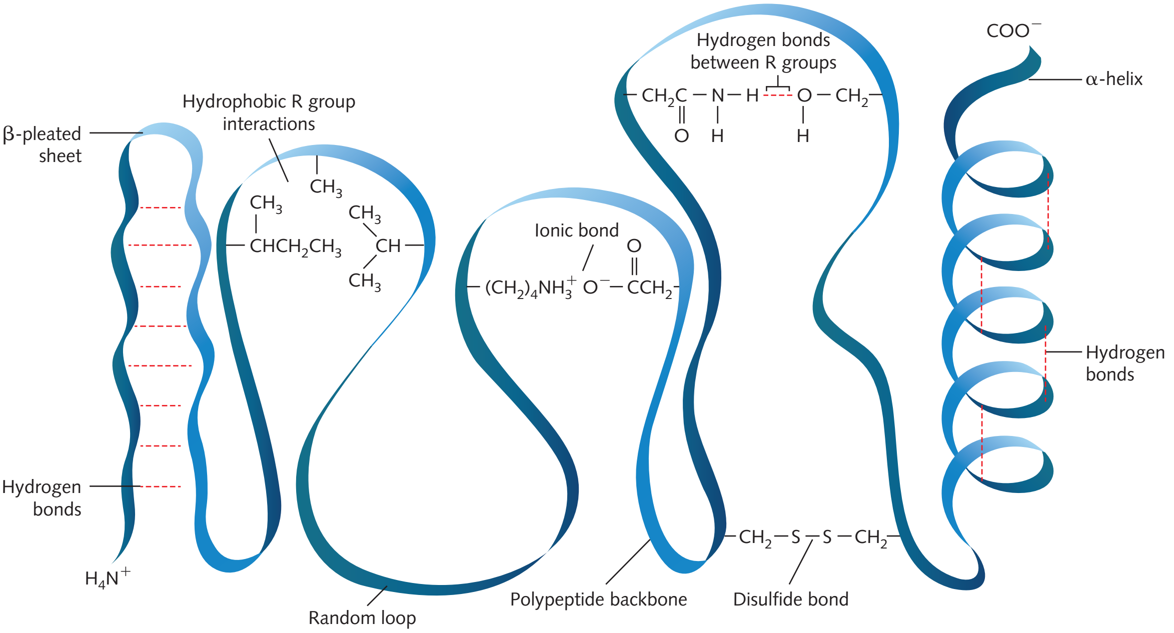

Figure 2.27 Secondary structures, such as α-helices and β-pleated sheets, form by hydrogen bonding within localised regions of the polypeptide. The polypeptide chain becomes folded, coiled or twisted in the protein.

Tertiary structure

Hydrophilic R groups of individual amino acids within the polypeptide chain attract hydrophilic R groups of other amino acids in other parts of the chain. Hydrophobic R groups attract other hydrophobic R groups, according to the chemical principle ‘like attracts like’. These interactions between the R groups of the amino acids cause the polypeptide chains to become folded, coiled or twisted into the protein's functional shape or **conformation**, described as the **tertiary structure** of the protein (Figure 2.28c).

The tertiary structure determines the **biological functionality** of a protein. Some proteins form long, closely packed fibres that are insoluble in water and form structural components of cells. For example, collagen is the fibrous protein that provides structure in connective tissue. Collagen is made up of three polypeptide chains that coil around one another to form a triple helix. Many collagen molecules pack together side by side to form fibrils that are arranged in different ways in different tissues to form collagen fibres.

Most proteins form spherical or globular molecules that are soluble in water and perform a variety of functional tasks. Enzymes are proteins that are mainly globular and the tertiary structure results in a spherical or ball-shaped structure. Depending on the way the protein folds into its tertiary structure, it has hollows on its surface called active sites. The substrates in a reaction fit into these active sites, thereby speeding up the reaction. This binding determines the specificity of an enzyme, as each enzyme can only speed up one particular reaction.

Receptor proteins on the surface or in the cytosol of target cells also have a tertiary structure with specific binding sites. This feature can be used in the development of highly specific drugs that will either stimulate the activity of the target or block its activity.

Quaternary structure

Many large, complex protein molecules consist of two or more polypeptide chains. The **quaternary structure** of a protein is formed when two or more polypeptides associate into the mature protein. A variety of hydrogen bonds, ionic bonds and covalent bonds hold the polypeptide chains together and give the overall shape to the molecule. Haemoglobin consists of four polypeptide chains – α_1, α_2, β_1 and β_2 (Figure 2.28d). Antibodies or immunoglobulins, involved in the immune system, consist of two heavy polypeptide chains and two light chains. (See Chapter 7 for more detail.) The enzymes Rubisco involved in photosynthesis, and both RNA and DNA polymersase, are proteins that have a quaternary structure.

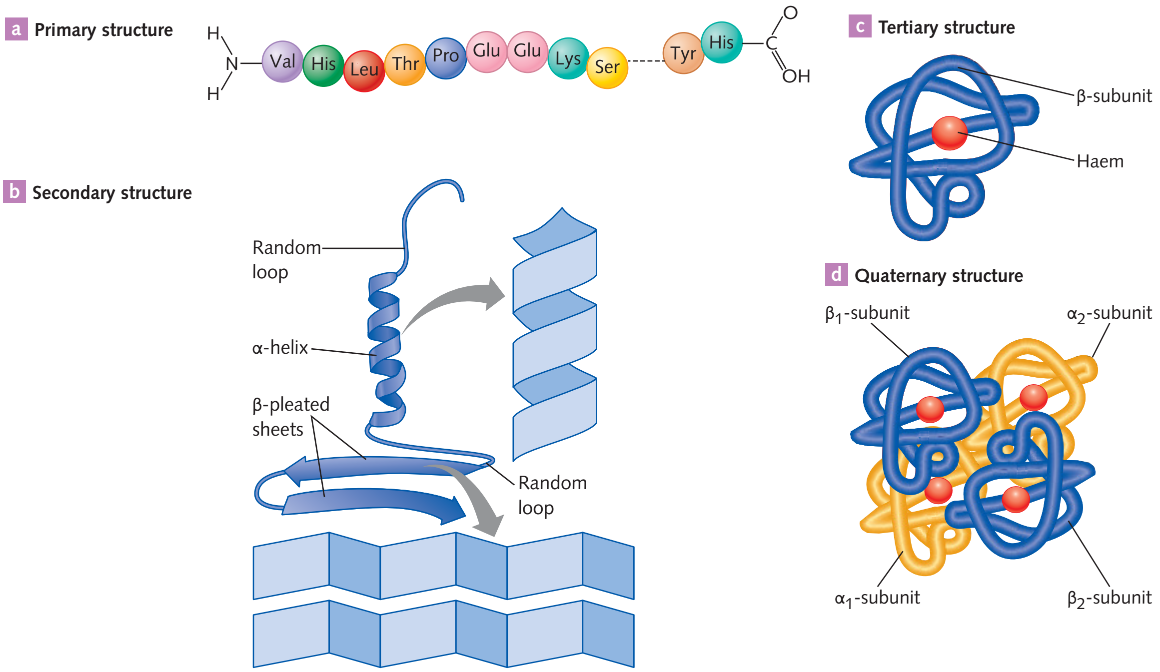

Figure 2.28 The four levels of organisation that give rise to the final protein structure of haemoglobin. a The primary structure consists of four chains of amino acids (two chains of 141 amino acids and two chains of 146 amino acids); b the secondary structure results from folding and coiling of the chains; c the tertiary structure is the conformation of the protein and d the quaternary structure consists of four polypeptide chains.

Changing the nature of proteins

A change to just one amino acid can alter the shape of the protein molecule so that it no longer functions properly. For instance, haemoglobin is made up of 574 amino acids. Some people with a different form of the gene, called an allele, produce haemoglobin with one different amino acid in one of the chains. This alters the shape of the haemoglobin molecule, leading to crescent-shaped rather than smooth disc-shaped red blood cells (Figure 2.29). These 'sickle cells' get stuck in blood vessels, obstructing blood flow and leading to the symptoms associated with sickle cell anaemia. Symptoms include episodes of severe pain, swelling of hands and feet and stroke (obstructed blood flow to the brain). Interestingly, the sickle shape protects red blood cells from infection by the malaria parasite, and this is probably why the allele persists throughout generations in populations that are exposed to malaria.

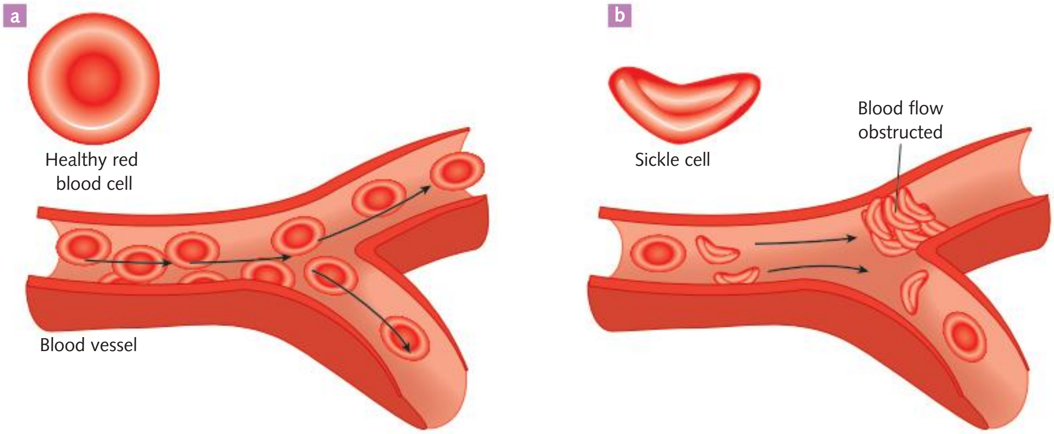

Figure 2.29 **Changing one amino acid alters the structure of the haemoglobin protein. a Correctly folded haemoglobin in healthy smooth disc-shaped red blood cells means that blood flows normally through vessels. b Crescent-shaped red blood cells called sickle cells get stuck in small blood vessels, obstructing blood flow.**

As well the sickle cell mutation, other mutations in the DNA code may result in amino acid changes that prevent polypeptides from folding correctly. Proteins may also lose their functional shape if they are exposed to high temperatures, concentrated salt solutions, or very acidic or alkaline conditions. These conditions can **denature** the protein molecules.

Cooking an egg causes the egg white to change from clear to white (Figure 2.30). Egg whites contain a protein called albumin. Heating breaks the bonds between different amino acids that give the protein

EXAM TIP
Often questions appear to have a lot of information that you don't know the first time you read it. Sometimes a question will describe a scenario to frame the real question. Think critically about how the scenario might apply to what you know about protein structure.

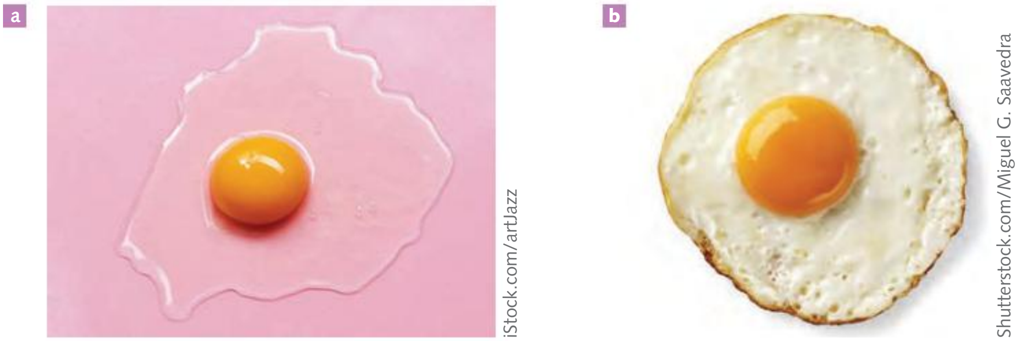

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Figure 2.30 **a Raw and b cooked egg. Albumin, the colourless protein of the egg 'white', is changed by heat.**

its 3D shape. The hydrophobic amino acids that are usually inside the protein become exposed to water molecules surrounding them in the egg white. The hydrophobic amino acids move to avoid the water molecules and clump together with other hydrophobic amino acids. This produces a solid protein network that turns the egg white from clear to white.

Types of proteins

The number of different proteins and their various modified versions in the human body is estimated to reach millions. This number will be refined with advancements in the technologies used to detect rare proteins. Proteins carry out many functions (Table 2.5). Proteins can:

» promote cellular motility, or movement
» provide structural support to cells and whole organisms
» transport molecules in and out of cells
» transmit signals within and between cells and organisms
» receive cellular signals and activate cellular responses to the signals
» help protect against attack by other organisms
» control the thousands of chemical reactions that maintain life processes (enzymes).

Table 2.5 The functional diversity of proteins

Type of protein	Function	Examples
Motility	Allow movement of cells and their organelles	Tubulin forms microtubules to move flagella, cilia, chromosomes and organelles. Actin and myosin work together to move muscles.
Structural	Provide support, strength and protection	Collagen supports body tissues. Fibroin makes a spider web stronger, weight for weight, than steel. Keratin forms nails and hair.
Transport	Carry molecules from one location to another or across plasma membranes	Haemoglobin carries oxygen to body cells. Porin forms a hydrophilic pore in the outer membrane of mitochondria for the passage of molecules.
Cellular signalling	Signal between different cell types; stimulation or inhibition	Insulin travels in the blood and binds to cell receptors to trigger the uptake of glucose. Endorphins activate nerve receptors to alleviate pain or stress. Cytokines signal between cells of the immune system to coordinate immune responses.
Cell-surface receptors	Receive signals such as hormones and growth factors, transmission of nerve impulse	Insulin receptors bind insulin to trigger the uptake of glucose by the cell. Rhodopsin in the retina membrane of the eye is a light-sensitive receptor that allows us to see in dim light.
Defence	Recognise and protect against foreign organisms	Antibodies cause foreign material to clump and be ingested by large white blood cells (macrophages). The castor oil plant produces ricin, a deadly toxin.
Enzymes	Catalyse, or actively assist, biochemical reactions	Catalase removes toxic hydrogen peroxide from cells by breaking it down into water and oxygen. DNA polymerase duplicates genetic information (DNA).

Enzymes

Enzymes are vital for life. They are proteins that **catalyse** or speed up the rate of every chemical reaction of the body, without themselves being changed in the process.

To catalyse biochemical reactions (chemical reactions in cells), enzymes locate molecules in the enzyme's active site – a pocket that fits the specific target molecule. The target molecule is called the **substrate** (Figure 4.5, p. 143.) Within the active site, the chemical and electrical properties of the enzyme apply pressure and tension to bend and twist the substrate, so that chemical bonds within the substrate are more easily broken down. Each enzyme has a specific substrate (either a single molecule or a small range of molecules) and produces specific **products** from the reaction. Importantly, the enzyme is not altered by the reaction, so it releases the products and is free to carry out further reactions. Enzymes are carefully controlled by regulatory processes to ensure that their functions in carrying out biochemical reactions occur only when they are needed.

In plants, enzymes are involved in building simple carbohydrates from inorganic materials in the process of photosynthesis. In all living cells, enzymes are essential in transforming energy in the process of cellular respiration. Without enzymes, all of the chemical reactions that sustain life would proceed at a very slow rate, so life as we know it would not be possible.

CONNECT

Enzymes and the biochemical pathways involved in photosynthesis and cellular respiration are discussed in detail in Chapters 4 and 5.

Proteome

The whole set of diverse proteins with different structures and functions produced by a cell, a tissue or an organism is called its **proteome**. The proteome of one cell type varies from that of another cell type because different genes are expressed in different cells depending on the function of the cell. Collectively, the proteins (including enzymes) that make up each cell's proteome are responsible for all of the functions and activities of that cell.

It is estimated that there are more than 100 000 different proteins in the human proteome. These proteins can be identified by a common laboratory technique called mass spectrometry. Humans can produce approximately 100 000 different proteins throughout their bodies, but each cell makes only a proportion of these. Antibodies are proteins that are produced by the immune system and help protect the body from unwanted invaders. A sperm cell's proteome contains 2000–2500 different proteins. This is the set of proteins that are necessary for the specific function of sperm, which is to deliver the male's genetic information to the oocyte (egg cell). Intestinal epithelial cells, which are the cells that line the gut, have a similar number of proteins in their proteome. However, the proteins they express are quite different from those of sperm cells and give them the capacity to absorb nutrients and keep harmful invaders out of the body.

Proteomics

Scientists often study single proteins of interest, one by one. However, in the last 20 years, new technologies and knowledge have led to a new field of study: **proteomics**. Proteomics is the study of proteomes – all the proteins in a cell, tissue or organ all at once. Proteomics is a dynamic field of research that is concerned with investigating the collection of proteins, their modifications and features, their subcellular locations, and the ways they interact with each other, in a particular cell type or tissue.

Functional proteomics particularly refers to the study of what proteins do in different cells or tissues. It can involve studying how proteins interact with each other in a specific tissue type, or how the collection of proteins in a tissue changes in response to particular conditions, such as during disease.

The combined expertise of computational biologists, mathematicians and molecular biologists has resulted in the development of powerful tools, techniques and databases for studying proteins, and this field of study is likely to expand greatly as better technologies are developed.

KEY CONCEPTS

- Proteins are a class of biological molecules with a diverse range of functions vital to cell structure, organisation and operation.
- Proteins consist of linear polypeptide chains of amino acids.
- There are 20 different amino acids that can be grouped according to their properties.
- Proteins fold into shapes that are defined by their amino acid sequence, and they exhibit four levels of structure in the course of folding into their proper shape.
- A protein's function depends on its shape.
- Heating proteins can cause them to unfold irreversibly.
- A proteome is the complete set of proteins in a cell, an organ or a tissue.

Concept questions 2.4

1 State the name of the monomers that make up polypeptides. What holds these monomers together?
2 List at least five types of proteins, state their functions and give an example of each.
3 Distinguish between hydrophilic and hydrophobic amino acids. Suggest where each is likely to be found in a folded protein and explain why.
4 Describe the basic structure of an amino acid molecule.
5 Describe how amino acids are linked together. Use the correct term for the process and name the small molecule that is released during the reaction.
6 Describe the four levels of protein structure.
7 What three types of folds are associated with the secondary structure of a polypeptide?
8 Define 'functional proteomics'.
9 Describe some potential benefits or uses of proteomics in medical research.

HOT challenge

10 Differentiate between genomics and proteomics.

WB 2.5.1 TELLING THE STORY OF THE PROTEIN SECRETORY PATHWAY PAGE 51

2.5 The protein secretory pathway

Proteins destined for secretion by cells (such as hormones) or expression in the endoplasmic reticulum, **Golgi apparatus** (or Golgi bodies), lysosomes or other membrane-bound organelles, can enter the **protein secretory pathway**. In the first step of this pathway, polypeptides that are made on endoplasmic reticulum-bound ribosomes are transferred through the endoplasmic reticulum membrane as they are synthesised and enter the lumen (interior) of the rough endoplasmic reticulum (Figure 2.31). Here, the polypeptides are assisted to fold in the correct way, then are sorted and transported to the Golgi apparatus in membrane-bound vesicles called **transport vesicles**. The Golgi apparatus is an organelle consisting of stacks of flattened pockets called **cisternae** (singular, cisterna) usually 4–8 cisternae per Golgi in a multicellular organism), held together by matrix proteins and microtubules of the cell's cytoskeleton. As proteins progress through the cisternae of the Golgi apparatus, they are modified by enzymes that may add or remove components until the protein is in its mature, functional form. In the last stage of progression through the Golgi apparatus, the proteins are concentrated and further packaged into secretory vesicles, which are then shuttled to the plasma membrane of the cell. Here, the secretory vesicles fuse with the plasma membrane as all intracellular organelle membranes are fluid and have the same basic structure as the plasma membrane. The secretory vesicle contents are released in the process of exocytosis exporting the contents into the extracellular environment. (Figure 2.31).

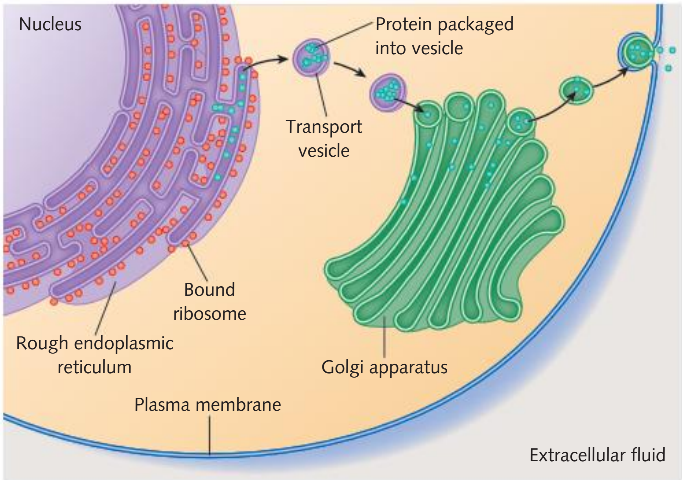

Figure 2.31 The protein secretory pathway packages proteins to be exported from the cell.

Proteins that have transmembrane domains or chemical attachments that allow them to be joined to plasma membranes stay membrane-bound through the process and end up tethered to the plasma membrane at the cell's surface. Some specialised cells secrete high concentrations of certain proteins. For example, plasma cells produce and secrete large numbers of antibody molecules that help to destroy pathogens. Plasma cells have abundant rough endoplasmic reticulum, which allow them to shuttle large amounts of antibodies through the protein secretory pathway.

CONNECT

Plasma cells and antibodies will be further discussed in Chapter 7.

Proteins are tagged with molecular labels so that they get to the right place in the cell to be secreted. This is analagous to how a letter is labelled with a postal address that is recognised by the post office so it gets sent to the right place.

KEY CONCEPTS

» Proteins destined for secretion can enter the protein secretory pathway.

» The rough endoplasmic reticulum, the Golgi apparatus and secretory vesicles modify, sort and package proteins for secretion from the cell and expression on the outside surface of the cell.

Concept questions 2.5

1 Starting at the ribosome construct a flow chart to show the steps of a protein that is secreted from the cell.

2 Describe how the endoplasmic reticulum and Golgi apparatus differ functionally.

HOT challenge

3 Secretory vesicles fuse with the plasma membrane to enable protein secretion as part of a protein secretory pathway. Interpret what this 'fusion' entails.

BRANCHING OUT

Biological knowledge and society: the speed gene

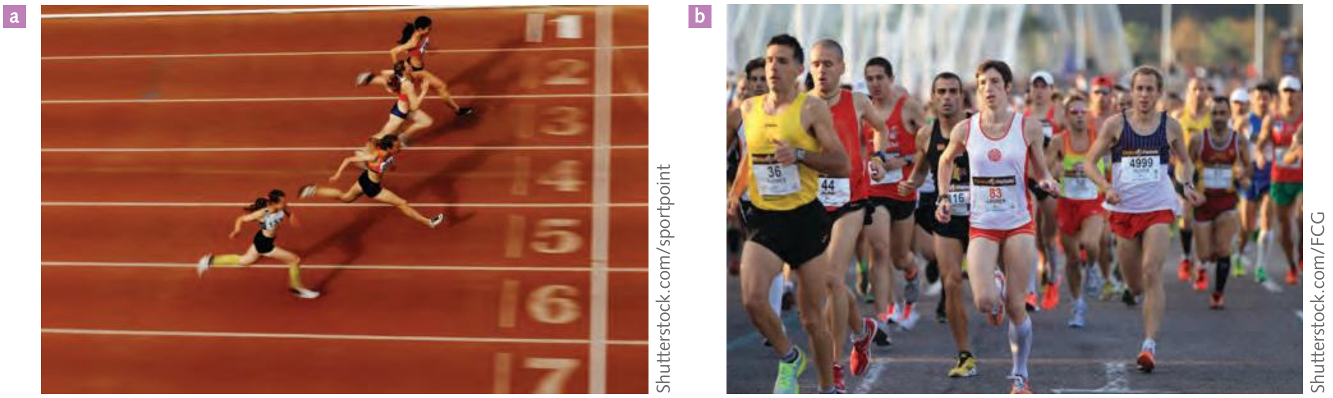

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Figure 2.32 Studies reveal genetic differences between **a** elite sprinters and **b** marathon runners.

The *ACTN3* gene on chromosome 11 has been called the 'speed gene' because it is correlated with elite athletic ability in sprint events. A controversy has arisen around genetic screening for the speed gene and concerns that it may lead to discrimination and the potential for 'designer athletes' and gene doping.

The genetics

The protein product of the *ACTN3* gene is alpha-actinin-3. It is expressed in fast-twitch muscle fibres where it connects actin protein chains to coordinate fast, repetitive and powerful muscle contractions

CONNECT

For more information on energy pathways used by cells, go to Chapter 5.

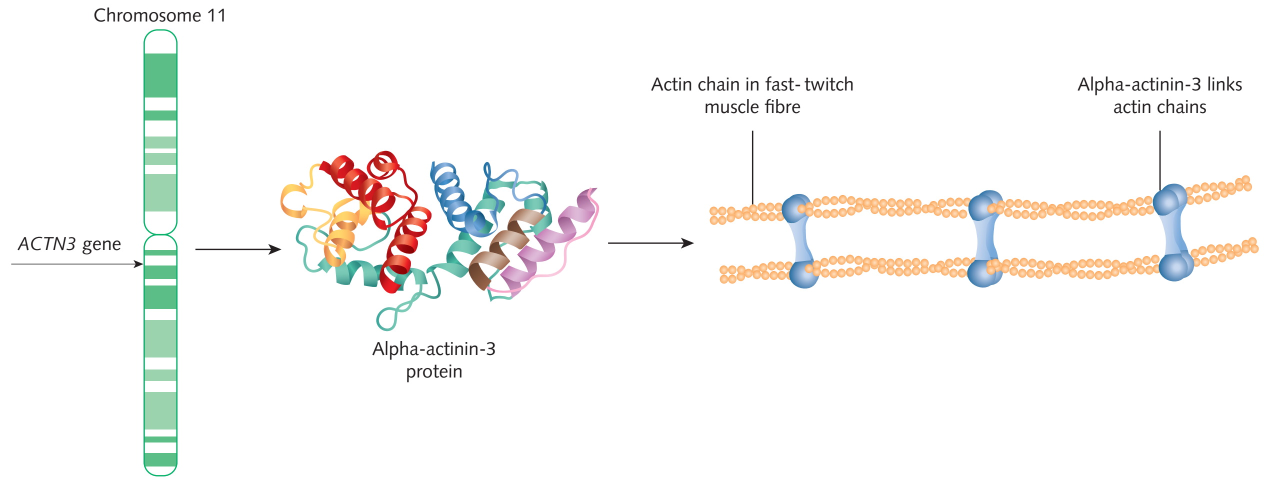


Figure 2.33 **Expression of the *ACTN3* gene in fast-twitch muscle fibres**

(Figure 2.33). These fast-twitch muscle fibres are powered by glucose and use anaerobic glycolysis pathways to provide energy in the form of ATP.

In 1999, Australian geneticist Professor Kathryn North and a team of scientists discovered a common mutation in the *ACTN3* gene. Two alleles for the *ACTN3* gene exist in the population: allele 577R codes for functional alpha-actinin-3, but a second allele, 577X, has a mutation that results in a premature stop codon so the protein is truncated (shortened). This mutant alpha-actinin-3 protein is not functional.

Having just one 577R allele means you can produce functional protein. This R variant codes for the dominant phenotype, while the X variant codes for the recessive phenotype. Inheriting two copies of the X variant means you cannot make functional alpha-actinin-3, as seen in 20% of the Australian population. There is no obvious effect if you do not make alpha-actinin-3; it does not cause disease.

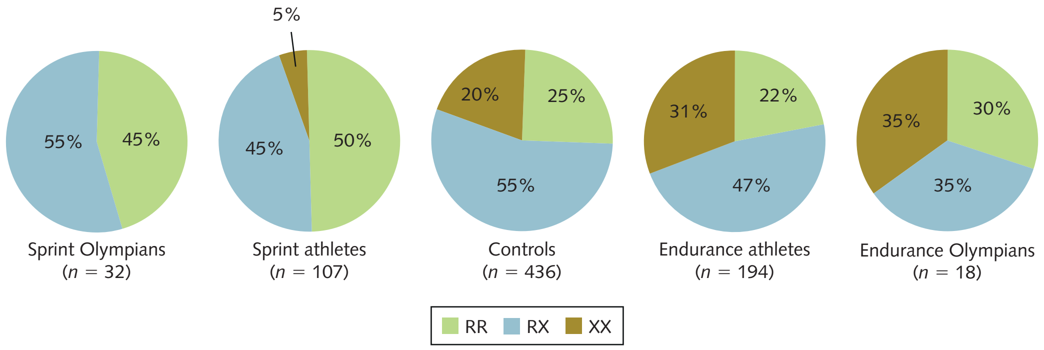


Figure 2.34 **The *ACTN3* allele status of Olympian sprint and endurance athletes, non-Olympian sprint and endurance athletes, and the general population (controls)**

A genetic test can be carried out to determine the *ACTN3* allele status of individuals. In 2003, Professor North collaborated on a study with the Australian Institute of Sport. The study involved determining the *ACTN3* allele status for 436 people in the general population as a control, 32 sprint Olympians, 107 sprint athletes, 194 endurance athletes and 18 endurance Olympians. The results are shown in Figure 2.34.

Alpha-actinin-3 influence on athletic performance

To study the effect of alpha-actinin-3, scientists generated a strain of *ACTN3* knock-out mice that could not produce the protein. The knock-out mice produced higher amounts of the protein alpha-actinin-2. This closely related protein is produced to replace the missing alpha-actinin-3. Table 2.6 shows results of further tests comparing the knock-out strain with controls.

Table 2.6 An overview of results comparing knock-out mice that cannot produce alpha-actinin-3 with control mice that do produce this protein (summarised from a paper by Berman and North, 2010)

Test	Knock-out mouse strain (no alpha-actinin-3) compared with control mouse strain
Weight	Slightly reduced
Muscle mass	Significantly reduced
Muscle fibre composition	Shift from fast muscle fibres towards having more slow oxidative fibres
Grip strength	Reduced by an average 6%
Endurance running	Run an average 33% further before exhaustion
Glycolysis enzymes	Elevated
Converting pyruvate to lactate through activity of lactate dehydrogenase (anaerobic pathway)	Decreased
Mitochondrial enzymes associated with aerobic respiration	Elevated
Oxidise fats for energy	Increased capacity

Many countries, including Australia, invest large amounts of money in programs to identify and train athletes. The Australian Institute of Sport provides programs to adolescents based on physical and psychological tests.

Many parents are also willing to invest in their children's sporting futures. Similar results to those seen in Figure 2.33 have been replicated in a number of independent studies around the world, so they are considered reliable. Companies such as 23andMe and Atlas Sports Genetics offer genetic tests to consumers. Atlas first recommends parents test children aged 0–8 years in order to provide early information on genetic predisposition for success in speed/power or endurance events. Critics are concerned that these companies are misrepresenting the science. Professor North cautions that the *ACTN3* gene in isolation is a poor predictor. The traits of successful athletes are complex, with hundreds of genes involved and the environment also playing an important role.

The Australian Law Reform Commission and the National Health and Medical Research Council released advice on the use of genetic information in sport (2013). They advised that 'there are concerns about the effect of genetic testing on individual athletes, especially when this involves children or young people. Inappropriate interpretation of test results could at best lead to incorrect advice about placement in sporting activities, and at worst could be detrimental to the physical or psychological health of an individual.'

Questions

1 Identify the bioethical issues.
2 Identify the biology that relates to these issues.
3 How can the results of the North (2003) study shown in Figure 2.33 be explained by the results of the Berman and North (2010) study shown in Table 2.6?
4 Refer to the results in Table 2.6. Discuss the altered muscle performance in knock-out mice and then formulate a hypothesis as to why an absence of alpha-actinin-3 leads to increased endurance performance and decreased sprint and power performance.
5 How ethically acceptable is it to select althletes based on genetic information? Frame your response in terms of one or more of the following ethical concepts: integrity, justice, beneficence, non-maleficence, respect.

References

Berman, Y., and North, K. (2010). A gene for speed: The emerging role of alpha-actinin-3 in muscle metabolism. Physiology. 25(4): 250–259.

National Health and Medical Research Council (2013). Use of genetic information in sport. Retrieved 19 October 2015 from https://www.nhmrc.gov.au/about-us/publications/use-genetic-information-sport

Online Key Concepts
Chapter 2: Summary
of key concepts

2 Summary of key concepts

2.1 Nucleic acids

KEY CONCEPTS

p. 39

- Nucleotides are monomers that are made up of a five-carbon pentose sugar, a phosphate group and a nitrogenous base.
- Nucleotide monomers make up the nucleic acids DNA and RNA.
- There are four different types of nitrogenous bases in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T).
- Nucleotides make up the strands of DNA, which are held together by hydrogen bonds between complementary bases.
- Bases pair according to the complementary base pairing rules: A always bonds to T; C always bonds to G.
- The two strands of DNA are antiparallel.
- In cells, DNA is organised into chromosomes.
- RNA is single-stranded.
- RNA nucleotides contain ribose sugar.
- RNA contains the base uracil (U) instead of the DNA base thymine (T).
- RNA strands are shorter than DNA strands.
- RNA plays a major role in gene expression.
- There are three main types of RNA: mRNA, tRNA and rRNA.

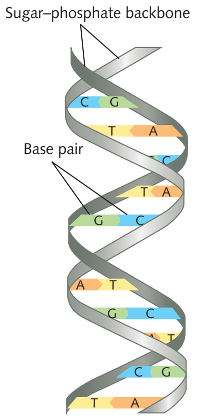


Figure 2.6 The DNA helix is a double-stranded molecule. Two strands are held together by hydrogen bonding between complementary nitrogen bases. An A–T pair is held together by two hydrogen bonds, whereas a G–C pair is held together by three hydrogen bonds

2.2 Gene expression

KEY CONCEPTS

p. 47

- Information in DNA is coded into groups of three nucleotides, or triplets, and each triplet corresponds to a specific amino acid.
- During transcription, mRNA is copied from template DNA.
- Pre-mRNA is processed by cutting, splicing and capping to become mature mRNA before it leaves the nucleus.
- Pre-mRNA can be alternatively spliced to generate different mRNA transcripts.
- mRNA is translated into a polypeptide by ribosomes in the cytosol or ribosomes bound to the endoplasmic reticulum.
- Translation is accomplished with the assistance of tRNA molecules that bring amino acids into position for incorporation into the polypeptide.

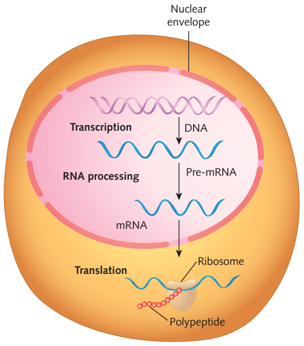


Figure 2.12 Gene expression relies on the processes of transcription and translation. During transcription, DNA is copied into pre-mRNA, which is processed into mature mRNA for export from the nucleus. During translation, the ribosome 'reads' the nucleotide sequence in the mRNA and forms the amino acid sequence of a polypeptide

2.3 Gene regulation

KEY CONCEPTS

p. 55

- Gene regulation is the process of switching gene expression on or off. The *trp* operon is an example of gene regulation in prokaryotes.
- In the *trp* operon, a regulatory gene codes for a repressor protein that blocks RNA polymerase from transcribing the *trp* operon.
- Tryptophan acts as an inducer that activates the repressor and consequently switches off expression of the *trp* operon genes.
- If there are low levels of tryptophan in the cell, the repressor will not bind to the operator, so the RNA polymerase will bind to the promoter and the five structural genes will be expressed. Attenuation is a second mechanism that controls tryptophan concentration in bacterial cells. It does not involve the repressor protein; instead it involves the leader region in the operon and results in transcription termination and a shortened (attenuated) mRNA transcript, and therefore no gene expression of the five structural genes.

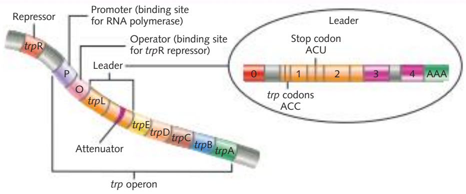


Figure 2.20 The structure of the *trp* operon, including the operator (the binding site for the repressor protein), the promoter (the binding site for RNA polymerase) and the five structural genes that code for proteins involved in tryptophan synthesis. The regulator gene *trp*R encodes the repressor protein and is located at another position on the chromosome of *E. coli*

2.4 Proteins

KEY CONCEPTS

p. 60

- Proteins are a class of biological molecules with a diverse range of functions vital to cell structure, organisation and operation.
- Proteins consist of linear polypeptide chains of amino acids.
- There are 20 different amino acids that can be grouped according to their properties.
- Proteins fold into shapes that are defined by their amino acid sequence, and they exhibit four levels of structure in the course of folding into their proper shape.
- A protein's function depends on its shape.
- Heating proteins can cause them to unfold irreversibly.
- A proteome is the complete set of proteins in a cell, an organ or a tissue.

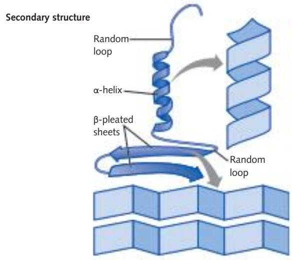


Figure 2.28 The four levels of organisation that give rise to the final protein structure of haemoglobin. **a** The primary structure consists of four chains of amino acids (two chains of 141 amino acids and two chains of 146 amino acids); **b** the secondary structure results from folding and coiling of the chains; **c** the tertiary structure is the conformation of the protein and **d** the quaternary structure consists of four polypeptide chains

2.5 The protein secretory pathway

KEY CONCEPTS

p. 64

- Proteins destined for secretion can enter the protein secretory pathway.
- The rough endoplasmic reticulum, the Golgi apparatus and secretory vesicles modify, sort and package proteins for secretion from the cell and expression on the outside surface of the cell.

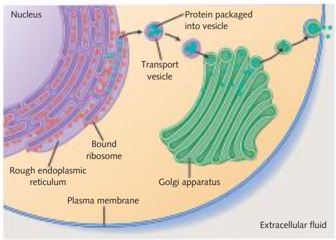


Figure 2.31 The protein secretory pathway packages proteins to be exported from the cell

WB

2.6.2
KEY TERMS
PAGE 53

2.6.1
SPOT THE ERRORS
PAGE 53

2 Chapter glossary

α-helix a type of secondary protein structure in which the polypeptide chain folds into a tight coil

allele a different version of the same gene (at the same locus) determined by small differences in the DNA sequence of the gene

alternative splicing a process in which one or more exons are removed with the introns to produce mRNA molecules of different length and sequence

amino acid a nitrogen-containing compound that is the monomer from which proteins are built

anticodon the three nucleotides in tRNA that bind to the complementary codon in mRNA according to base-pairing rules, resulting in the addition of a specific amino acid to the polypeptide chain

antiparallel parallel but orientated in the opposite direction

attenuation a mechanism for reducing expression of the *trp* operon when tryptophan levels are high, by preventing completion of transcription of the structural genes, not by stopping initiation of transcription by a repressor protein

β-pleated sheet a type of secondary protein structure in which segments of the polypeptide chain bond side by side into a flattened assembly

base pair two complementary nitrogen bases linked by hydrogen bonding

biological functionality the function of a protein

catalyse to speed up a biochemical reaction by using an enzyme

chromosome a thread-like structure made of nucleic acids and proteins that encode genetic information

cisterna a flattened membrane disc that makes up the Golgi apparatus and endoplasmic reticulum

codon a group of three nucleotides in mRNA that specifies an amino acid

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T (or U in RNA) and C pairs with G

condensation polymerisation a reaction in which monomers are linked together into a polymer with the release of a small molecule, such as water, as a by-product

conformation the proper or functional shape of a protein

degenerate a property of the genetic code in which most amino acids are encoded by two or more codons

denature to permanently change the molecular structure of a protein or DNA

deoxyribonucleic acid (DNA) the information molecule that is the basis of an organism's genetic material

DNA polymerase the enzyme that catalyses the bonding of nucleotides to form new strands of DNA

endoplasmic reticulum an organelle made up of a network of membranous tubules involved in protein synthesis and folding for secretion

enzyme a specific protein catalyst that increases the rate of a biochemical reaction within the cell by lowering the amount of energy required for the reaction to proceed

exon a segment of DNA or RNA containing information that codes for a polypeptide or part of a polypeptide

functional proteomics the study of how proteins work together in different cells or tissues, or under different circumstances

gene a segment of DNA in a chromosome that codes for a polypeptide; comprises the promoter, exons and introns

gene expression the process by which the information in a gene is turned into a polypeptide

gene regulation the process by which gene expression is switched on or off

genetic code the complete set of mRNA codons and the corresponding amino acids they specify

genome the complete sequence of DNA in a single (haploid) set of an organism's chromosomes, including nuclear, mitochondrial and chloroplast DNA

Golgi apparatus a collection of membranes that package and store substances into vesicles in preparation for their release from the cell

histone a protein that binds and packages DNA in eukaryotic chromosomes

hydrogen bond a weak chemical bond between a hydrogen atom on one molecule and a more electronegative element, usually an oxygen or nitrogen atom, on another molecule

hydrophilic describes substances such as polar molecules and ionic compounds that dissolve readily in water

hydrophobic describes substances such as non-polar molecules that are insoluble in water

inducer a signalling molecule that switches on expression of a gene

intron a segment of DNA within a gene or pre-mRNA that does not code for a polypeptide and interrupts the sequence of a gene

leader region of the operon codes for the leader sequence in the mRNA including the attenuator region which can fold back on itself forming a hairpin loop that blocks the RNA polymerase thereby terminating transcription of the structural genes prematurely

messenger RNA (mRNA) RNA copied from DNA that conveys the instructions needed for polypeptide synthesis from the nucleus to the cytoplasm

non-coding region DNA that does not encode a protein sequence

non-template strand the DNA strand that has the same sequence of nucleotides as the mRNA (except it has T instead of U)

nucleic acid a large, linear polymer built from nucleotide monomers bonded together; includes DNA and RNA

nucleosome a histone with a length of DNA coiled around it

nucleotide the monomer, or building block, of DNA and RNA, consisting of sugar, phosphate and a nitrogen base

operator a segment of DNA to which a protein binds, usually to switch off gene expression

operon a group of genes that are expressed as a single unit

peptide bond a chemical bond that links two amino acids in a chain

phosphodiester bond a chemical bond that links two nucleotides in a growing chain

plasmid a small, circular DNA structure independent of the chromosome in prokaryotic cells

poly-A tail a chain of 100–200 adenine nucleotides added at the 3′ end of an mRNA strand

polypeptide a linear polymer built from amino acid monomers

polyribosome a chain of ribosomes formed by attaching to and translating from a single mRNA strand

pre-mRNA an unprocessed RNA strand that is transcribed directly from the DNA

primary structure the linear sequence of amino acids that makes up a polypeptide chain

product the outputs of a chemical reaction that are formed from the reactants or inputs

promoter region a segment of DNA to which RNA polymerase binds to begin transcription

protein a polymer made up of amino acid monomers; may consist of a single polymer chain or many polymers bonded together into a functional molecule

protein secretory pathway the pathway through which cells package proteins into vesicles for release into the extracellular environment

proteome the complete set of proteins produced by a cell, a tissue, or an organism

proteomics the study of proteomes

quaternary structure the structure formed when two or more polypeptides associate into a mature protein

random coil a secondary protein structure in which the polypeptide chain does not fold into a specified arrangement

regulatory gene a gene whose product switches on or switches off expression of one or more other genes

repression a mechanism for reducing the expression of the *trp* operon when levels of tryptophan are high by blocking transcription

repressor protein a protein that binds to DNA to prevent RNA polymerase attaching or transcribing; essentially shutting off gene expression

ribonucleic acid (RNA) a type of nucleic acid consisting of a single strand of nucleotides; has essential roles in protein synthesis

ribosomal RNA (rRNA) an RNA strand that serves as a structural component of a ribosome

ribosome a small structure consisting of RNA and proteins where amino acids are joined to form polypeptides

RNA polymerase the enzyme that catalyses the synthesis of RNA

rough endoplasmic reticulum endoplasmic reticulum with ribosomes attached

secondary structure the localised folding of a polypeptide chain when neighbouring amino acids bond to each other to form α-helices, β-pleated sheets or random loops

semi-conservative replication the replication of DNA in which the product contains one original and one newly made strand

structural gene a gene that codes for tRNA, rRNA or a polypeptide other than a regulatory molecule

substrate the substance that an enzyme acts on

subunit a distinct component of a biological particle; in proteins, it refers to each polypeptide that contributes to the quaternary structure

template a pattern that can be used for making many more copies

template strand a strand of DNA that is copied during DNA or RNA synthesis

tertiary structure the overall three-dimensional shape of a completely folded polypeptide

transcribe to copy DNA into mRNA

transcription the process by which DNA is copied into mRNA

transcription factor a protein that binds to DNA to control the rate of transcription from a gene

transfer RNA (tRNA) an RNA molecule that transports an amino acid to the ribosome for assembly into a polypeptide

translation the process of turning the nucleotide sequence of mRNA into the amino acid sequence of a polypeptide

transport vesicle a small membrane-bound sac containing protein that is transported from the Golgi apparatus to the plasma membrane for release into the extracellular environment

triplet a set of three nucleotide codes

2 Chapter review

Remembering

1 Why is nitrogen (N) considered to be an essential element for all living things?

2 List two ways that different tRNAs are the same, and two ways they are different.

3 On an A3 sheet of paper, outline the main steps in protein synthesis. Include transcription, translation, gene regulation, primary and secondary structures of α-helices, β-pleated sheets, or random coils, tertiary and quaternary formation of proteins and final examples of product.

4 Structural genes and regulatory genes have different functions.
   a Define the main functions of the different types of genes.
   b Explain how the genes operate to affect the processes that they are involved in.

5 What are the 5′ and 3′ caps, and what do they achieve for the mature mRNA?

6 How are DNA and RNA related?

7 How does the antiparallel structure of DNA determine the template strand?

8 Do all codons code for an amino acid? Explain your answer.

Understanding

9 Explain the relationship in the sequence between the triplets in the non-template strand of DNA, the mRNA codons and the tRNA anticodons.

10 All amino acids contain the same two functional groups. How do the 20 amino acids differ from one another?

11 How many different types of polypeptide can be constructed from just five amino acids?

12 Polymers result when bonds between monomers are formed with the removal of water. Suggest a way that the bonds between monomers could be broken. Justify your answer.

13 Explain how some proteins are located in the cytosol, whereas others are secreted by the cell.

14 Explain why the ability to control which genes are expressed is important:
   a during cell differentiation
   b in mature cells.

15 Explain why successful attenuation relies upon the synchronisation between transcription and translation.

Applying

16 Egg white is rich in the globular protein albumin. When heated, this liquid becomes a white opaque solid. Use your knowledge of protein structure to explain this observation.

17 Antibodies are proteins of the immune system. Antibodies contain many disulfide bridges, which are strong bonds between two sulfur atoms within a protein. Suggest why this feature of antibodies might be beneficial during a fever.

18 Histones are predominantly made of positively charged amino acids.
   a Would these proteins be soluble or insoluble in water? Explain why.
   b Describe the properties of histone proteins that enables them to package DNA into chromosomes.

19 The *ara* operon of *E. coli* contains three genes that import and digest the five-carbon sugar arabinose (Figure 2.35).

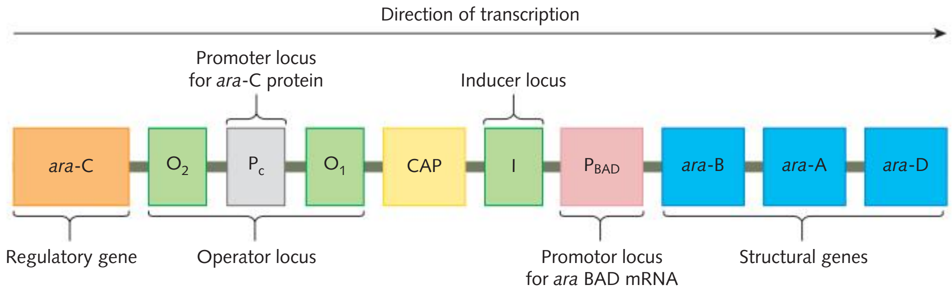


Figure 2.35 Structural elements of the *ara* operon

The *ara* operon is under the control of a protein called *ara*-C. Gene expression from the operon is normally switched off. If arabinose is present, gene expression from the operon is switched on.

a Use your understanding of the *trp* operon (pp. 55–56) to describe the mechanisms regulating gene expression at the *ara* operon.

b Use annotated diagrams to demonstrate the *ara* operon of *E. coli* and this system of gene regulation in the absence and presence of arabinose.

20 Figure 2.36 shows diagrams of two mammalian cells: a spermatid (a sperm in the process of development) and a mature sperm cell.

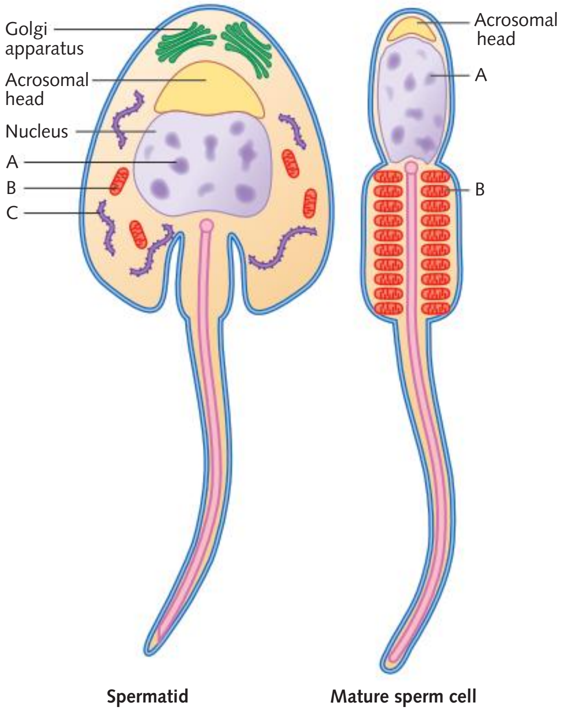


Figure 2.36 Immature and mature sperm cells

a Name the organelles labelled A, B and C.

b The Golgi apparatus and the ribosomes no longer exist in the mature sperm cell. Explain why.

c i What is the function of the ribosomes in this process of development?

ii What is the function of the Golgi apparatus in this process of development?

iii What is the function of structure C in this process of development?

Analysing

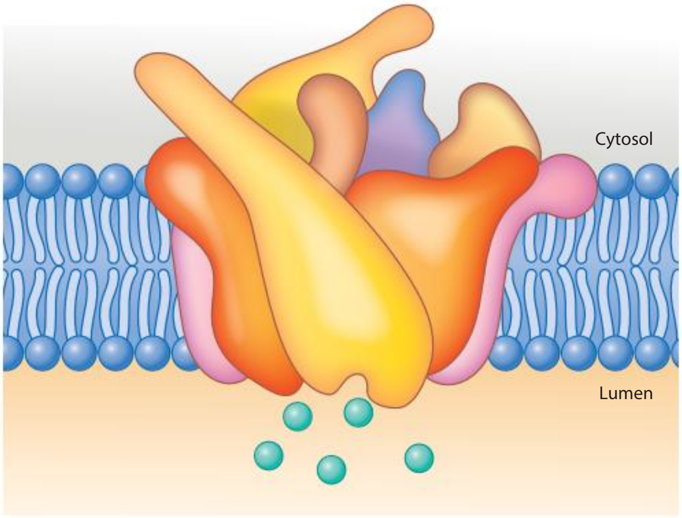


Figure 2.37 A calcium channel protein

21 Figure 2.37 shows a calcium channel protein embedded in the membrane of the endoplasmic reticulum. The protein transports calcium ions from the cytosol side of the membrane to inside the lumen of the endoplasmic reticulum.

- a What sort of technique might have been used to determine the structure of the protein?
- b What type of secondary structure dominates the protein?
- c What properties might the amino acids have that are on the face of the protein embedded in the membrane?
- d What properties might the amino acids have that line the inside of the channel?
- e Each polypeptide chain in the protein is coloured differently. How many polypeptides make up the mature protein, and what level of protein structure is this?

Evaluating

22 Rats have two forms of the muscle protein troponin T. One form comprises four exons, called W, X, Alpha and Z. The other also comprises four exons, called W, X, Beta and Z. However, rats have only one copy of the troponin T gene with five exons. What might explain these observations? Draw an annotated diagram to support your explanation.

23 Figures 2.38 refers to a type of protein called an enzyme. Evaluate the graphs in Figure 2.39 in terms of what is being depicted by Figure 2.38.

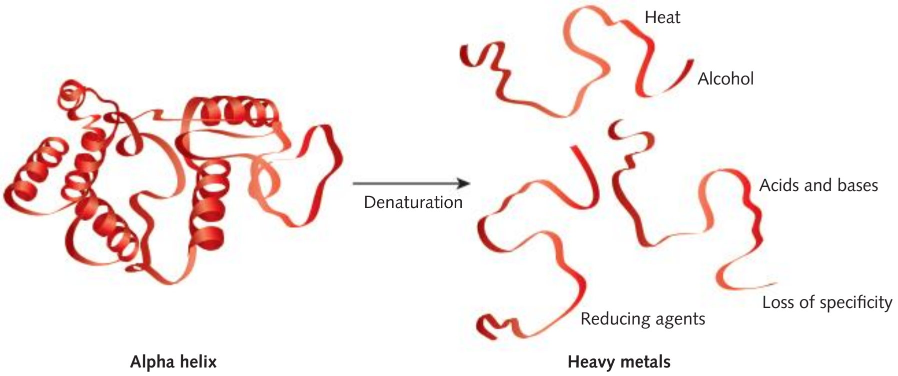


Figure 2.38

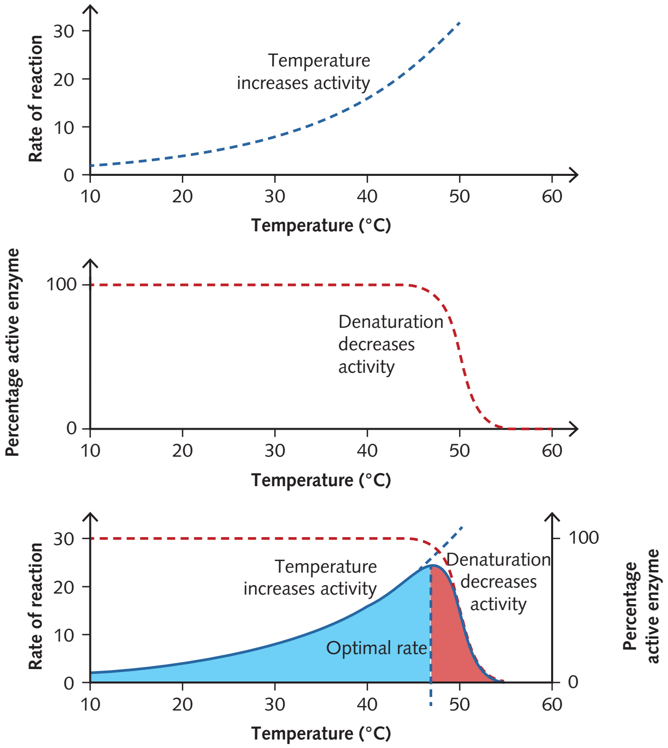


Figure 2.39

Creating

24 Scientists are concerned with identifying proteins involved in the progression of liver cancer. What sort of methodology could they use, and how might the results inform their research?

25 The nucleotide base sequence of a strand of DNA that codes for a specific amino acid is GGAATGCTCGACATC.

- **a** What is the base sequence of the complementary strand of mRNA?
- **b** How many amino acids are coded for by the strand of mRNA? List them.
- **c** What does the last codon code for and what does this mean?

26 Splicing joins two exon sequences. The following are steps in the production of a mature mRNA. Place them in the correct order and put them in a flow chart.

- Addition of 5′ cap
- Transport to cytoplasm
- Initiation of transcription
- Splicing
- Addition of poly-A tail

3

DNA manipulation techniques and applications

By the end of this chapter you will have covered the following material.

Key knowledge

DNA manipulation techniques and applications

- the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA, pp. 84–86
- the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome, pp. 87–92
- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling, pp. 92–104
- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin, pp. 105–15
- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease, pp. 115–118

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation, pp. 96–98; 109–111
- predict possible outcomes, pp. 96–98; 109–111

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation, pp. 96–98
- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated, pp. 96–98; 109–111
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications, pp. 96–98; 109–111

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks, pp. 96–98; 109–111

»

- apply relevant occupational health and safety guidelines while undertaking practical investigations, pp. 96–98; 109–111
- demonstrate ethical conduct when undertaking and reporting investigations, pp. 96–98; 109–111

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources, pp. 96–98
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs, pp. 96–98; 109–111
- plot graphs involving two variables that show linear and non-linear relationships, pp. 96–98

Analyse and evaluate data and investigation methods

- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors, pp. 96–98

Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation, pp. 102–104; 117

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement, pp. 96–98; 109–111
- discuss relevant biological information, ideas, concepts theories and models and the connections between them, pp. 109–111
- analyse and evaluate bioethical issues using relevant approaches to bioethics and ethical concepts, including the influence of social, economic, legal and political factors relevant to the selected issue, pp. 102–104; 117

To access resources below, visit www.nelsonnet.com.au

Online map:
- Chapter 3 map (p. 78)

Online Key Terms:
- Chapter 3 flashcards (p. 80)

Weblinks:
- CRISPR: The hacking tool that modifies DNA (p. 88
- Polymerase chain reaction (p. 94)

Online Worksheets:
- CRISPR: The hacking tool that modifies DNA (p. 88
- Polymerase chain reaction (p. 94)

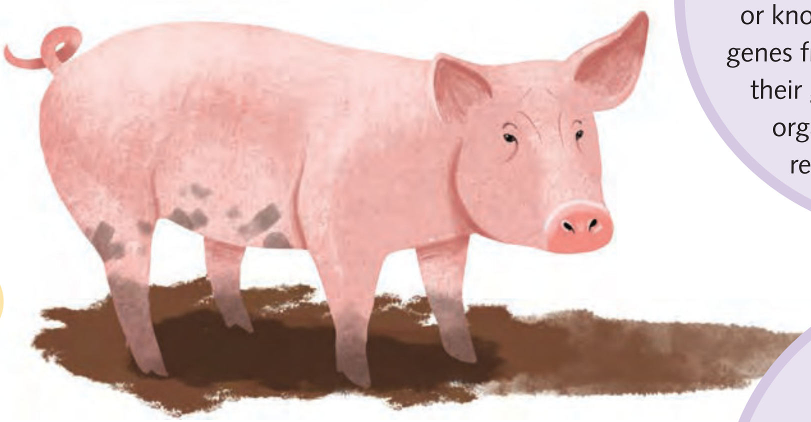
Video:
- CRISPR: (p. 87)

Online Key Concepts:
- Chapter 3: Summary of key concepts (p. 120)

Online Chapter Map
Chapter 3 map

3 DNA manipulation techniques and applications

The structure of DNA and the processes of transcription and translation have led to DNA manipulation techniques to make new combinations of genes within a genome.



3.1 Genetically modified organisms

p. 81

Genetically modified organisms have had their genomes modified by genetic engineering. Transgenic or knock-in organisms have had genes from other species added to their genomes, and knock-out organisms have had genes removed or inactivated.

3.2 Enzymes for modifying DNA

p. 84

Endonuclease cuts DNA at known sites to make sticky or blunt ends, ligase glues DNA back together and polymerase makes new copies of DNA.

3.3 CRISPR-Cas9

p. 87

The CRISPR-Cas9 enzyme makes precise cuts in DNA so segments or single bases can be replaced. CRISPR is a technique widely used in cancer treatment, gene therapy and agriculture.

3.4 Amplifying DNA

p. 92

DNA polymerase is a bacterial enzyme that can make many copies to amplify DNA. The polymerase chain reaction increases the amount of DNA for analysis in techniques such as gel electrophoresis.

3.5 Gel electrophoresis

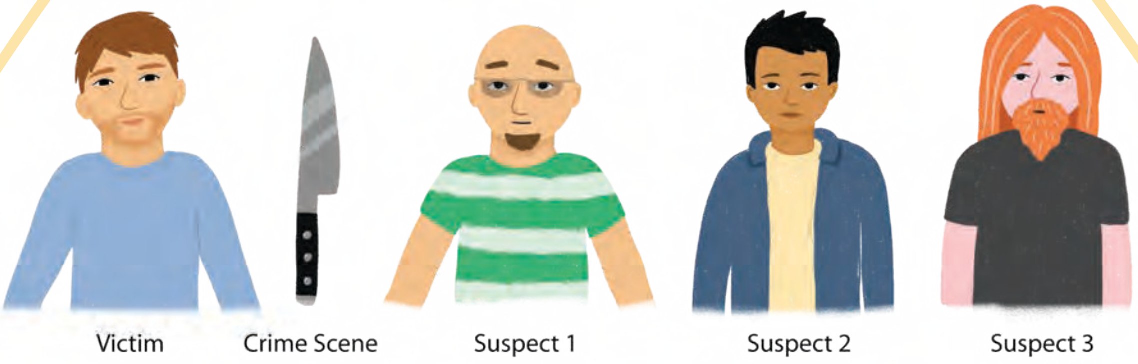
p. 95

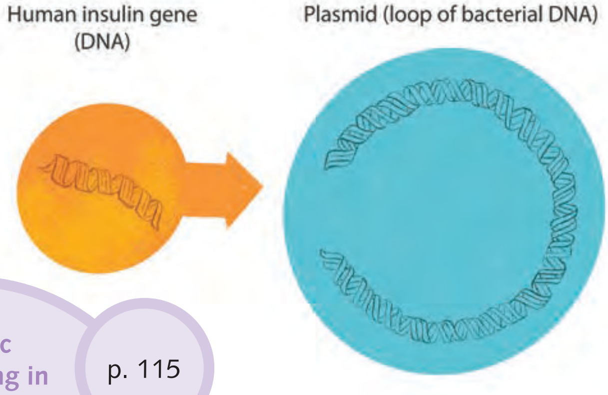
Gel electrophoresis sorts DNA into fragments according to size. Unique patterns emerge by running DNA through a charged gel. This DNA profile is unique to every individual except identical twins and triplets.

3.6 DNA profiling

p. 98

DNA profiling is used to identify people by their DNA. This is used in criminal investigations, identifying human remains and disaster victims and establishing parentage of children. DNA does not lie!





3.7 Recombinant plasmids and human insulin

p. 105

Recombinant DNA technology is used to insert the human insulin gene into the *E. coli* bacteria genome. So, we can use bacteria rather than animals to produce insulin for human use.

3.8 Genetic engineering in agriculture

p. 115

Genetic engineering can increase yield and disease resistance in crops to help the world prepare for an increasing global population.

Do humans have the right to alter the genomes of other organisms? If genetic modification of other organisms aids human survival, does it make it acceptable? These are some of the bioethical questions you need to consider.

Online Key Terms
Chapter 3 Flashcards

Know your key terms

agarose gel
annealing
antibiotic selection
bacteriophage
bioethics
biotechnology
blunt end
Cas9 protein
CRISPR-Cas9
crRNA (CRISPR RNA)
DNA ligase
DNA profiling
DNA sequencing
frameshift mutation
gel electrophoresis
gene cloning
genetic engineering
genetically modified organism (GMO)
knock-in organism
knock-out organism
molecular size marker
polymerase chain reaction (PCR)
polymorphism
primer
recombinant DNA technology
recombinant plasmid
reporter gene
restriction digest reaction
restriction endonuclease
restriction enzyme
restriction fragment
restriction site
short tandem repeat (STR)
single guide RNA (sgRNA)
sticky end
transformation
transgenic (TGO)
vector
wild type

Remember

REMEMBER
PAGE 58

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1. DNA is an information molecule that is the basis of an organism's genetic material.
2. DNA is made up of two strands of nucleotides linked together through complementary base pairing.
3. Genes are segments of DNA that code for proteins.
4. DNA is copied into two identical copies in the process of DNA replication.

Less than a century after the discovery of the structure of DNA by Watson and Crick in 1953, the 'gene revolution' has completely changed agriculture, medicine, global health and scientific research. Researchers now have an extensive toolkit of technologies to modify DNA. The current and potential applications of these technologies are vast and hold great promise. For example, rice has been nutritionally enhanced to reduce malnourishment in developing countries (Figure 3.1), insulin is now made in bulk so that type 1 diabetes is no longer a fatal disease, and scientists now have hundreds of animal models to study diseases and their potential treatments. In this chapter, you will explore basic and more recently developed techniques such as CRISPR-Cas9, which have been borrowed from Biology for use in a wide range of technological and medical applications, such as changing the genetic sequence of organisms.

Science Photo Library/US Department of Agriculture/Scott Bauer

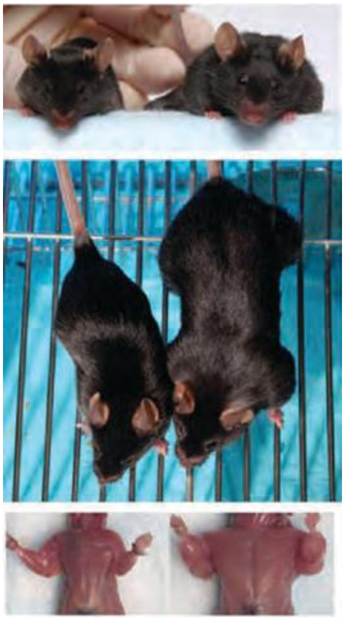
Figure 3.1 Genetically modified rice (left) compared to normal rice (right) has been developed to aid the digestion of its nutrients for humans and animals, as well as reduce the amount of phosphate run-off on the surrounding land, reducing the negative effects on the environment.

3.1

Genetically modified organisms

The term **genetic engineering** means changing the genetic sequence of an organism through human use of modern **biotechnology** techniques. Such genetically engineered organisms are called **genetically modified organisms (GMOs)**.

The term 'genetic engineering' applies to a range of techniques and processes for investigating and modifying DNA, genes and genomes of species. Scientists can use genetic engineering to switch genes on or off, remove genes and introduce genes from one species into another (Figure 3.2). Common genetic modifications include knock-out, knock-in and transgenic organisms.



Johns Hopkins Medicine/Se-Jin lee

Figure 3.2 A comparison of body size between normal mice (left), and mice genetically engineered (right) so that the gene myostatin is deleted and the gene follistatin is overproduced. These genes control muscle growth.

Transgenic organisms

Inserting DNA from one organism into the genome of another unrelated of a different species produces a **transgenic organisms (TGO)**.

Genes inserted into transgenic organisms are inserted into a locus that is known to be available for transcription all the time, allowing strong, constant expression across different tissues and not disrupting other genes. Scientists may also insert genes randomly, although by chance this may sometimes affect tumour suppressor genes, proto-oncogenes (genes involved in cell growth that promote cancer when mutated) or other genes that are important for normal development or function. The genes may be inserted with a promoter that drives strong expression, or a promoter or extra domain that allows gene expression to be manipulated (switched on or off); for example, by treatment with a drug.

Figure 3.3 summarises transgenic, knock-out and knock-in organisms.

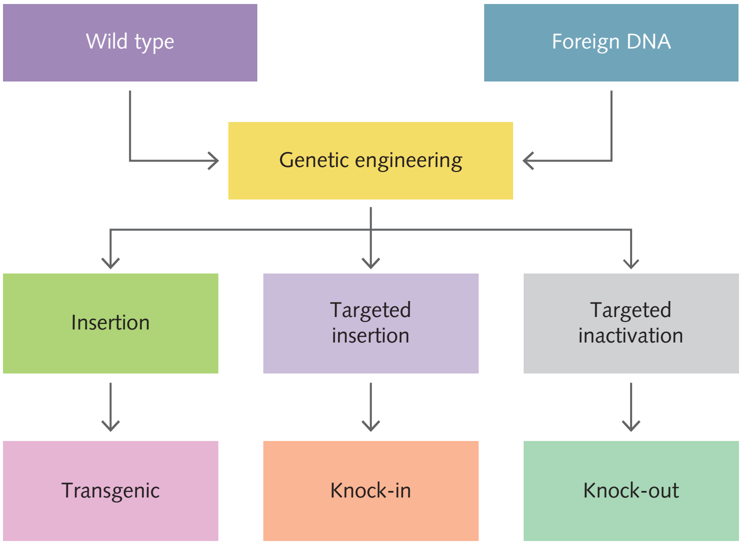


Figure 3.3 Genetically modified organisms include knock-out, knock-in and transgenic organisms.

Knock-out organisms

Knock-out organisms are produced by cutting out genes or gene segments to prevent their expression or the proper functioning of particular gene products.

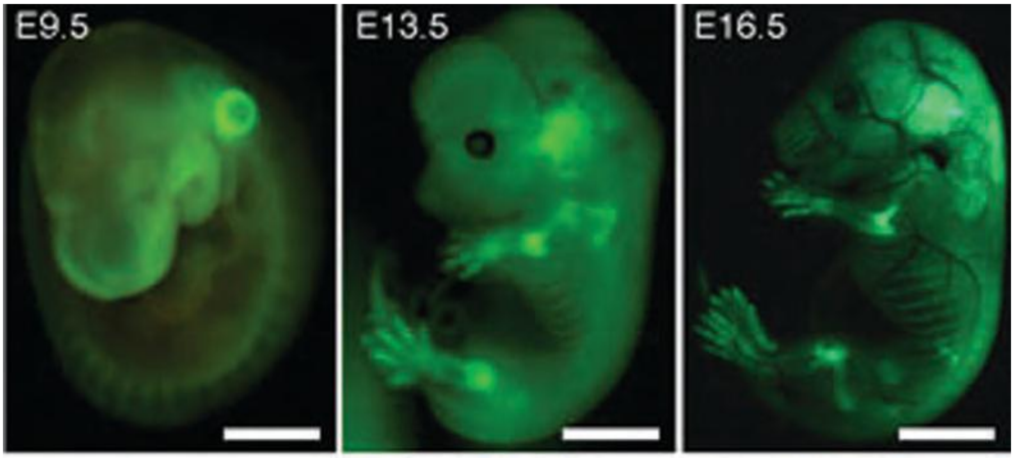
This technique is commonly used in research laboratories to study the functions of the knocked-out gene in animal models, especially mice. Scientists can study biological processes or disease models in the knock-out animals and infer the functions of the gene from the differences between the knock-out animals and control (called **wild type**) animals. Studies of this type can provide information about the activities of homologous genes in humans that cannot usually be gathered from *in vitro* tissue culture techniques, but instead can only be studied accurately when the gene is acting in the context of the whole animal.

WB
3.1
GENETICALLY MODIFIED ORGANISMS
PAGE 59

Knock-in organisms

Knock-in organisms are produced by inserting genes into a specific locus so that they are controlled by a particular promoter in the organism's genome.

Scientists use this technique to insert a **reporter gene**, such as that for green fluorescent protein, into the locus of a gene whose pattern of expression is unknown. The reporter gene will be expressed under the control of the promoter of the gene of interest and its characteristics are easily identified in the organism. Organisms that express green fluorescent protein show bright green fluorescence (Figure 3.4)



Reprinted by permission from Springer Nature: Nature Communications: Wwp2 is essential for palatogenesis mediated by the interaction between Sox9 and mediator subunit 2, Figure 1 ©, Yukio Nakamura et al, © 2011

Figure 3.4 Expression of Sox9, which is important for normal skeletal development, in mouse embryos at different days of embryonic development (for example, E9.5 = embryonic day 9.5), measured by knocking green fluorescent protein into the Sox9 locus. The expression of green fluorescent protein is therefore under the control of the Sox9 promoter.

when exposed to ultraviolet light. The green fluorescence can be measured in cells and tissues to give information about the location and timing of expression of the gene of interest. Inserting the reporter gene can sometimes inhibit the activity of the gene of interest, in which case the knock-in organism is also a knock-out organism for the gene.

Are all GMOs also TGOs?

All GMOs have their genes altered in some way- by addition of genes from other species, modification of their genetic material, or by 'knock in or out' of genes. Many genetically modified organisms are transgenic (TGOs) as they have one or more genes of another species genetically recombined into their genome. However, there are some GMOs which are not transgenic (non-transgenic) as their genome has been modified by gain of genetic material from the same species or loss of their own genes or genetic material (eg.non-allergenic cats in which the gene for a saliva protein has been silenced so they no longer cause allergic reactions in people that are hypersensitive to cats). Therefore it can be stated that "All TGOs are GMOs, but not all GMOs are TGOs".

KEY CONCEPTS

» Genetic engineering refers to the use of living things to make new products or systems by switching genes on or off, removing genes or introducing genes from one species into another.
» Organisms that are altered or produced by genetic engineering techniques are known as genetically modified organisms (GMOs).
» Genetically modified organisms include knock-out organisms, knock-in organisms and transgenic organisms.
» Knock-out organisms have had a gene deleted or its function interrupted.
» Knock-in organisms have had a gene or fragment of DNA inserted into a particular locus in the genome.
» Transgenic organisms have had a gene or fragment of DNA inserted into a non-specific locus.

Concept questions 3.1

1 List three types of genetic modification.
2 How has genome sequencing made genetic modification possible?
3 What type of genetically modified organism is a knock-in mouse and is this the same as a transgenic organism?
4 What does switching genes on or off mean?
5 Define 'knock-out organism'. Give three examples.
6 What is the difference between a specific locus and a non-specific locus when discussing transgenic organisms?
7 Define:
   a genetic engineering
   b genetic modification
   c transgenic organism
   d gene locus
   e promoter gene
   f reporter gene.

HOT Challenge

8 Figure 3.4 demonstrates expression of the Sox9 locus in embryonic skeletal development of mice. Why have the researchers used a reporter gene that codes for green fluorescence protein and how does this relate to the promoter gene in operation at this locus?

3.2 Enzymes for modifying DNA

3.2
ENZYMES FOR
MODIFYING DNA
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Genetic engineers use different tools for specific purposes. Genetic engineering, also called biotechnology, has its own set of specialised tools, which are mostly derived from other organisms. Some of the fundamental tools in biotechnology are enzymes for synthesising, cutting and pasting DNA.

DNA Polymerase to synthesise DNA

All life forms must be able to produce identical copies of DNA. The class of enzymes that is responsible for this is the DNA polymerases, which catalyse the formation of new DNA molecules from free nucleotides and a template DNA strand. In biotechnology, polymerases are used to:

- make copies of DNA
- read the sequence of DNA fragments
- detect single nucleotide differences
- amplify whole genomes
- diagnose medical conditions
- artificially synthesise new DNA fragments without a template strand.

The **polymerase chain reaction (PCR)** is a common biotechnology technique that has been in use since 1985. The key enzyme used in PCR is polymerase. PCR produces billions of copies of a section of DNA, which can subsequently be manipulated by other enzymes such as endonucleases for cutting and ligases for joining.

CONNECT

The polymerase chain reaction is discussed in detail on page 93.

Endonucleases to cut DNA

One of the essential requirements in genetic engineering is the ability to cut segments of DNA at known sequences. The cutting tools used are enzymes known as **restriction endonucleases** ('endo' within, 'nuclease' an enzyme that cleaves or cuts nucleic acids) or **restriction enzymes**. These are like 'molecular scissors', cutting DNA molecules into smaller pieces, called **restriction fragments**, in a controlled way. DNA cut with restriction enzymes is often said to be 'digested' by the enzymes. Restriction enzymes only cut specific sequences of DNA, known as **restriction sites** or recognition sequences. Different restriction endonucleases have different restriction sites, although some endonucleases do share restriction sites with other endonucleases. Most recognition sequences are palindromes of their complementary sequence; that is, the sequence of the non-template strand in the 5′–3′ direction is the same as the sequence from 5′ to 3′ on the complementary strand.

Endonucleases occur naturally in bacteria, where they cleave 'foreign' DNA that enters from invading viruses, thus destroying any potential threat. They are an important mechanism of immunity for bacteria. Endonucleases are named according to the bacterial strain from which they are derived. The first endonuclease isolated from *Escherichia coli* strain RY13 was thus named EcoRI. Table 3.1 identifies a number of common endonucleases and their bacterial sources.

Table 3.1 Common endonucleases and their restriction sites

Enzyme	Bacterial source	Restriction site	After cutting
EcoRI	*Escherichia coli*	5′G↓AATTC3′ 3′CTTAA↑G5′	5′G AATTC3′ 3′CTTAA G5′
HindIII	*Haemophilus influenzae*	5′A↓AGCTT3′ 3′TTCGA↑A5′	5′A AGCTT3′ 3′TTCGA A5′
AluI	*Arthrobacter luteus*	5′AG↓CT3′ 3′TC↑GA5′	5′AG CT3′ 3′TC GA5′
BamHI	*Bacillus amyloliquefaciens H*	5′G↓GATCC3′ 3′CCTAG↑G5′	5′G GATCC3′ 3′CCTAG G5′

To date, about 3000 different endonucleases have been identified. Although each endonuclease recognises a specific sequence of 4–8 nucleotide base pairs (bp) of the double-stranded DNA, multiple enzymes isolated from different organisms can recognise the same sequence. Endonucleases bind to their restriction site and cut the double-stranded DNA at that point. The cuts may form either overhanging steps, called **sticky ends**, which leave some nucleotides exposed (Figure 3.5a), or **blunt ends** (Figure 3.5b), in which the cut has occurred at the same position in each strand of the DNA and there are no overlapping strands. Spontaneous hydrogen bonding between overhanging nucleotides helps restriction fragments with sticky ends bind to other DNA fragments with complementary sticky ends.

In molecular biology, endonucleases are used in **restriction digest reactions**, in which the substrate may be a plasmid, genomic DNA or a PCR product.

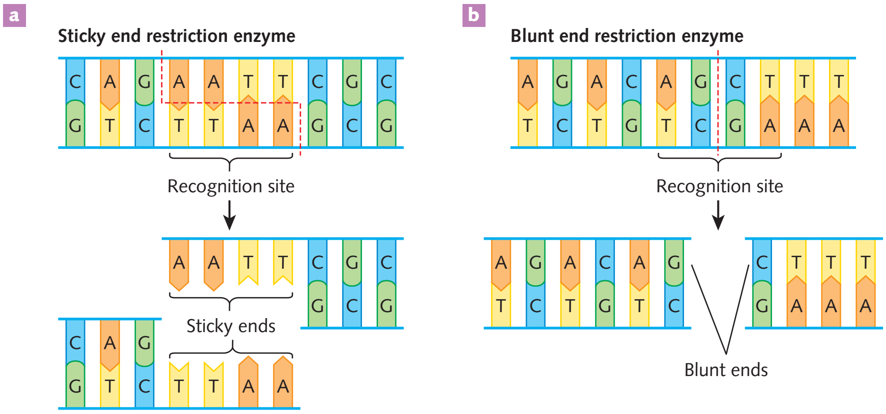


Figure 3.5 **a** Sticky ends produced by cutting DNA with the endonuclease EcoRI. **b** Blunt ends produced by cutting DNA with the endonuclease AluI.

Ligase to join DNA

Sometimes, molecular biologists want to combine two samples of DNA; for example, they might want to insert a piece of DNA into a plasmid (a small circular piece of bacterial DNA). **DNA ligase** is an enzyme used to join different pieces of DNA together. DNA ligase acts by forming a phosphodiester bond between the two fragments of DNA. It joins the 3′ end of one nucleotide with the 5′ phosphate end of another nucleotide. DNA ligase requires magnesium ions and ATP for its activity, so DNA ligation is an energy-consuming reaction.

The process of joining two strands of DNA together using DNA ligase is more successful if the two strands can be brought together. If the restriction enzymes used to cut the DNA generate sticky ends, two DNA fragments that have been cut with the same enzyme will have identical sticky ends and thus the complementary bases will be exposed. This means that if the ends of the two strands come into contact with each other by chance, their nucleotides will form hydrogen bonds at the sticky ends and remain in place, leaving just a nick in the DNA backbone to be ligated. DNA ligase can then be used to recombine these two fragments by creating a covalent phosphodiester bond between them, even if they are from two unrelated organisms. For example, EcoRI can be used to cut both human DNA and bacterial plasmid DNA, leaving sticky ends that are complementary and able to bond to each other (Figure 3.6).

CONNECT

The application of these techniques to make insulin is discussed on page 105.

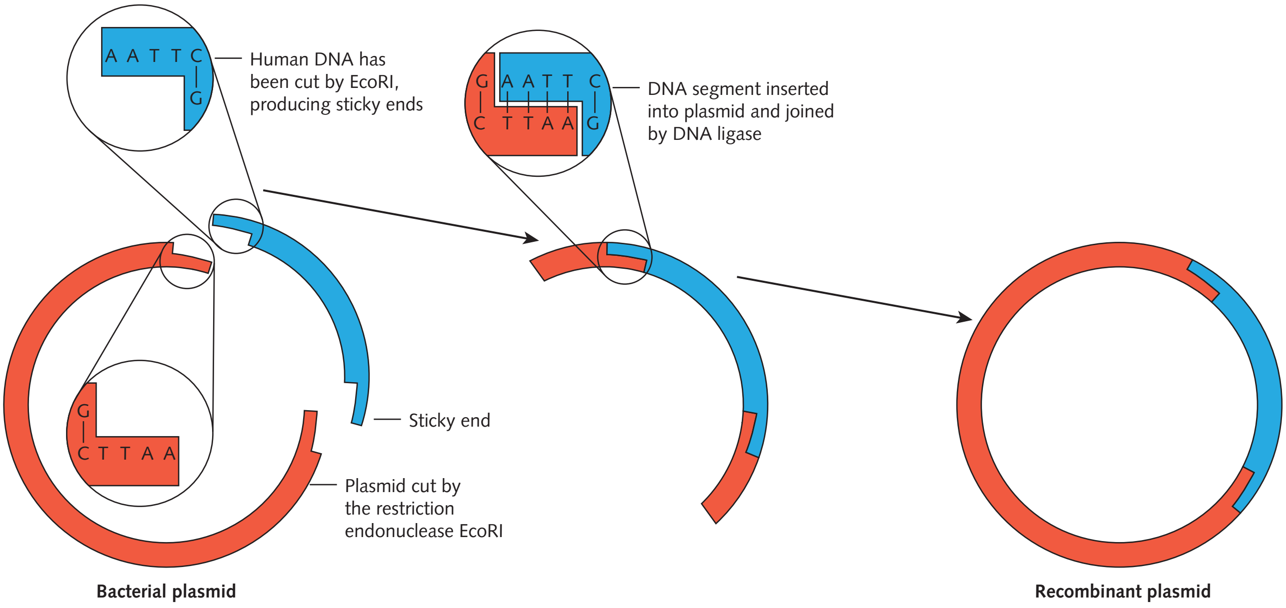


Figure 3.6 DNA ligases join DNA inserted from a foreign source that has complementary sticky ends.

Fragments with blunt ends can also be joined by DNA ligase, but this process is much less efficient. Sticky end ligation also ensures the joined DNA fragments are the right orientation when joined. The technology that recombines DNA from different sources to modify the DNA sequence is called **recombinant DNA technology**.

KEY CONCEPTS

- DNA polymerases catalyse the formation of new DNA molecules from free nucleotides and a template DNA strand.
- The technology that recombines DNA from different sources to modify the DNA sequence is called recombinant DNA technology.
- The cutting tools used are enzymes known as restriction endonucleases, or restriction enzymes.
- Endonucleases can generate blunt or sticky ends.
- DNA ligase is an enzyme used to join different pieces of DNA together.
- Complementary sticky ends help DNA strands bind to each other via hydrogen bonding.

Concept questions 3.2

1 State the function of:
  a DNA polymerase
  b restriction endonuclease.
2 What is the difference between sticky and blunt ends created by restriction enzymes?
3 What process completes the formation of restriction fragments?
4 Which restriction enzymes listed in Table 3.1 'cut' fragments with sticky ends and which 'cut' fragments with blunt ends?
5 DNA ligases act by forming phosphodiester bonds between fragments of DNA. Explain how the following terms are connected: ligase, phosphodiester bond, 5′ phosphate end, 3′ hydroxyl end, nucleotide, DNA fragment and hydrogen bonds.

HOT Challenge

6 Restriction endonucleases were first isolated from bacteria. What is their specific use in bacteria? Why are they applicable for use in different species of living organisms?

3.3 CRISPR-Cas9

WB
3.3
CRISPR-CAS9
PAGE 60

Video
CRISPR

New biotechnological tools are continually being developed as our understanding of biological mechanisms grows. An example is the recent development of the CRISPR (clustered regularly interspaced short palindromic repeats) (pronounced 'crisper') genome editing system, which is based on a bacterial defence mechanism.

Getty Images/Miguel Riopa

Figure 3.7 **Professors Emmanuelle Charpentier (left) and Jennifer Doudna (right) demonstrated how the bacterial immune defence mechanism CRISPR-Cas9 could be applied to precisely edit the genome.**

In 2012, scientists Professor Jennifer Doudna and Professor Emmanuelle Charpentier (Figure 3.7) demonstrated for the first time that a bacterial antiviral immune defence mechanism could be harnessed to apply breaks in specific sites of double-stranded DNA to edit genomes. The resulting **CRISPR-Cas9** genome editing system has revolutionised the capacity to modify DNA. Charpentier and Doudna were awarded the Nobel Prize in Chemistry in 2020 for their work.

Function of CRISPR-Cas9 in bacteria

Like animals and plants, bacteria are susceptible to infection by viruses, specifically called **bacteriophages**. To protect themselves against these bacteriophages, bacteria slowly evolved the CRISPR-Cas9 system which functions as a primitive adaptive immune system. On infection by a virus (bacteriophage), bacteria collect small fragments of the bacteriophage DNA called protospacers and integrate (insert) them into certain regions of their bacterial genome. These regions are called CRISPR loci. The protospacers are integrated into the bacterium's CRISPR gene and become a spacer. Throughout the CRISPR loci, viral sequences derived from previous exposure to viruses are interspersed with short repeats of host bacterial DNA. Every time the bacterial strain is exposed to a new viral infection, a new piece of viral DNA is integrated at the 5′ end of the CRISPR locus within the bacterium's genome. Therefore, if you read the CRISPR locus from the 5′ end to the 3′ end, you will get a chronological record of previous viral infections that the bacteria has encountered. Transcription of the CRISPR spacers together with some of the CRISPR palindrome repeats of DNA either side of the spacer produces an RNA molecule called **crRNA (CRISPR RNA)** that is complementary to the integrated fragments (spacers) of viral DNA. This crRNA binds to **Cas9 proteins** to produce a CRISPR-Cas9 complex. This complex then scans the bacterial cell for any future infecting bacteriophage DNA that is complementary to that in the crRNA. The Cas9 will only cut out the target sequence if it recognises a very short (2–6 nucleotides) sequence adjacent to the spacer called a PAM (protospacer adjacent motif). If located, the Cas9 endonuclease proteins cleave the phosphate-sugar backbone of the bacteriophage DNA, inactivating and destroying it. As the same DNA sequence occurs in both the viral DNA and the bacteria's spacers in the CRISPR locus, PAM is an important tool to distinguish self from non-self. Much like receiving an immunisation, the CRISPR system provides the bacterial cell with a 'memory' of previous bacteriophage infections that it uses to defend itself against reinfection.

Biotechnology applications of CRISPR-Cas9

Online Weblink
CRISPR: The hacking tool that modifies DN

Online Worksheet
CRISPR: The hacking tool that modifies DN

In biotechnology applications of CRISPR-Cas9, the bacteria-derived Cas9 enzyme (the molecular scissors) is directed by a short piece of RNA called a **single guide RNA**. **sg(RNA)** has a complementary target site in genomic DNA, rather than the crRNAs generated from transcription of the CRISPR locus that occurs in the bacterial CRISPR-Cas9 system. Synthetic sgRNA is produced by scientists that has a complementary spacer to the target DNA that they wish to cut. A Cas9 enzyme is added that has a PAM (protospacer adjacent motif) detector. PAM is a short 2-6 nucleotide segment found next to the targeted DNA that guides the Cas9 to cut at a precise location just before PAM. When injected into cells by scientists, the Cas9 will find the target PAM sequence, check whether the sgRNA aligns with the DNA, and then cut the selected sequence of DNA. It creates a double-strand break (which would naturally target bacteriophage DNA for destruction inside infected bacterial cells). The eukaryotic host cell's machinery tries to repair the break. The repair mechanism is error prone and this often results in nucleotides being inserted or deleted, which causes a **frameshift mutation** that interferes with translation of the targeted gene and results in a knock-out organism. If a donor DNA fragment has been added to the reaction, the DNA repair machinery may incorporate it at the target site, resulting in a knock-in organism (Figure 3.8).

The CRISPR-Cas9 system is a revolutionary biotechnological tool because:

- » its use of specific guide RNA sequences (around 20 bases long) can direct the complex to exactly the desired location in the genome
- » it can cut double-stranded DNA at any desired site.

This technique has greatly simplified the processes involved in genetically modifying organisms.

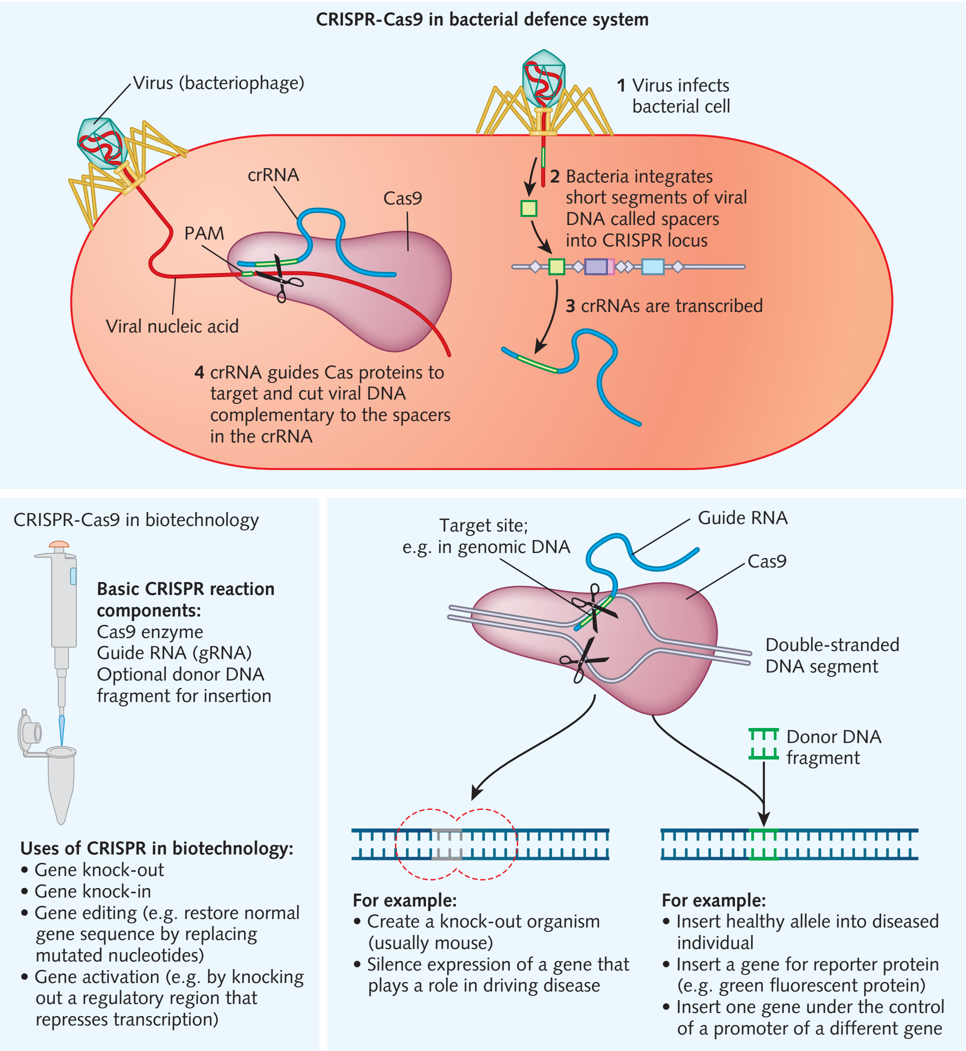


Figure 3.8 The CRISPR-Cas9 system in bacteria and its application to genetically modify DNA. A short molecule of RNA (guide RNA) binds to the target gene and guides the Cas9 enzyme to the gene. The Cas9 enzyme cleaves the DNA and the bacterial cell tries to repair the break. This can result in a frameshift mutation – deletion or insertion of a gene – if a donor DNA fragment is incorporated.

Applications of CRISPR technology

The CRISPR-Cas9 system has revolutionised the capacity for scientists to modify DNA of previously intractable organisms. Imagine being able to apply precise gene editing to awaken the immune cells of a patient with an aggressive form of cancer that switches the immune system off. This approach would transform cancer treatment and is an intense subject of scientific research using mouse models of cancer.

The CRISPR-Cas9 system can also be applied to create a knock-out mouse to decipher the function of a gene. Using CRISPR-Cas9, a new mouse strain takes around three months to make, instead of one or two years.

Current and potential applications of the CRISPR-Cas9 genome editing system are extensive. Some examples are described here.

Editing faulty alleles in genetic diseases

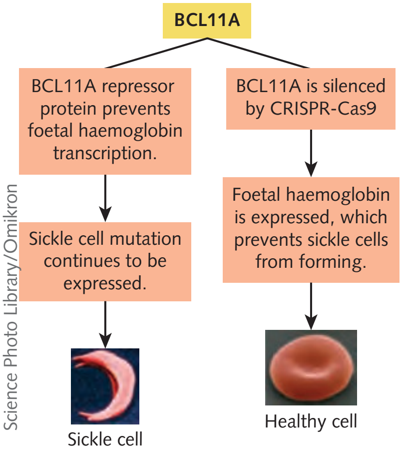
Sickle cell anaemia is a blood disease caused by a genetic mutation that produces an abnormal form of haemoglobin (the molecule that carries oxygen around the body, Figure 2.28, p. 61). The red blood cells are distorted. They are shaped like a sickle, rather than the smooth disc of healthy red blood cells. The rigid sickle cells clump together and obstruct blood flow in small blood vessels, causing organ damage and severe pain. Sickle cell anaemia can cause stroke, organ failure and sometimes death.

Gene therapy is a promising approach for treating the disease. Researchers have applied CRISPR-Cas9 using two different approaches to try to cure the underlying genetic cause. Both approaches are based on the same overall idea. First, blood from the patient is taken into the laboratory where the haemopoietic stem cells (the precursor cells of blood cells) are edited by using the CRISPR-Cas9 system. After careful tests to ensure the genetic modification is correct, the edited cells can then be transfused back into the patient's bloodstream.

The first approach was developed at Stanford University in the United States. CRISPR-Cas9 was used to induce a double-stranded break in the β-globulin gene of haemoglobin. A donor fragment of DNA containing the normal healthy version of the gene was added into the reaction and the DNA repair machinery incorporated it into the target site. The edited cells then transcribed the normal version of haemoglobin and had healthy disc-shaped red blood cells. This method of applying CRISPR-Cas9 to cure sickle cell anaemia is still in the preclinical stages of research.

The second approach uses CRISPR-Cas9 to introduce a mutation in a gene encoding a transcription factor called BCL11A. BCL11A represses haemoglobin F, a form of haemoglobin expressed in foetuses but repressed in adults. By silencing BCL11A, the 'molecular handbrake' is released and haemoglobin F is transcribed. Haemoglobin F interferes with the polymerisation of the sickle haemoglobin molecule, thus preventing the formation of sickle cells (Figure 3.9). This approach was inspired by the observation that carriers of the sickle cell mutation who also had a mutation in the BCL11A gene were resistant to sickle cell disease.

In 2019, two companies, CRISPR Therapeutics and Vertex Pharmaceuticals, performed clinical trials together on this CRISPR-Cas9 therapy (called CTX001). The initial four-month trial assessed safety and efficacy in one patient with sickle cell anaemia. The preliminary results were encouraging. The patient did not experience any vaso-occlusive crises (when sickle cells get stuck in blood vessels) and expressed 46% foetal haemoglobin



Science Photo Library/Omikron

Science Photo Library/Eye of Science

Figure 3.9 In adults, foetal haemoglobin is not expressed because it is repressed by the repressor protein BCL11A. Scientists used CRISPR-Cas9 to target BCL11A, silencing its expression and allowing foetal haemoglobin to be produced in adult cells.

(25–30% foetal haemoglobin is considered to cure a patient with sickle cell disease). In the next stage of the clinical trial, more patients will be enrolled in the study and will be followed for a longer time after the treatment (two years) to assess safety and efficacy.

CRISPR in agriculture

CRISPR-Cas9 enables scientists to quickly and precisely insert or delete the desired traits to improve yield, tolerate environmental stress and resist disease. Since 2013, CRISPR-Cas9 has been applied to crops such as rice, wheat, corn, tomatoes and mushrooms. In the US, plant biologist Yinong Yang from Pennsylvania State University applied CRISPR-Cas9 to the white button mushroom genome, knocking out one of the genes that encodes polyphenol oxidase (PPO). PPO is an enzyme that causes browning. By deleting one of the genes that encodes PPO, the activity of the enzyme was reduced by 30%. Therefore, the CRISPR-edited white button mushrooms have a longer shelf life and are resistant to browning commonly caused by mechanical handling (Figure 3.10).

In 2016, a letter addressed to Yinong Yang from the US Department of Agriculture (USDA) made headlines about genetically modified organisms. In the letter, the USDA confirmed that they did not require the genetically edited mushrooms to pass through the agency's regulatory process because the CRISPR-mushrooms did not contain any foreign DNA integrated into the mushroom genome. Therefore, the USDA did not consider it necessary to regulate the mushrooms, and gave permission for the mushrooms to be grown and sold in the US. This opened public debate and dialogue about newer technologies that make GMOs and prompted the US government to review the regulatory process to consider newer technologies such as CRISPR-Cas9.

Getty Images/Jose A. Bernat Bacete

Figure 3.10 White *Agaricus bisporus* mushrooms that have been modified by CRISPR to reduce browning

Using CRISPR to make new mouse strains

The mouse genome is similar to the human genome and many symptoms of human disease can be replicated in mice. This makes the mouse a useful animal model in the laboratory. Scientists commonly knock out genes in mice to study the function of the deleted gene. Traditional methods of making a knock-out mouse were laborious and time consuming. Despite this, at the time, the technique of genetically modifying embryonic stem cells and injecting them into mouse embryos was groundbreaking. This technology led to the 2007 Nobel Prize in Physiology or Medicine being awarded to Mario Capecchi, Martin Evans and Oliver Smithies. In 2013, the discovery that the CRISPR-Cas9 system could be harnessed to edit the mammalian genome meant that efficient, targeted modifications of the mouse genome were now possible. What would take one or more years with traditional approaches now would take only a few months.

CONNECT

The steps involved in CRISPR technology are explained on page 88.

Keeping up with CRISPR

The sudden arrival of CRISPR technology, and its vast potential applications, has thrown up major ethical considerations about its use. Organisms, including humans, with heritable genome alterations can be produced relatively easily, but specific legal frameworks and a clear pathway towards translation of the technology have yet to be established.

Controversial use of CRISPR on human embryos

In November 2018, Chinese scientist He Jiankui announced to the international scientific community that he and his colleagues had applied CRISPR-Cas9 to edit the genome of twin baby girls born that month. The scientists used CRISPR-Cas9 to target the gene *CCR5* to inhibit its expression. Edited embryos were produced by *in vitro* fertilisation (IVF) and then implanted into a woman, who gave birth to the baby girls. CCR5 is a protein expressed on the surface of immune cells. The human immunodeficiency virus (HIV) uses CCR5 as a receptor to enter T cells (a type of white blood cell), where it replicates and then goes on to infect other T cells. Therefore, CCR5 can be considered a 'door handle' for HIV entry into T cells. He Jiankui targeted CCR5 because the father of the girls had HIV. Silencing CCR5 meant that HIV had no 'door handle' to allow the entry into the babies' T cells and they could not become infected with HIV. Effectively, the genome modification conferred resistance to HIV.

The announcement of the world's first 'CRISPR babies' made news headlines around the world. It sparked intense controversy and in many cases horror. Immediately following the announcement, a World Health Organization (WHO) expert Advisory Committee on Human Genome Editing examined the ethical and technical consequences of editing the human genome, particularly germline cells (eggs and sperm and their precursors, responsible for passing on genetic material to the next generation). The committee recommended that all countries inhibit any application of human genome modifications until they could vigorously consider the ethical and biological implications of such work.

In late 2019, He Jiankui was sentenced to three years in prison and a fine of 3 million yuan for practising medicine without a license, fabricating ethical review documents and violating Chinese law regarding assistive reproductive technology.

Policies and laws lag behind the pace of CRISPR technology

A US National Academies of Sciences, Engineering, and Medicine committee, which consisted of bioethicists, scientists, lawyers and patient advocates, suggested that strict regulations were needed to monitor CRISPR-Cas9 technology. However, both the WHO committee and the US committee made their recommendations after the announcement of the CRISPR babies. This highlights how policies and regulations lag behind the pace of CRISPR technology.

In agriculture, CRISPR-Cas9 modified plants, such as Yinong Yang's white button mushrooms, were not subject to the same regulatory process as plants genetically modified by traditional methods. Additionally, they did not have to be labelled 'transgenic' because foreign DNA had not been introduced into the mushroom genome. Should CRISPR-Cas9 modified plants be subject to regulation like other DNA manipulation approaches? Or is it reasonable that because foreign DNA is not inserted, they should be allowed to be grown and sold without approval? These are questions being considered by different regulatory bodies around the world. For instance, in 2018, the Court of Justice of the European Union ruled that CRISPR-edited crops should be subject to the same regulations as traditionally genetically modified organisms.

Bioethical considerations for CRISPR-Cas9 technology

CRISPR-Cas9 technology holds enormous promise and potential for developing new treatments for disease, improving crops in agriculture and developing new mouse strains and disease models for scientific research, but it also raises serious ethical questions. In the case of the CRISPR babies, the genetic change to the germline (sperm and egg cells) means that the edited genetic material will be passed down from generation to generation. In this situation, the genetic change was intended to benefit human health and prevent disease. Therefore, it could be considered beneficial to pass on a genetic change that will confer resistance to a disease such as HIV. By this logic, it might be possible in the future to cure human disease, if it is deemed safe. However, we do not know if it is possible to predict the full implications and perhaps unanticipated consequences of such a genetic change over a whole human lifespan, or several generations.

We need to consider whether editing the human genome is altering the human species. Consider the potential application of CRISPR-Cas9 gene editing to enhance a desired trait for a non-therapeutic purpose. We need to consider that genome editing might only be available to the wealthy and would therefore increase the disparity in access to health care between the rich and poor. Could these issues be managed through policy and laws?

There is a long list of ethical considerations surrounding CRISPR-Cas9 technology. **Bioethics** is the study of such ethical issues that arise from advances in biology, such as CRISPR-Cas9. Bioethics committees examine bioethical issues that emerge because of advancements made in biological research. These committees are made up of experts and people from various disciplines, including ethics, philosophy, genetics, politics, psychiatry, law, medicine, scientific research and teaching. Together, they consider the ethical questions that emerge as a result of biological and medical research, aiming to inform policy and public debate.

KEY CONCEPTS

- The CRISPR system is a bacterial immune defence mechanism against viral infection.
- CRISPR-Cas9 is applied to edit genomes to precisely create knock-out or knock-in genetic modifications.
- Applications of CRISPR-Cas9 technology include editing faulty alleles in disease, improving agriculture and making new mouse strains for scientific research.
- The use of CRISPR-Cas9 technology raises serious bioethical questions.

Concept questions 3.3

1 CRISPR is an acronym. What does each letter stand for?
2 Define 'palindromic repeat' and provide an example.
3 What is the CRISPR locus?
4 crRNAs are complementary to the integrated fragments of viral DNA in a bacterium. Where did this viral DNA come from?
5 'crRNAs form a complex with CRISPR-associated 9 (Cas9) proteins, which are able to cleave (cut) DNA.' Interpret this statement by explaining each of the terms and the overall meaning.

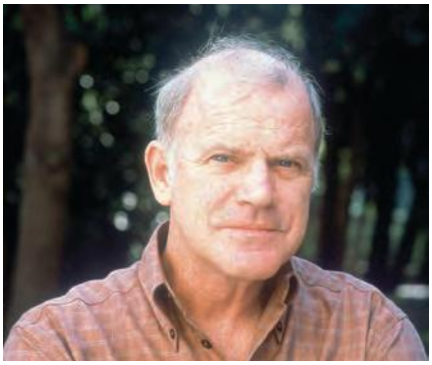
HOT Challenge

6 CRISPR-Cas9 technology raises many issues that need to be resolved. Choose one of the biotechnology applications of this form of 'DNA scissors' and discuss the reasons why the application is useful for humans but is ethically complex.

WB 3.4 AMPLIFYING DNA PAGE 63

3.4 Amplifying DNA

Each eukaryotic somatic cell has only two copies of a gene of interest and prokaryotic cells have only one copy. This small amount of DNA poses a problem for scientists wishing to work with it. Similarly, only a small sample of DNA may be available for analysis; for example, at a crime scene or in preserved bones. The first step in DNA analysis or in genetic engineering is to make enough copies of the DNA to be able to work effectively. Biotechnologists have an important tool to do this: the polymerase chain reaction (PCR). PCR is used to amplify, or make many copies of, a specific sequence of DNA. It uses DNA polymerase that is usually derived from *Thermus aquaticus*, a thermophilic species of bacteria that thrives in hot conditions such as the geothermal springs of the Yellowstone National Park, US. *T. aquaticus* has evolved to withstand these hot conditions, and so its DNA polymerase (called Taq polymerase) remains stable during the high temperature cycles of the PCR process. The US biochemist who invented PCR, Kary Mullis (Figure 3.11), was awarded the 1993 Nobel Prize in Chemistry for his work.



Alamy Stock Photo/Agence Opale/Basso Cannarsa

Figure 3.11 Kary Mullis was awarded the 1993 Nobel Prize in Chemistry for his work inventing the polymerase chain reaction.

Polymerase chain reaction

A number of components are required for PCR:

- the DNA that is to be copied (the template)
- DNA polymerase (the enzyme)
- a buffer solution that contains salts and other chemicals that help the polymerase to function (called cofactors)
- a supply of the four nucleotides (A, T, C, G) from which to build the new DNA molecules
- two **primers** – short sequences (about 20 nucleotides) of single-stranded DNA, complementary to the nucleotide sequences at either end of the DNA section that is to be copied.

The primers are necessary as a starting point from which the DNA polymerase can add new DNA nucleotides. DNA polymerase can only extend a DNA strand from an existing nucleotide; it cannot create a new complementary strand without primers to begin extending from. The nucleotide composition of the primer determines its annealing (joining) temperature because it determines the number of hydrogen bonds that form between the primer and template strand. Because G–C complementary nucleotides pair with three hydrogen bonds, they require more kinetic energy (a higher temperature) to separate than do A–T nucleotides, which pair with two hydrogen bonds.

PCR has three steps (Figure 3.12).

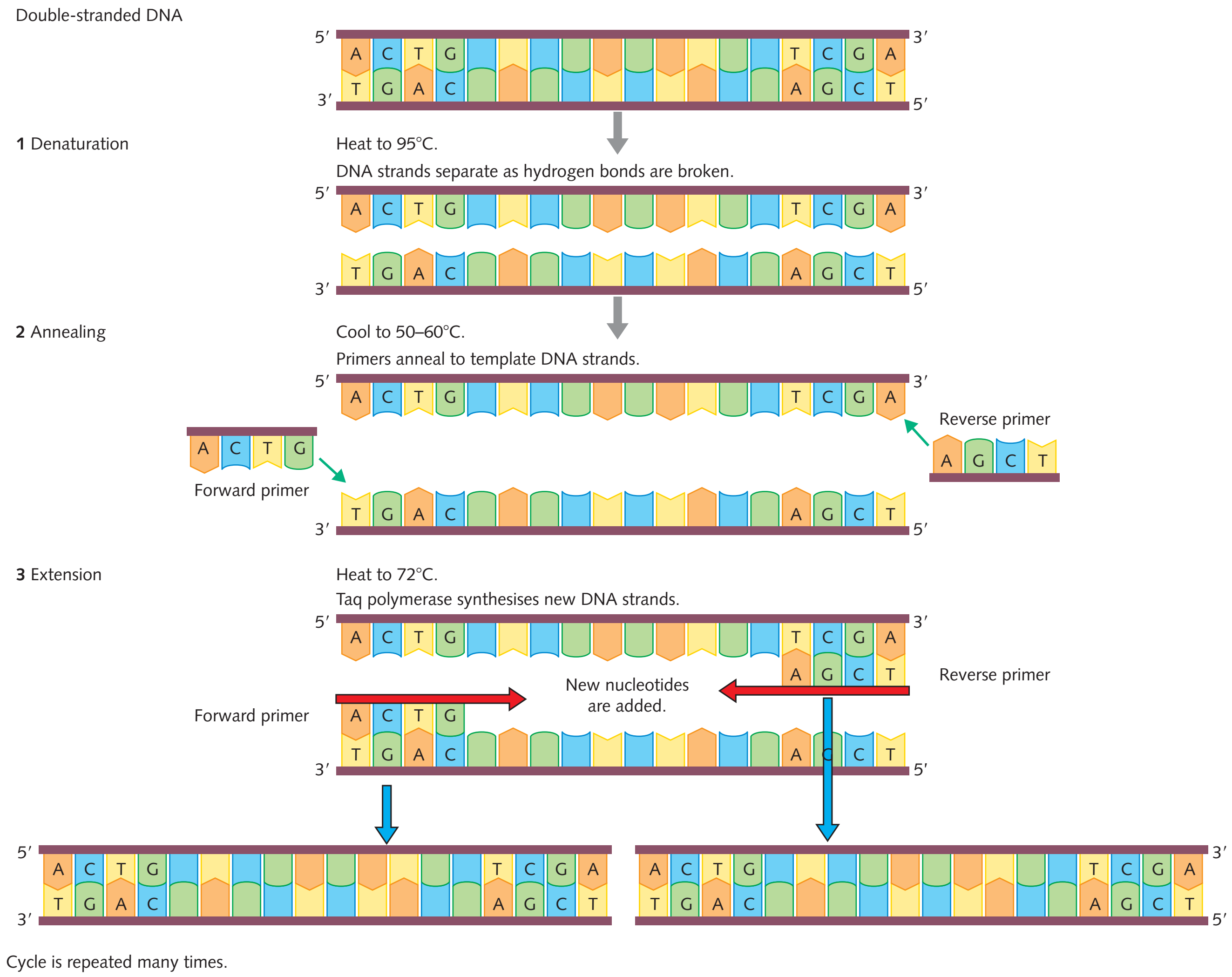


Figure 3.12 Amplifying DNA using PCR

Weblink
Polymerase Chain Reaction

Online Worksheet
Polymerase Chain Reaction

1 Denaturation: the double-stranded DNA is heated to 95°C. This breaks the hydrogen bonds between the bases and causes the two strands to denature.
2 **Annealing**: the temperature is reduced to 50–60°C, allowing the primers to join to complementary sequences on opposite ends of each strand: either genomic DNA in the first cycle or PCR products generated during the previous cycle. The reduced temperature is necessary to allow base pairing and the formation of hydrogen bonds.
3 Extension: the temperature is raised to 72°C, the optimum temperature for the particular DNA polymerase used in PCR. Starting from the primers, new DNA strands are synthesised in the 5′ to 3′ direction, using DNA polymerase and the available nucleotides. At the end of this phase, there are two copies of the double-stranded DNA. Each copy consists of one 'parent' strand and one new strand.

This cycle is repeated until enough DNA has been produced. Each cycle doubles the number of DNA strands; therefore, in just 20 cycles more than one million copies of target DNA will be produced ($2^{20} = 1\,048\,576$ copies of DNA). There are usually 30–35 cycles in a PCR program (to produce $2^{35} = 3.44 \times 10^{10}$ copies of DNA), which take place in instruments called thermal cyclers (Figure 3.13).

EXAM TIP
Read the question carefully and answer what is being asked. Only discuss the specific aspect of PCR that the question is asking about. The number of marks will indicate the depth required in the answer.


Science Photo Library/Philippe Psaila

Figure 3.13 A thermal cycler, in which the PCR is carried out as a programmable automated process. The thermal cycler raises and lowers the temperature of the samples, which allows denaturation, annealing and extension to take place in a controlled way.

KEY CONCEPTS

- » PCR is a process that amplifies a specific DNA sequence for analysis.
- » The sequence of the primers determines the DNA sequence to be amplified.
- » The steps involved in PCR are denaturation, annealing and extension. These steps are repeated many times to yield a large number of identical DNA molecules.

Concept questions 3.4

1 What does DNA amplification mean and why is it used in biotechnology?
2 State the components of a PCR reaction.
3 List the three main steps of PCR.
4 What specific task in this process do primers perform?
5 If you start with five copies of a DNA region, how many copies will be produced if your sample goes through 30 cycles of PCR?

HOT Challenge

6 At each stage of the PCR process, the temperature is adjusted to enable certain steps to occur.
   a Draw up a table that summarises the name of each step, the temperature at which the step occurs, and the actual process facilitated.
   b In your table, state where and how the properties associated with hydrogen bonding are vital and how the optimal temperature for operation of the DNA polymerase is 72°C and not 95°C.

3.5 Gel electrophoresis

WB
3.5 GEL ELECTROPHORESIS
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DNA molecules are too small to see. One way to visualise them is to separate the fragments according to size by gel electrophoresis. Alternatively, scientists use a DNA probe to identify fragments, or analyse the nucleotide sequence by **DNA sequencing**.

Gel electrophoresis method

Gel electrophoresis separates fragments of DNA according to their size and charge. DNA has an overall negative charge due to the phosphate groups on its backbone. Gel electrophoresis makes use of this property to separate DNA fragments within an **agarose gel**. The agarose gel is melted and poured into a flat mould to cool. Wells are formed by placing a plastic comb into the gel as it sets, creating indentations into which DNA samples can be loaded.

The gel is placed in a tray filled with buffer solution (Figure 3.14), and positive and negative electrodes are attached at each end of the gel. When the electric current runs, the DNA fragments are repelled from the negative electrode and move towards the positive electrode at the other end. The gel acts as a large sponge through which the DNA strands move while under the influence of the electric current. Smaller strands can move faster than the larger strands through the gel matrix. Therefore, this method separates DNA strands based on their size.

DNA itself will not be visible in the gel. To view the separated DNA fragments, a fluorescent DNA-binding dye such as ethidium bromide is added to the agarose gel before it sets. The dye binds to DNA and fluoresces under ultraviolet light, showing a pattern of bands that can then be photographed. Each band on the gel contains millions of pieces of DNA of the same size. The bands can also be cut out and the DNA purified to yield a solution of DNA fragments of the required size.

The position of bands on an agarose gel depends on the size of DNA fragments in each band; the smaller the fragments, the further they move in a given time. To determine the size of a given piece of DNA, molecular biologists use standards called **molecular size markers**, or molecular weight ladders. These are pieces of DNA with a known number of base pairs. They are used to determine the size of the separated DNA fragments by comparing their location along the gel. Figure 3.15 shows four markers in the molecular marker (calibration) lane: 1700 bp, 1000 bp, 500 bp and 200 bp (bp = base pairs).

Science Source/Sigrid Gombert

Figure 3.14 A researcher loads genetic material from coral into an agarose electrophoresis gel apparatus.

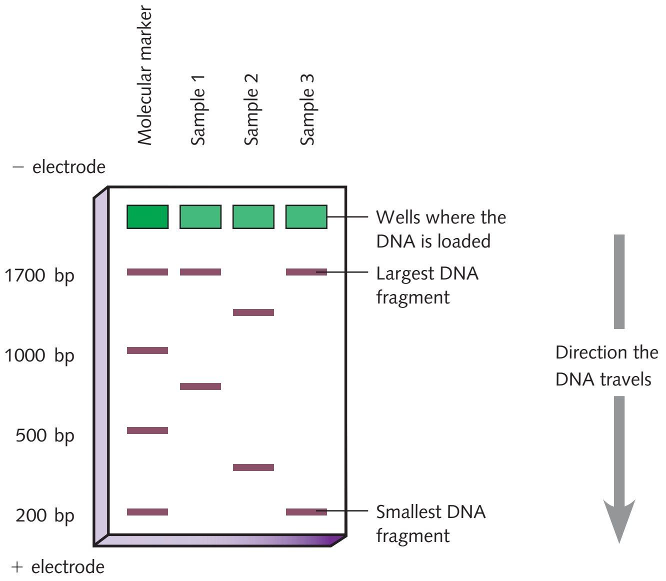

Figure 3.15 Standards are molecular markers of known size that are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel.

KEY CONCEPTS

- Gel electrophoresis separates DNA molecules by size.
- Negatively charged DNA travels through a gel matrix towards a positive electrode.
- DNA is visualised with a DNA binding dye.
- The sizes of DNA fragments can be estimated by comparing their movement through the gel with that of fragments of known sizes, referred to as molecular size markers.

Concept questions 3.5

1 What gives DNA its negative charge?
2 How can you estimate the size of a DNA fragment by gel electrophoresis?
3 Why is a standard used?
4 What does 700 bp mean?
5 Name two applications of DNA sequencing.

HOT Challenge

6 In gel electrophoresis, what is the main property of the DNA fragments (not the negative charge) that is used to separate the mixture into the different fragments?

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INVESTIGATION 3.1

Effect of restriction digestion enzymes on lambda DNA

Restriction digestion is the process of cutting DNA molecules into smaller pieces with special enzymes called restriction endonucleases (or restriction enzymes). These enzymes recognise specific sequences in the DNA molecule (e.g. EcoRI recognises GAATTC) wherever that sequence occurs in the DNA. Lambda DNA is a common DNA substrate extracted from a bacteriophage.

Aims

1 To use restriction enzymes to cut DNA into respective fragments
2 To analyse your restriction digestion by gel electrophoresis

Time requirement

55 minutes

Materials

Restriction digestion

- Lambda DNA (1 μg) (8 μL)
- Restriction digestion buffer (20 μL)
- EcoRI enzyme (1 μL)
- HindIII enzyme (1 μL)
- BamHI enzyme (1 μL)
- Sterile nuclease-free water (0.2 mL)
- 4 × 0.5 mL sterile microtubes
- Variable (5–50 μL) micropipette
- Microtube rack
- Variable (0.5–10 μL) micropipette
- Sterile pipette tips
- Water bath
- Micro centrifuge (optional)
- Lab coat
- Safety glasses
- Gloves
- Ruler

Electrophoresis

- TBE buffer (25 mL)
- 0.8% agarose gel with 2 μL Midori Green safe stain (for pre-staining technique)
- Loading dye (50 μL)
- Electrophoresis chamber (blueGel^{TM})
- Power supply 100 V (if using an alternative to blueGel^{TM})
- Blue light transilluminator (optional)

Note: the above measurements are based on using a blueGel^{TM} electrophoresis apparatus. If using an alternative electrophoresis chamber, increase TBE quantities based on chamber size.

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
TBE buffer can irritate the skin on contact.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

Restriction digestion

1 Label four 500 μL (0.5 mL) microtubes H, E, B and C, as shown in Figure 3.16.

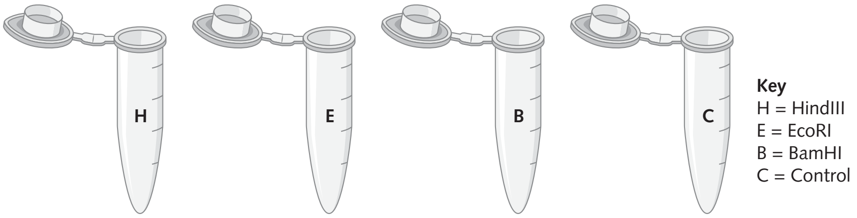


Figure 3.16 Label your microtubes H (HindIII), E (EcoRI), B (BamHI) and C (control).

2 Using a variable micropipette, add 42 μL of nuclease-free water to each of the microtubes.
3 Add 2 μL of lambda DNA to each of the microtubes.
4 Using a fresh micropipette tip, add 5 μL of buffer to each of the microtubes.
5 Using a fresh micropipette tip for each sample, add 1 μL of HindIII enzyme to microtube H, 1 μL of EcoRI enzyme to microtube E, 1 μL of BamHI enzyme to microtube B and 1 μL of nuclease-free water to microtube C.
6 Mix the samples thoroughly by pipetting up and down a few times until the solutions have an even consistency. Use a fresh micropipette tip for each sample. To collect the liquid at the base of the tubes, close the lids and spin them with a microcentrifuge.
7 Place the microtubes in a 37°C water bath for 10 minutes.

Analysing your digestion by gel electrophoresis

1 Remove the four microtubes from the water bath and add 10 μL of loading dye to each sample.
2 Mix the samples thoroughly by pipetting up and down a few times until the solutions have an even consistency. Use a different pipette for each solution. To collect the liquid at the base of the tubes, close the lids and spin them with a microcentrifuge.
3 Place the prepared 0.8% agarose gel into the gel electrophoresis chamber. Make sure that the wells are at the top or negative electrode section of the chamber.
4 Pour TBE buffer into the gel electrophoresis chamber. Make sure you completely cover the surface of the gel.
5 Using a sterile pipette for each sample, load 10 μL of each sample into the wells near the negative electrode and note the specific lanes in which the different samples were loaded.
6 Carefully place the lid on the gel chamber, turn it on and let the gel run for 30 minutes. Turn on the built-in blue light to visualise DNA band separation if using a blueGel™ electrophoresis chamber.

Note: If using a gel electrophoresis chamber that requires an external power supply, carefully plug the positive and negative electrodes into the gel box without dislodging the gel. The negative end should be connected to the end closest to the DNA samples. Plug in the power source (set at 100 V), turn it on and let the gel run.

7 After 30 minutes, turn the power supply off and visualise your results by either turning on the blue light or transferring to a blue light transilluminator.

Note: If you did not use the Midori Green stain, then DNA will not be visible until the gel has been soaked in methylene blue or equivalent for up to 24 hours.

Results

1 How many cuts did each restriction enzyme make?
2 Copy the results table into your logbook. Measure the distance in millimetres and fill in the results table.
3 Graph your results for the HindIII digest to determine the sizes of the EcoRI and BamHI digests.
4 Do those fragments add up to the size of lambda DNA? If not, provide possible explanation(s) as to why not.

Analysis of restriction digests of DNA

HindIII		EcoRI			BamHI		
Distance (mm)	**Size (bp)**	**Distance (mm)**	**Calculated bp**	**Size (bp)**	**Distance (mm)**	**Calculated bp**	**Size (bp)**
	23 130			21 226			16 841
	9416						
	6557						
	4361						
	2322						
	2027						

Discussion

1 Why was 1 μL of nuclease-free water added to microtube C in step 5?
2 Why did you incubate the restriction digests at 37°C?
3 What is the purpose of the dye?
4 What would occur if the gel electrophoresis chamber was filled with distilled water instead of TBE buffer?
5 Explain why DNA samples must be loaded at the negative end of a gel electrophoresis chamber.
6 What would occur if the electrodes in the electrophoresis chamber were reversed?

Conclusion

Write a conclusion for your investigation, including a short discussion of your results.

Taking it further

Investigate where restriction enzymes are used and how they assist in medical disease diagnosis.

WB
3.6
DNA PROFILING
PAGE 70

3.6 DNA profiling

Most DNA is identical from one person to the next apart from the different alleles for characteristics such as eye and hair colour. However, some regions in our genome show high variability – called **polymorphisms**. Each of us inherits a unique combination of polymorphisms from our parents, which can be examined to create a DNA profile. In **DNA profiling**, DNA is extracted from a sample of body fluid, skin cells, hair root or blood. The amount of DNA obtained is usually very small so amplification of the DNA is necessary by the process of PCR (polymerase chain reaction) to obtain millions of genetically idetical copies of the DNA target segment. This can then be used to carry out the process of gel electrophoresis to sort the DNA fragments for DNA profiling. The segments that are amplified and sorted in a gel are often short tandem repeats (STRs) which are discussed later. DNA profiling is used to help solve crimes, determine family relationships and identify human remains and disaster victims.

Solving crimes

Television shows tend to oversimplify and exaggerate the scientific processes involved in forensic investigations. However, DNA profiling is a crucial scientific technique applied to solve crimes. We leave cells behind everywhere we go, so at a crime scene typical pieces of evidence include skin cells and hair.

DNA profiles from samples obtained from crime scenes, convicted offenders, items from missing people and unidentified bodies are added to DNA databases. DNA databases can help to solve crimes, including cold cases, and identify missing people, disaster victims or human remains. The first example of a DNA database was in 1986, after a young woman, Dawn Ashworth, was murdered in Leicestershire, UK. In the investigation, police collected blood samples from the males in the town (about 4000 men). At the time, this was an informal database, and the police applied DNA fingerprinting to try and solve the crime. No match was found. However, a man was overheard saying that he had been paid to provide false samples for another man, Colin Pitchfork. When Colin Pitchfork's DNA was collected and analysed, it matched that found at the crime scene. He was arrested in 1987.

Today, most developed countries have a DNA database. The Australian National Criminal Investigation DNA Database (NCIDD) managed by CrimTrac was established in 2001 and has more than 1.2 million DNA profiles. Although DNA databases are valuable, especially in solving crimes, their use raises ethical questions, especially about privacy. For example, do the benefits to society outweigh a person's right to privacy in having their DNA profile stored? Should everyone give a DNA sample? How long should information from a person's DNA profile be stored?

Determining family relationships

DNA profiling can be used to determine if people are related to each other. Paternity testing usually aims to determine if someone is the biological parent of an individual. In New York, there are buses similar to the one shown in Figure 3.17 – mobile units for collecting DNA samples from citizens wanting to establish relatedness. Demand for tests in the US to establish parentage is estimated at 500 000 per year. People using the service have discovered that they have half-sisters or half-brothers in other parts of the country. In other cases, men have discovered that they are, or are not, the fathers of their children.

There are no mobile clinics in Australia, but more people are having DNA tests to establish the parentage of their children. Men may want to confirm that they are the father of their children. Women may want to ensure they came home from hospital with the right baby or confirm the father of their child. More than 10 000 tests are ordered from laboratories registered in Australia every year. It is a simple process. A DNA kit is ordered online so that DNA samples of parents and children can be collected in the home and mailed to DNA testing laboratories. The results arrive in the mail a few weeks later.

There are concerns about using the tests, including the:

- possibility of contamination when collecting DNA
- interested party not knowing how to interpret the results of the test
- issue of consent if DNA is collected without a child's or parent's knowledge.

Occasionally the issue of paternity arises in cases where child support is being sought from a man who claims he is not the father of a child. In such cases, the court orders a DNA test to be conducted if paternity cannot be determined in any other way and if evidence places the paternity in question. Legal tests are required to comply with the Family Law Act, so samples must be collected and tracked from a registered collection centre and sent to specified testing laboratories. The results of non-legal tests are not admissible in a court of law.

Shutterstock.com/Tommy Liggett

Figure 3.17 A DNA bus in New York is popular with people who want to establish relatedness, including legal paternity.

Identifying human remains and disaster victims

After a disaster, such as an aeroplane crash, where there are unidentified victims, family members will voluntarily submit a DNA sample of their own to reduce the time taken to identify bodies by DNA profiling. This approach was used after the 2014 Malaysia Airlines plane crash in the Ukraine to identify the bodies of victims from countries all over the world.

Short tandem repeats construct DNA profiles

In Chapter 2, you learned that not all your DNA codes for polypeptides – 99% of the genome is non-coding. Non-coding regions of the genome contain inherited sequences of DNA that vary between individuals (polymorphisms). The polymorphisms used to construct a DNA profile for an individual are **short tandem repeats (STRs)**. STRs are segments of DNA that contain repeats of 2–6 nucleotides in tandem, such as AGATAGATAGATAGATAGAT. The human genome has many of these STRs at fixed locations on our chromosomes.

The number of repeats we have at each STR region is how we vary from one another. Each person has two copies (alleles) of each STR region, one inherited from their mother and one inherited from their father. Each can have a different number of repeats at the same region, increasing the specificity of a person's profile. The information can be entered into a forensic database, and used to indicate the number of repeats in two alleles. For example, in Figure 3.18, the numbers recorded would be 5, 10 and 12, 7. STR loci refers to the STR locations on a chromosome. What makes DNA profiling such a powerful tool is that multiple STR loci are examined simultaneously to obtain a profile, which is then compared with a reference sample. For example, a profile obtained from the DNA at a crime scene would be compared with samples from a database or a suspect. Similarly, a profile obtained from unidentified human remains could be compared with a sample obtained from a family member.

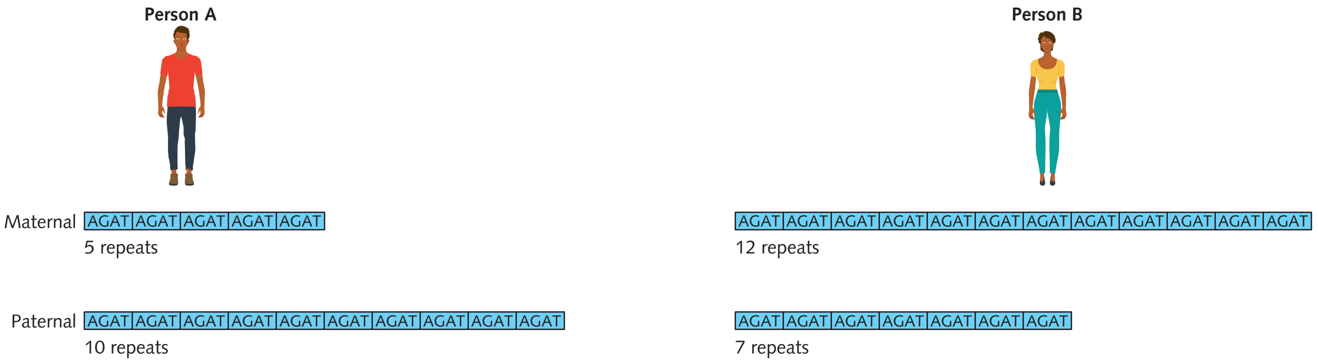
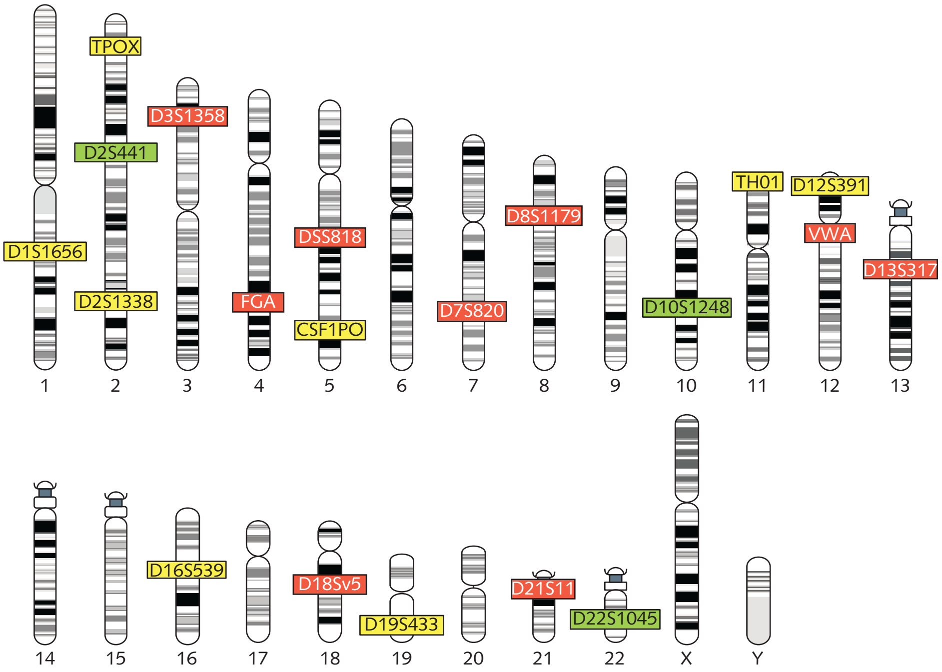


Figure 3.18 The number of repeats at each STR loci varies from person to person.

In modern DNA analysis, a minimum of 10 gene loci (called CODIS) are used in forensic analysis, with 13 used in 2017, and increased to 20 by 2021. If only a small number of STRs at different gene loci are used in forensic analysis, there is a chance that two people might have the same DNA profile. With todays technology using a greater number of gene loci, the chance is greatly reduced and the chance of correctly identify the culprit is greatly increased.

Different countries use different STR DNA profiling systems, based on the population size of that country. For example, the US originally used 13 loci, while Australia used nine. In 2013, Australia increased the number to 18. In 2017, the US added an additional seven STR loci for a total of 20 STR markers. In both countries, this includes the Amelogenin gene (although it is not an STR), which is located on the X and Y chromosomes and used as a gender marker. The gender of a person can be determined from this marker because on the X chromosome the Amelogenin gene is six base pairs shorter than on the Y chromosome. The STR loci used in Australia are shown in Figure 3.19.



Genome Research Limited https://www.yourgenome.org/facts/what-is-a-dna-fingerprint CC-BY 4.0 https://creativecommons.org/licenses/by/4.0/

Figure 3.19 **Chromosomal location of the 17 STRs (not including Amelogenin gene) used to construct a DNA profile in Australia. Those shaded in pink are the original nine STR loci used in Australia. Those shaded in yellow are the eight STR loci added in 2013. Green shading represents the additional STR loci used in the US but not in Australia.**

How DNA profiles are produced today

Today, DNA profiling does not use restriction enzymes as in the original DNA fingerprinting method. Rather, it uses the PCR reaction to generate many copies of specific STR regions for analysis.

Type of DNA used to obtain profiles

First, DNA is extracted from the samples, usually skin cells, hair roots, cheek swabs (from the inner cheek) or blood. Two types of DNA can be used for DNA profiling. Commonly, genomic DNA is used (chromosomal DNA, making up most of a person's DNA). However, if there is not enough nuclear DNA available to create a profile, mitochondrial DNA (mtDNA), which can be more resistant to degradation, is used. Mitochondria are the cell organelles responsible for energy production. They contain a single circular chromosome inherited from the mother. There are several hundred mitochondria in each cell and so there might be hundreds or thousands of copies of mtDNA per cell. Because it is maternally inherited, mtDNA is identical between siblings and maternal relatives.

Amplification and visualisation of STRs

Once DNA has been extracted from a sample, PCR is used to amplify the specific STR sequences. PCR amplification is useful when analysing DNA from crime scenes, where only a small amount of DNA might be recovered.

As you learned on page 93, primers bind to complementary sequences of DNA and mark the starting point from which DNA polymerase will add new nucleotides. The primers used in the PCR reaction are designed to amplify the STR sequences of interest for profiling. Primers for each STR are tagged with a specific fluorescent molecule.

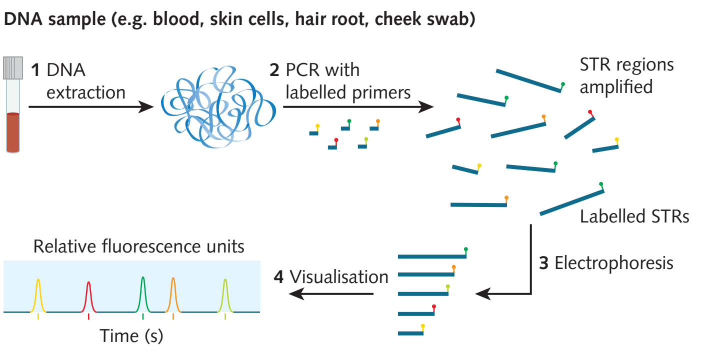


Figure 3.20 1–3: The steps involved in obtaining an electropherogram: DNA extraction is followed by PCR and electrophoresis. 4: The vertical axis (fluorescence) of the electropherogram is a measurement of the amount of DNA present. The horizontal axis (time) identifies the size of the STR alleles.

Once the STR sequences have been amplified, they are separated according to size by electrophoresis. Lasers are then used to excite the fluorescent molecules attached to the primers. This enables visualisation of the PCR-amplified DNA fragments that have fluorescent primers incorporated. Fluorescence measurements are collected by a detector and converted to a series of coloured peaks called an electropherogram. From this, the length of each STR sequence can be determined to give the DNA profile. This process is illustrated in Figure 3.20.

An example of an electropherogram is shown in Figure 3.21. Each peak represents an allele and, because there are two chromosomes, each individual has two alleles at each STR locus. For example, in this individual, at the STR locus D5S818, one allele has 11 repeats and the other allele has 12 repeats. This individual has two identical alleles of 11 repeats at D16S539. As the two alleles of 11 repeats at D16S539 are exactly the same length, they occur at exactly the same position on the electropherogram. The height of the peak (amount of DNA) is double the height as if only one allele was present because there is twice the amount of DNA present.

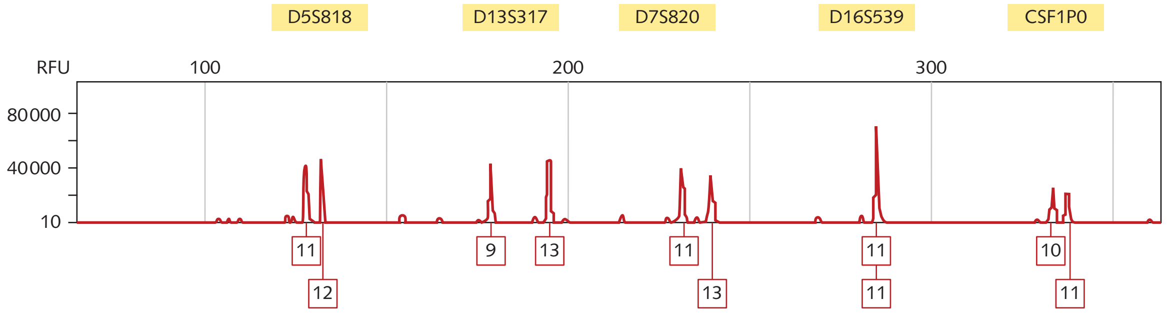


Figure 3.21 An electropherogram of five STRs

ACTIVITY 3.1

DNA profile analysis

Aim

To analyse DNA profiles and consider issues surrounding use of this technology

Task 1: Paternity case study 1

A legal paternity test was ordered by a judge to determine whether a man should pay child support. A DNA sample was collected from the mother, the child and the man. Each DNA sample was treated separately to produce a DNA profile for each individual. DNA was extracted from cells and PCR was used to amplify chosen STR regions, using sequence-specific primers. These primers are complementary to sequences of DNA that flank the STR region. Figure 3.22 shows the THO1 alleles inherited by an individual. Each individual has two alleles for each STR, one inherited from each parent. This individual has inherited the alleles THO1-6 (THO1 allele with 6 repeats of TCAT) and THO1-5 (THO1 allele with five repeats of TCAT). This individual's genotype can be written as THO1-5,6.

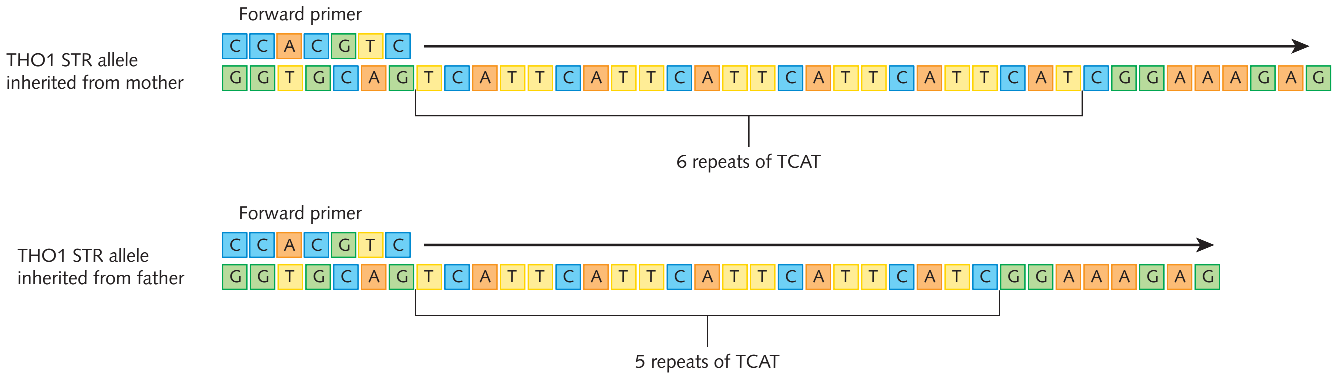


Figure 3.22 The THO1 alleles for an individual can be amplified using polymerase chain reaction (PCR). Forward and reverse primers are designed to complement sequences flanking the STR region so the specific STR region is amplified. Forward primers are shown here.

After PCR has been performed, the DNA samples are subjected to gel electrophoresis. Table 3.3 provides results for the STRs inherited by the child, mother and possible father. Only three STR regions have been amplified for each individual. Table 3.2 lists the STRs used in DNA profiling and the known alleles of these STRs. This information can be compared with the STRs inherited by the child, mother and possible father to determine paternity.

Use the data in Tables 3.2 and 3.3 to calculate the length of DNA fragments generated after PCR for each individual. Then construct an image of the resultant gel electrophoresis run for each individual. Label your gel to indicate which individual's DNA profile is shown in each lane.

Questions

1 What can you tell the judge presiding over this court case?
2 Can you be certain of the results of paternity using this test?
3 What might increase the certainty of these results?

Table 3.2 Variations found in STR loci used in DNA profiling

STR name	Locus	Repeat	Number of known alleles of this STR	Variation in number of repeats that can be found (known alleles of this STR)	Variation in length of DNA fragments (nucleotides)
TPOX	2p25.3	GAAT	15	4–16	12–64
D5S818	5q23.2	AGAT	15	7–18	28–72
CSF1PO	5q33.1	TAGA	20	5–16	20–64
D7S820	7q21.11	GATA	30	5–16	20–64
THO1	11p15.5	TCAT	20	3–14	12–56
D13S317	13q31.1	TATC	17	5–16	20–64

Table 3.3 The STR alleles inherited by three individuals (results shown for three STR regions only)

Individual	STR alleles inherited		
Child	TPOX-4,12	D5S818-7,7	THO1-5,14
Mother	TPOX—12,16	D5S818-7,12	THO1-3,14
Man who the mother claims is the father	TPOX-4,9	D5S818-7,10	THO1-5,6

Task 2: Paternity case study 2

A man (male 2) returned home to Australia after working overseas for 10 years. On his return he became aware that the girlfriend he had dated up until leaving had a 10-year-old son. He suspected that he might be the biological father of this child. However, the mother was happily married to another man (male 1), who she claimed was the biological father of the child. Male 2 ordered a DNA testing kit and managed to secretly collect a DNA sample from the child, mother and husband. He sent these for testing, along with his own DNA. A gel with seven STR regions is shown in Figure 3.23. The STRs inherited by the son from his mother are highlighted.

Questions

1. Use the gel results to determine which male is the biological father.
2. Is it ethically sound to order DNA tests without the consent of all individuals concerned?
3. Can you think of issues that may emerge given the results of this test?

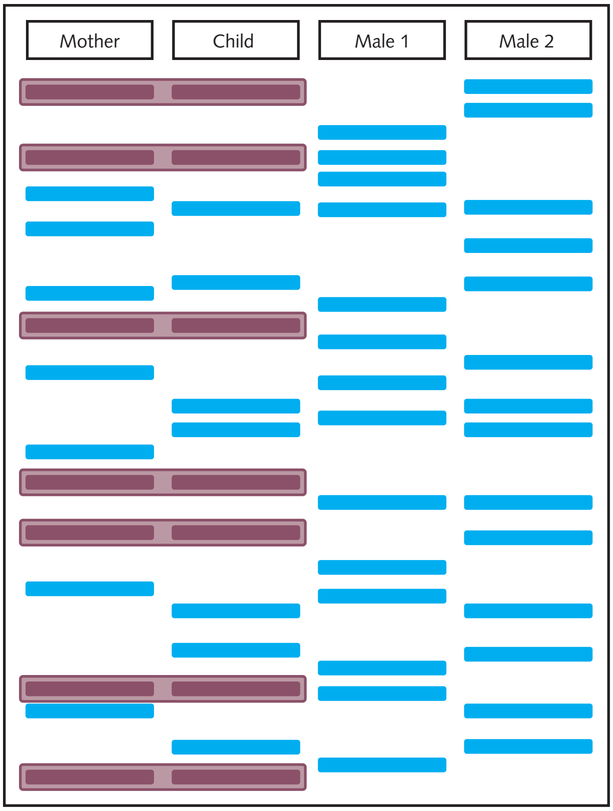


Figure 3.23 This gel electrophoresis run reveals the DNA profiles of the four individuals in case study 2. Primers for seven STR regions were used to generate these DNA profiles.

KEY CONCEPTS

- DNA profiling is used to solve crimes, determine family relationships and identify human samples.
- By examining STRs in non-coding regions of DNA, a profile can be obtained that can be compared to a database of samples given by family members.

Concept questions 3.6

1. What samples of human organisms can be collected for DNA profiling?
2. Polymorphisms are regions in our DNA of high variation. What does this mean?
3. STR is an acronym. What do the letters stand for and what do STRs mean?
4. How are STRs used to perform the process of DNA profiling?
5. There are standard STR markers used for DNA profiling in various countries. How does this enable DNA profiling to occur if the whole genome is not being examined?

HOT Challenge

6. How easy is it to challenge DNA profiling? List the reasons for and against as to how one could do it or not.

3.7 Recombinant plasmids and human insulin

Biotechnology applications are used to produce recombinant insulin to treat people with diabetes. Insulin is a peptide hormone that promotes uptake of sugar from the bloodstream and its storage in muscle or adipose tissue. Insulin is essential for normal metabolism; without insulin, the body relies on fat as an energy source. This can result in the build-up of dangerous substances in the blood, which can be life-threatening.

Type 1 diabetes is an autoimmune disease in which the insulin-producing cells of the pancreas come under attack from the body's own immune system and are unable to produce sufficient insulin (Figure 3.24). People with type 1 diabetes must inject insulin up to four times a day. Before insulin became available, people who had diabetes would likely die.

In 1889, German researchers Oskar Minkowski and Joseph Von Mering discovered that when they removed the pancreas from dogs, the dogs developed symptoms of diabetes. In the years that followed, the chemical responsible for diabetes, insulin, was described. In 1921, Canadian scientists Frederick Banting and Charles Best removed insulin from the pancreas of a dog, and used this insulin to keep another dog with diabetes alive. Soon after this striking observation, Frederick Banting and Charles Best worked together with another Canadian scientist, John Macleod, to purify insulin. Insulin was further purified by James Collip. In 1922, a 14-year-old boy in Toronto dying from diabetes became the first person to receive insulin injections, which was eventually successful. This work led to the 1923 Nobel Prize in Physiology or Medicine being awarded to Frederick Banting and John Macleod.

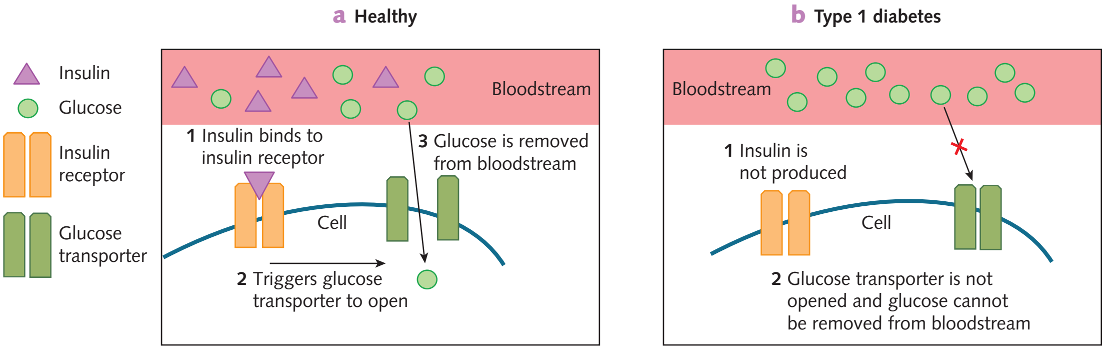


Figure 3.24 a Under healthy conditions, insulin binding to the insulin receptor activates glucose transporters to uptake glucose from the bloodstream into cells, maintaining healthy glucose levels. b In type 1 diabetes, insulin is not produced and glucose cannot be taken up by cells from the bloodstream (right).

Recombinant DNA technology: a game changer for insulin production

The first synthetic human insulin was produced in 1978 by using *E. coli* bacteria. Bacteria can be grown quickly and in large batches in controlled environments. Bacterial cells can be lysed (ruptured) and proteins extracted from their cytosol. Genetic engineering of *E. coli* has allowed the human insulin gene to be inserted into bacteria, replicated to vast numbers as the bacteria replicate, and expressed by the bacteria to produce the functional insulin protein. Insulin is then extracted, purified and distributed as the injectable therapy that is a lifesaver for many people worldwide. This process is summarised in Figure 3.25.

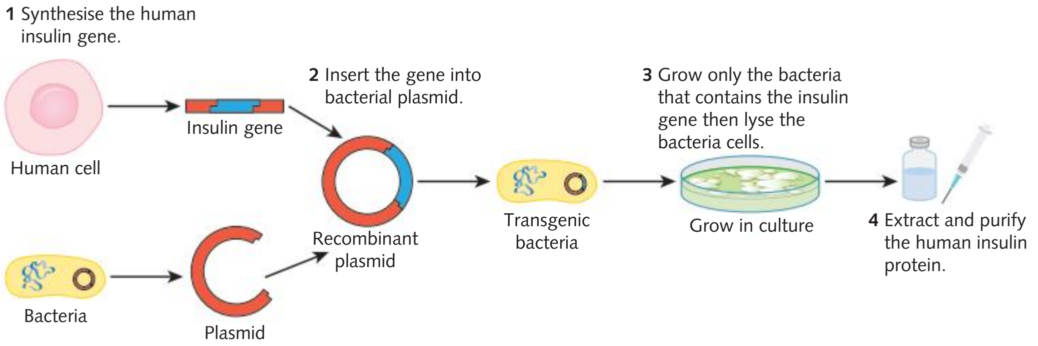


Figure 3.25 The steps involved in producing insulin in bacterial cells

In 1982, Humulin became the first approved product made by recombinant DNA technology. Today, three kinds of Humulin are used to manage diabetes (Figure 3.26). Each kind acts over a specific amount of time, which is affected by factors such as diet and exercise. Humulin R U-100 (100 units of insulin per millilitre) provides a short-acting insulin peak, which manages increased blood sugar levels that occur after a meal (within 30 minutes). Humulin N has a longer period of activity, and is used to manage blood sugar levels between large meals (e.g. breakfast and lunch) or overnight. Humulin 70 combines the short effects of Humulin R U-100 with the longer lasting 'intermediate' Humulin N to manage blood sugar levels after meals, throughout the day and during the night. People with diabetes work with their doctor to find the best type of insulin to manage their blood sugar levels.

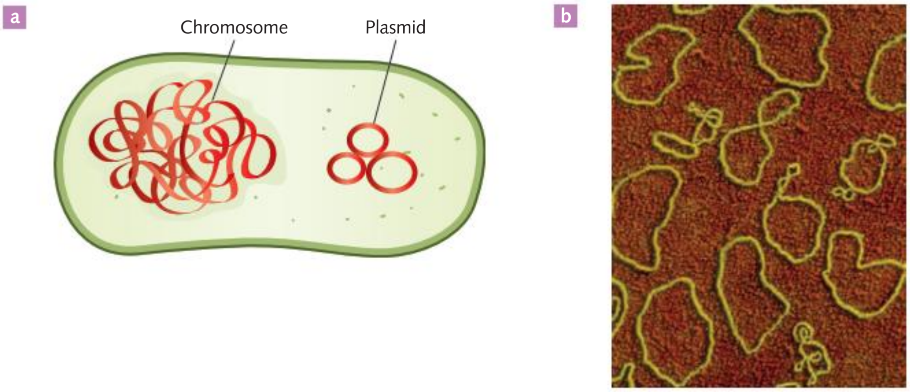
Science Photo Library/Sue Trainor/Scott Camazine

Figure 3.26 Humulin N and Humulin R U-100 are two of the three kinds of Humulin that are used to manage type 1 diabetes. The other kind is a mix of the two.

WB 3.7.1 RECOMBINANT PLASMIDS PAGE 73

Recombinant plasmids

Before the human insulin gene can be used in genetic engineering, it must usually be copied, or amplified, to produce enough to work with; for example, by PCR. Short linear DNA fragments produced by PCR or cut out from a chromosome by restriction endonucleases are unstable. They do not survive long in cells or a test tube and can lose base pairs from the ends through enzymatic or mechanical degradation. They are also often too small to manipulate in the laboratory. For this reason, scientists use bacterial plasmids – circular pieces of DNA that reproduce independently of the bacterial chromosome (Figure 3.27).



Alamy Stock Photo/Science Photo Library

Figure 3.27 **a** A bacterial cell containing bacterial DNA and plasmids; **b** a transmission electron micrograph of bacterial plasmids from *Escherichia coli*

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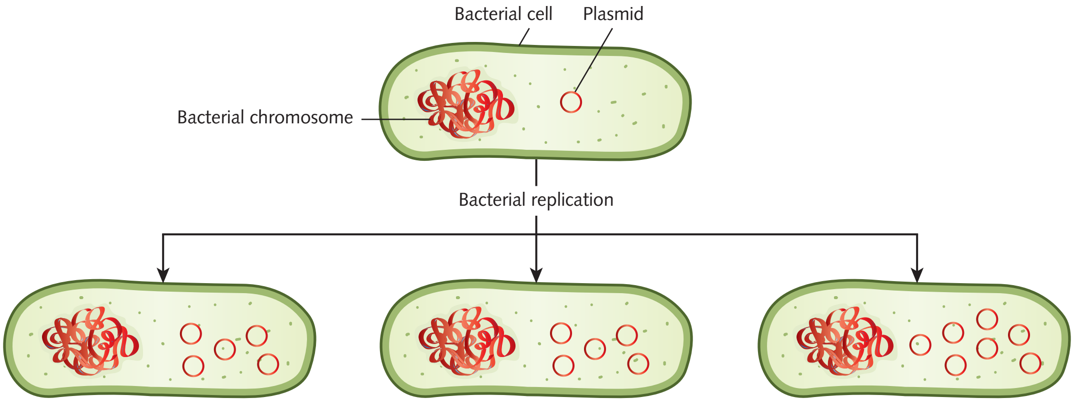


Figure 3.28 **Plasmids are copied when bacterial cells replicate.**

DNA fragments, such as PCR products amplified from a gene of interest, can be inserted into plasmids that have been cut open, and then the plasmid can be closed again by ligases, incorporating the DNA fragment. A plasmid that has incorporated a DNA fragment is called a **recombinant plasmid**.

In biotechnology, plasmids are ideal for DNA fragment **vectors** (the plasmid will carry the DNA or gene into a bacterium). Plasmids are copied many times within the bacterial cells and are copied when the bacteria replicate, and this also copies any DNA fragments inserted into them (Figure 3.28). Because they are circular, plasmids are much more stable than linear fragments. Their stability also allows them to survive the harsh conditions that are used to rupture the bacterial cells and purify the plasmid DNA. They are small enough to be distinguished from the main bacterial chromosome, but large enough to be extracted and manipulated in the laboratory. Plasmids can also be easily engineered to carry a number of different genes or DNA elements such as promoters and restriction sites, making them ideal tools for manipulation of DNA fragments. Promoter regions on plasmids can allow genes to be expressed in prokaryotes or eukaryotes depending on the application.

An alternative to using PCR to generate many copies of a DNA sequence is to insert the DNA into bacteria. This process is called **gene cloning** and it has many advantages. Gene cloning allows replication of larger segments of DNA, and it permits the analysis of any gene and associated proteins encoded in the DNA sequence in an environment where they are active.

Plasmids are used to insert DNA into the bacteria. The key to using plasmids as DNA copiers lies in our ability to incorporate foreign genes into plasmid DNA and in their ability to replicate in bacteria. A number of steps are involved in this process (Figure 3.29).

1. Plasmids are extracted from bacteria by rupturing the cell walls. A restriction enzyme is used to cut the plasmid DNA to produce sticky or blunt ends.
2. The same restriction enzyme is used to cut the DNA of the gene to be inserted so that both pieces of DNA have complementary sticky ends.
3. DNA ligase binds the ‘foreign’ DNA fragment into the plasmid DNA. After binding, the DNA fragment becomes a permanent part of the recombinant plasmid.
4. The recombinant plasmids are added to a bacterial culture. They are taken up by some bacteria, in which they replicate. In the normal process of growth and division, bacteria replicate the plasmid, and thus numerous copies of the incorporated foreign DNA are made.

Only a small percentage of the bacteria take up the recombinant plasmids; others simply seal up without taking up the plasmid. The process of bacteria taking up the plasmid is called **transformation**. After transformation, the bacterial cells that contain recombinant plasmids have to be isolated from most of the cells in the colony, which have not taken up plasmids.

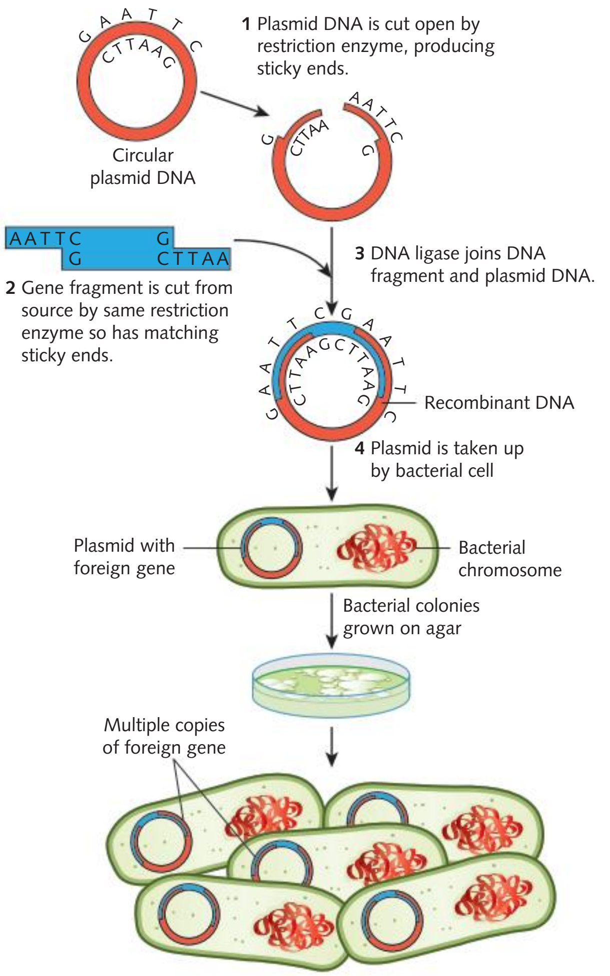


Figure 3.29 **Transformation: a foreign gene is inserted into plasmid DNA to produce a recombinant plasmid. This is introduced into bacteria, where it can make multiple copies of itself. When bacteria take up the plasmid, they are transformed. Note: the plasmid is not normally as large as shown here. It has been magnified in this diagram.**

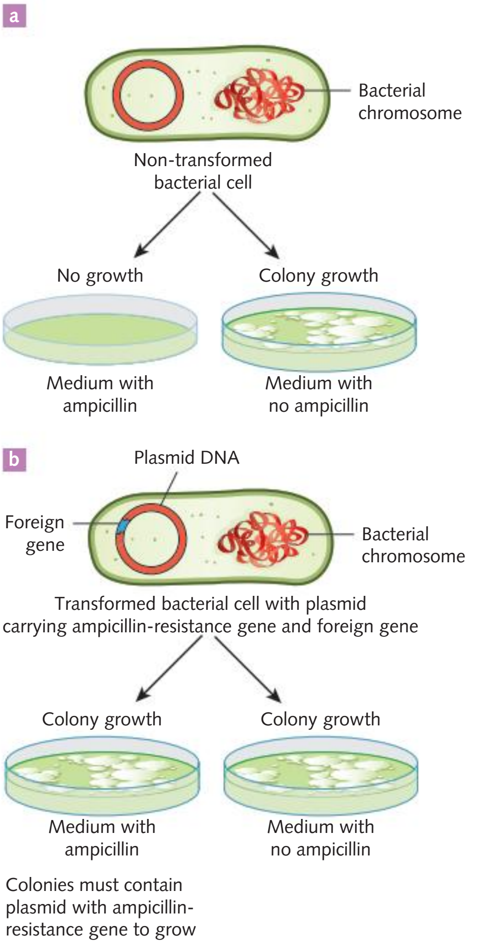


Figure 3.30 **Antibiotic selection of transformed bacteria. a Non-transformed bacteria cannot grow on media supplemented with ampicillin, but grow well on normal media. b Transformed bacteria can grow equally well on either medium. The plate without ampicillin provides a positive control condition.**

Plasmid DNA often contains genes for resistance to an antibiotic, such as ampicillin. Bacteria that have been transformed with the plasmid can grow and multiply on a medium that is supplemented with ampicillin because they are resistant to it. The bacteria without the plasmid do not grow because they are sensitive to the antibiotic ampicillin (Figure 3.30). This process is called **antibiotic selection** and is an important component of many biotechnology techniques.

Plasmids are very useful vectors in genetic engineering. Vectors in this context are agents that can deliver a piece of foreign DNA into a host cell. Other types of vectors include recombinant viruses and liposomes, which are synthetic spherical vesicles encased by a phospholipid bilayer that can encapsulate the DNA to be delivered.

The bacteria with antibiotic resistance are then selected and grown in culture. To study the gene of interest, scientists isolate and analyse the plasmids. This technique of bacterial transformation is also used to insert genes that code for useful proteins into bacteria so that the bacteria will then make the protein for human use.

Transformation is also used to produce human growth hormone, which is used to treat people with a certain form of dwarfism. Before this technique, the hormone was extracted from the pituitary glands of human corpses.

Developed by Southern Biological

INVESTIGATION 3.2

Bacterial transformation

DNA can mutate spontaneously or after an error occurs in DNA replication. Biotechnologists have developed methods of controlled DNA mutation, such as intentionally mutating DNA to alter how the cell behaves. It is also possible to transfer DNA from one organism into another. This method, called genetic transformation, uses an engineered molecule of DNA to transfer a gene or genes from one organism to another so that the organism is capable of producing the protein encoded by the transformed gene.

Aim

To perform a bacterial transformation using the green fluorescent protein plasmid pGreen

Time requirement

50 minutes

Materials

- *E. coli* MM294
- pGreen plasmid (10 µL)
- 2 agar plates containing Luria broth
- 2 agar plates containing Luria broth with ampicillin
- Sterile Luria broth (10 mL)
- Sterile 50 mM $CaCl_2$ (10 mL)
- Sterile water (20 mL)
- 2 sterile transformation tubes
- 3 sterile 1 mL transfer pipettes
- 10 µL micropipette
- 3 sterile disposable inoculation loops
- 4 sterile disposable inoculation spreaders
- Sterile tips for 10 µL micropipette
- Water bath
- Ice bath
- Fine point marker pen
- Stopwatch
- Test-tube rack
- Adhesive tape (to seal plates)
- Thermometer
- Incubator
- Lab coat
- Safety glasses
- Gloves
- Disinfectant
- UV light

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Some bacteria may cause disease, so assume them to be pathogenic. (Note: *E. coli* MM294 is a commonly used laboratory strain of *E. coli* and is safe to use in schools.)	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash your hands thoroughly at the end of the investigation. Decontaminate benches before and after the investigation. Flood any spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open agar plates once they are securely taped. Dispose of agar plates appropriately after autoclaving.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach, wipe off the residue. Ensure your lab coat sleeves are rolled down and wear gloves.

Method

Note: To use aseptic technique, wipe your bench down with ethanol (or bleach), and keep your work near the Bunsen burner to waft potential contaminants away from your materials.

Preparing the transformation solution

1 Label the transformation tubes '+' (+ plasmid) and '–' (– plasmid) (Figure 3.31). Keep the tubes cold by placing them upright in the ice bath. Keep the tubes capped at all times except when in use.

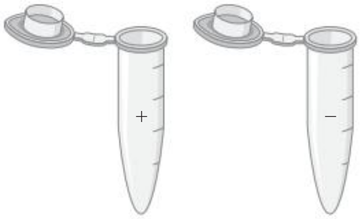


Figure 3.31 Label the transformation tubes '+' and '–'.

2 Add 250 µL (0.25 mL) of ice cold $CaCl_2$ solution to each transformation tube, using a sterile transfer pipette. Maintain the temperature by placing the tubes back into the ice bath.

Suspending the bacteria

1 Use a sterile inoculation loop to transfer a single colony of *E. coli* from the starter plate to the ice cold $CaCl_2$ solution in the '+ plasmid' transformation tube. To dislodge the *E. coli* cells from the loop, spin the loop rapidly in the solution. Check whether your *E.coli* has transferred successfully – it should be visible in your tube.
2 Suspend the *E. coli* in the $CaCl_2$ solution by drawing the solution in and out of a pipette by squeezing and releasing the bulb several times. Do not incorporate air bubbles in the liquid or allow any liquid to splash up the sides of the tube. The solution should start to become milky white as the cell mass is suspended. To check that there are no lumps or particles in the tube, hold it up to the light; then return the tube to the ice.
3 Repeat steps 1 and 2 to transfer a single colony of *E. coli* from the starter plate to the ice cold $CaCl_2$ solution in the '– Plasmid' transformation tube.

Adding the plasmid

1 Your teacher or lab technician will bring the plasmid to your workstation. Using a micropipette, transfer 10 µL (0.01 mL) of plasmid solution to the '+ plasmid' transformation tube. Add the plasmid directly to the liquid in the tube without allowing it to touch the sides.
2 Immediately return the tube to the ice bath and mix the plasmid into the bacterial suspension by placing a sterile inoculation loop into the liquid and rapidly spinning it. Incubate the two tubes on ice for 15 minutes.
3 Label the two agar plates containing Luria broth 'LB + plasmid' and 'LB – plasmid'. Label the two agar plates containing Luria broth with ampicillin 'LB/amp + plasmid' and 'LB/amp – plasmid' (Figure 3.32).

Heat shock

1 After 15 minutes of incubation, remove the two tubes from the ice bath and transfer both of them to a warm water bath (42°C). Hold them in the bath for 90 seconds and make sure the tube caps do not become fully submerged in the water. Gently agitate the tubes while they are warming up in the water.
2 Immediately transfer the tubes to the ice bath when the time is up. Allow the tubes to rest in the ice bath for at least 1 minute before continuing.

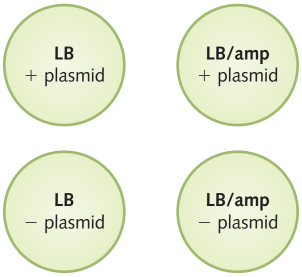


Figure 3.32

Recovery

1 Using a sterile plastic pipette, transfer 250 µL (0.25 mL) of Luria broth to each tube. Mix the liquids at the base of each tube by gently grasping the top and tapping the base with your finger.
2 Allow the tubes to recover for 10 minutes in a microtube rack at room temperature.

Plate inoculation

1 Use a sterile plastic pipette to transfer two drops of liquid from the '+ plasmid' tube to the 'LB + plasmid' plate. Use a sterile spreader to quickly spread the liquid evenly over the plate.
2 Use a sterile plastic pipette to transfer two drops of liquid from the '+ plasmid' tube to the 'LB/amp + plasmid' plate. Use a sterile spreader to quickly spread the liquid evenly over the plate.
3 Use a sterile plastic pipette to transfer two drops of liquid from the '– plasmid' tube to the 'LB – plasmid' plate. Use a sterile spreader to quickly spread the liquid evenly over the plate.
4 Use a sterile plastic pipette to transfer two drops of liquid from the '– plasmid' tube to the 'LB/amp – plasmid' plate. Use a sterile spreader to quickly spread the liquid evenly over the plate (Figure 3.33).

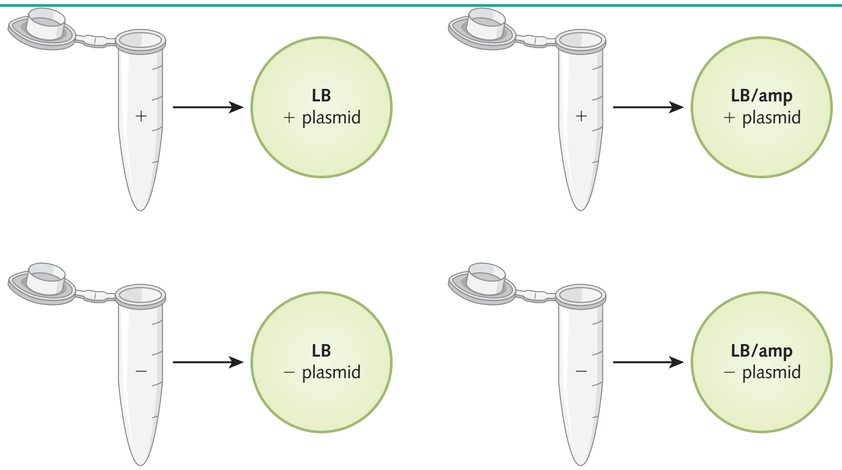


Figure 3.33

5 Secure the lid of each Petri dish to its base with tape. Leave the plates to rest on the bench for 5 minutes and then place them upside down (agar on top) in a 33°C incubator for 24–36 hours. Inspect the growth after this time. You should see either a bacterial lawn, single colonies, or no growth on the individual plates. Take the plates into a dark room to observe evidence of fluorescence in the transformed colonies. Use of a UV light may enhance the fluorescence.
6 To count the number of individual colonies, mark the lid of the Petri dish above the colony with a marker as you count it. If cell growth is too dense to count individual colonies, mark that plate as a lawn. Record your results in the results table.

Results

1 Copy the results table into your logbook. Record the results of your experiment in the table.

Bacteria colony results

Plate	Result
LB + plasmid	
LB – plasmid	
LB/amp + plasmid	
LB/amp – plasmid	

2 What growth and phenotypes can you observe?
3 Describe what you see on your plates when you look at your plates under UV light.

Discussion

1 Why is the plasmid solution placed on ice for 5 minutes?
2 Which plate is the control in this experiment? Explain your answer.
3 Explain the function of the Luria broth.
4 What is the purpose of incubating the cells at room temperature?
5 Explain what a plasmid is.
6 Explain how the DNA plasmid is put in the bacteria. What is the advantage of being able to do this? (Consider what the plasmid DNA allows the bacteria to do.)
7 Explain how we are able to identify that the plasmid DNA is in the bacteria.

Conclusion

Write a conclusion of your investigation, including a short discussion of your results.

Taking it further

Investigate how genetic engineering and bacterial transformation enables the advancement of medical treatments for example in insulin creation.

Production of human insulin

Human insulin is a small protein made up of two separate polypeptide chains: the A chain and the B chain. Each chain is coded for by a different gene. Once the two chains are synthesised, they join together by two disulfide bonds to form a functional insulin molecule.

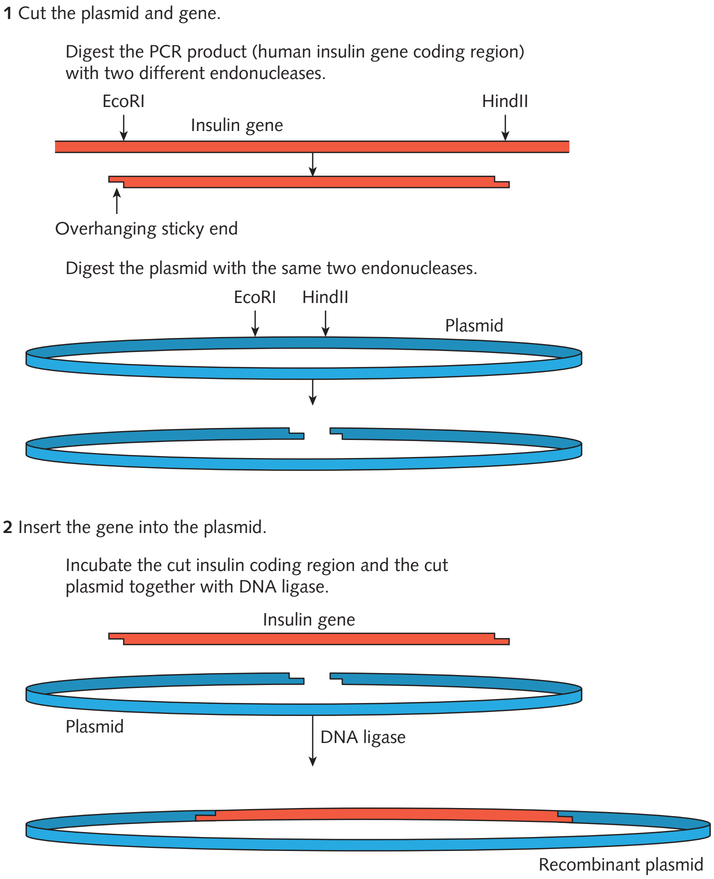
One of the main methods of commercial production of human insulin involves the insertion of the two genes for the two different polypeptide chains into two different plasmids. Each chain is produced separately in different cultures, however use the same bacterial species. The insulin genes are inserted into the cut plasmid next to another inserted gene, this time for β-galactosidase protein. The gene for the protein β-galactosidase has had the STOP codon removed. This gene is used to detect successful insertion and assists in gene regulation, enabling the two genes (β-galactosidase and insulin) to be seamlessly translated together to make a fusion protein. Once produced, the insulin protein is removed from the fusion protein. Expression of each gene from the two bacterial cultures, from bacteria that were successfully transformed, results in the two different polypeptide chains being produced. The proteins are then extracted, purified and mixed together to produce functional insulin.

WB
3.7.2
SYNTHESISING THE HUMAN INSULIN GENE
PAGE 74

Preparing the insulin genes for insertion

WB
3.7.3
PLASMID X
PAGE 75

Before inserting the DNA fragments containing the insulin coding sequences of the two genes, A and B, into a plasmid, the human insulin genes must be copied and produced in adequate quantities to be manipulated. This can be done by PCR, but first some alterations need to be made to the PCR template sequence so the genes can ultimately be expressed in bacteria. Bacterial DNA does not contain introns, and bacteria do not have the machinery to splice them out of mRNA. For a eukaryotic gene such as the insulin gene to be expressed in *E. coli*, the inserted DNA must contain the exons (coding regions) only; introns must be removed before PCR and insertion in the plasmids. In this situation the nucleic acid template for PCR amplification is mRNA molecules (exons only). These are first converted back into DNA strands, now called copy DNA (cDNA). Primers for PCR must be designed so that they bind on either side of the cDNA region to be amplified. One primer binds to the template strand and the other to the complementary strand of the cDNA. The PCR reaction then proceeds and produces billions of copies of the insulin genes for the A and B polypeptide chains.


1 Cut the plasmid and gene.

Digest the PCR product (human insulin gene coding region) with two different endonucleases.

Digest the plasmid with the same two endonucleases.

2 Insert the gene into the plasmid.

Incubate the cut insulin coding region and the cut plasmid together with DNA ligase.

Figure 3.34 Inserting the human insulin gene into a plasmid

Creating recombinant plasmids carrying the two insulin genes

Once the human insulin genes have been amplified by PCR, they can be inserted into two different plasmid vectors located in two different bacterial cultures. The PCR products (the insulin genes) are cut with two different endonucleases that give them different sticky ends at either side. The plasmid can be cut with the same two endonucleases, and so the sticky ends of the insulin genes bond with the complementary ends of the plasmid and the genes slot into the plasmids in the correct orientation. The two insulin genes are inserted next to a gene for β-galactosidase protein. Since DNA ligase 'glues' the insulin genes into the plasmids, the two types of recombinant plasmids are complete. The steps involved in creating the recombinant plasmid with an insulin gene inserted are summarised in Figure 3.34.

Quality control of the recombinant plasmid

DNA ligation in the laboratory is not usually 100% efficient. Following the restriction digest reaction and the ligation reaction, four possible DNA fragments may be found in the tube. These are the:

- plasmid with the insulin gene incorporated into it (the recombinant plasmid that is the desired product)
- cut PCR product (insulin gene), which has not been inserted into the plasmid
- cut plasmid, in a linear conformation
- plasmid, with cut ends re-ligated to themselves.

The recombinant plasmid containing the insulin gene will be used to insert the gene into bacteria. This is separated from the other DNA fragments by DNA gel electrophoresis. The digested PCR product, digested plasmid and uncut plasmid are run in parallel wells as controls to compare sizes. The circular plasmid runs slightly further than the linear plasmid because it is more compact. The recombinant plasmid travels the shortest distance because it is the longest fragment. It can be cut out of the gel and purified out of the agarose. The DNA sequence of the region of the plasmid containing the inserted insulin gene is checked to ensure the insulin gene is inserted in the correct orientation and no mutations have arisen during the PCR reaction used to amplify the gene. The sequence is compared to public genomic databases to check that it aligns and that there are no mismatches.

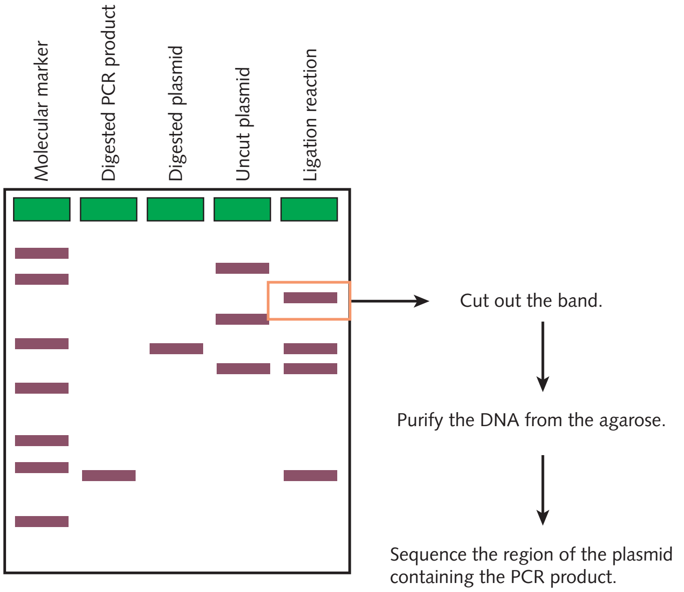


Figure 3.35 **Isolating the recombinant plasmid by gel electrophoresis**

Sequencing the regions where the DNA fragment joins the plasmid can show whether the DNA fragment has been inserted in the correct orientation. This is especially important when the ligation reaction has joined blunt ends. Together these steps ensure that the DNA being cloned is correct. A single base pair mismatch can completely change the function of a gene product.

Transformation of bacterial cells and selection of transformed bacteria

Once the recombinant plasmids containing the human insulin genes A and B are purified, they are ready to be inserted into *E. coli* cells. Bacteria are kept ice-cold to halt their metabolism, and their plasma membranes are compromised by chemicals or electrical pulses so that DNA of the recominant plasmids can be taken up more readily. Heat shock is used to increase the likelihood of uptake of the plasmid by the bacterial cells. It is difficult for the recombinant plasmids to enter the bacterial cells despite all these techniques to make them competent. Only a few in a thousand will be transformed. Since only the transformed cells are wanted, a selection process using an antibiotic is used. The cells are put in a selection medium containing an antibiotic. Because the plasmids encoding the insulin genes also code for a gene for antibiotic resistance, only cells that have taken up the plasmid can survive in the presence of the antibiotic, and will grow on agar plates containing that antibiotic. Another selection process involves β-galactosidase. β-galactosidase is an enzyme that can break down lactose as well as a molecule known as X-gal. When broken down, X-gal changes from a white to a bright indigo blue. This colour change is used to detect successful insertion of the gene, and is therefore used as another selection method. These colonies can be picked off the agar and the selected cells rapidly reproduce in two large separate tanks. While growing, the transformed bacteria containing the recombinant plasmids express the two insulin polypeptide chains, A and B. Once the cells reach an optimal density in the tanks, they can be filtered out and lysed to rupture their plasma membranes and release the insulin proteins. These steps are summarised in Figure 3.36. Once the fusion proteins A and B are produced in each tank, collected and purified, the insulin polypeptides A and B will be removed from the proteins and when mixed, they will combine together joining with disulfide bonds to produce functional insulin. The protein is harvested and purified, and then packaged into insulin pens for medicinal use (Figure 3.37).

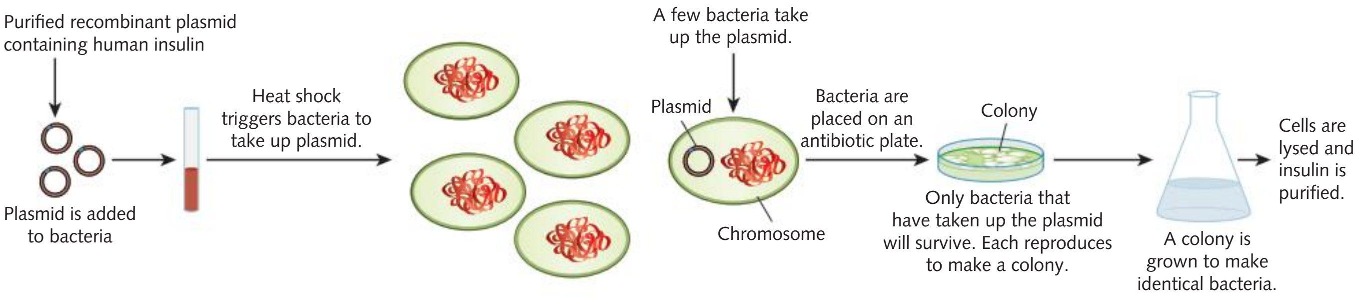


Figure 3.36 The steps involved in transformation of *E. coli* bacteria with the plasmid containing one of the two genes human insulin gene


age-fotostock/N. Aubrier

Figure 3.37 A person with diabetes using an insulin pen to deliver a dose of insulin

Islet transplantation: a cell-based therapy for type 1 diabetes

The production of recombinant human insulin has meant that type 1 diabetes is no longer a fatal disease. However, ongoing research aims to lower or completely remove the need for insulin injections. In Australia, islet transplantation is a cell-based treatment for type 1 diabetes. Pancreatic islets are groups of cells in the pancreas. They include beta cells, which produce insulin. In islet transplantation, islets are purified from the pancreas of a deceased donor, then infused into the recipient (the patient) through the portal vein into the liver by using a catheter (a thin, flexible tube). X-rays and ultrasound help to guide the catheter from an incision in the upper abdomen to the portal vein into the liver. The islets establish in the liver and, in the following weeks, new blood vessels form and connect the transplanted islets to the recipient's blood vessels. The beta cells produce insulin and release it into the bloodstream.

People with type 1 diabetes who want an islet transplant need to satisfy certain criteria. For example, they must have had diabetes for 5 years or more and be over the age of 18. The major criterion is that the person has severe hypoglycaemic unawareness. This means that they are unable to detect when they have a large drop in blood sugar, because they do not secrete the hormone epinephrine (which generates the characteristic symptoms of low blood sugar).

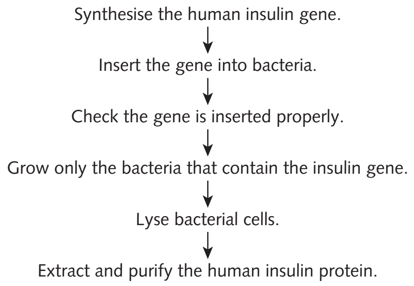
Although islet transplantation is very successful at managing type 1 diabetes, it also has several challenges. For example, recipients must take immunosuppressants (drugs that suppress the immune system) to prevent their immune cells from attacking and destroying the transplanted islets. Immunosuppressants have side effects that affect quality of life. Current research aims to find ways to suppress the immune response without the need for immunosuppressive drugs.

KEY CONCEPTS

- Gene cloning is an alternative to PCR for generating many copies of DNA. It uses bacterial plasmids to produce many copies of a gene.
- A DNA fragment can be inserted into a plasmid and replicated as the bacteria carrying the plasmid divide. The plasmid is then called a recombinant plasmid.
- Recombinant plasmids can be used to produce insulin for use by people with type 1 diabetes.
- Recombinant plasmids usually have an antibiotic resistance gene added that allows them to be selected. Only the plasmid-carrying bacteria can grow in the presence of the antibiotic.

Concept questions 3.7

1 List the steps involved in synthesising a recombinant plasmid.
2 Recombinant plasmids are called 'vectors'. What does this mean in this application?
3 Define 'transformation' in the context of genetic engineering.
4 Gene cloning is used as an alternative to PCR. How are they different?
5 The gene for human insulin can be inserted into a plasmid and replicated as the bacteria carrying the now recombinant plasmid divide. The following flow chart summarises the steps in making insulin for use in humans. Copy the flow chart and add notes describing each step.



HOT Challenge

6 Transformation is not 100% effective. What can biotechnologists do to ensure that the plasmids of transformed bacteria they are dealing with are only the ones that have taken up the foreign DNA (e.g. the insulin gene)?

3.8 Genetic engineering in agriculture

WB
3.8.1
LITERATURE REVIEW
P. 77

One of the most common applications of genetic engineering is the use of GMOs in agriculture, such as growing crops, managing the pasture of livestock, and aquaculture (farming fish). Genetic engineering provides ways to meet the great, and growing, global demand for food and other agricultural products by:

- increasing yield
- decreasing pesticide use
- improving food quality
- providing disease resistance
- increasing shelf life
- increasing nutritional value
- increasing tolerance to environmental stress (such as drought).

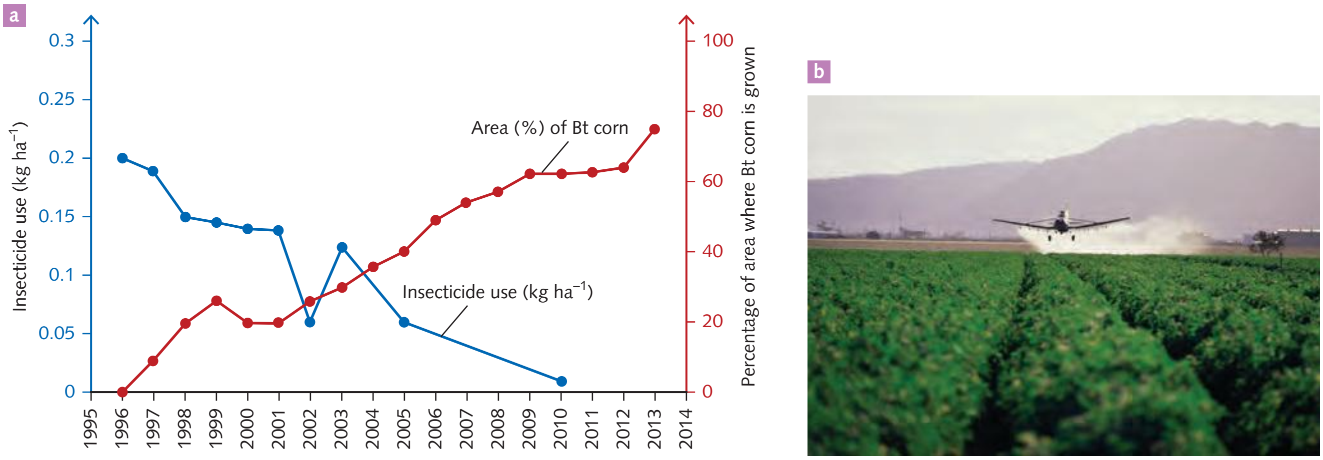
The process used for most of these applications is transformation – the process of transferring a gene from one species and into another to obtain a desired characteristic, producing knock-in or transgenic GMOs. The use of GMOs is controversial and, before their widespread use, there are extensive investigations into their potential impacts on the environment, and the possibility they may be toxic or cause allergies. Ethical considerations are important, particularly animal welfare. Here we will discuss some examples of GMOs currently used or being developed for use in agriculture to increase yields and provide resistance against disease.

Inserting genes to protect crops against pests and disease: Bt crops

The United Nations estimates that 20–40% of global crops are lost to insects pests each year. Insecticides are widely used to kill insects and improve crop yields (Figure 3.38). However, insecticides are a potential risk to humans and the environment.

In the mid-1990s, new GMO crops gave plants intrinsic resistance to insects and avoided the need for insecticides. These crops, including cotton, corn, sweet corn and potatoes, were engineered to express genes taken from the bacterium *Bacillus thuringiensis* (Bt), which encode toxins harmful to a range of common crop pests. The Bt genes were engineered to be controlled by promoters from the plants that resulted in the Bt genes being expressed in the appropriate plant tissues, at the appropriate stage of plant development. The US Environmental Protection Agency found that the Bt protein behaves like a normal dietary protein and is not toxic when ingested, even in high doses. Over time, with increased use of Bt corn, insecticide use in the US decreased (Figure 3.38a).

After 20 years, patents expire and generic brands using the same technology begin to appear. Because the Bt technology is so commercially important, the company that sells it (Bayer) has developed new improvements to the genetic modifications, which produce new intellectual property and new patents, preventing generic copies from taking a significant share of the market. Bt cotton, in its third iteration, now contains three transgenes from *Bacillus thuringiensis*: *cry1Ac*, *cry2Ab* and *vip3A*. The proteins encoded by these genes are lethal to common Lepidopteran pests (moths and butterflies) when ingested. Resistance of the pests to the Bt crops is minimised by having the three proteins, as the chances of the pests developing resistance to all three is much lower.



American Association for the Advancement of Science

Alamy Stock Photo/redbrickstock.com

Figure 3.38 **Pesticide application in agriculture. a In the US, insecticide use (blue) is inversely correlated with the use of Bt corn (red) i.e. insecticide use decreased over time as Bt corn use increased. b Insecticide being applied to crops.**

Inserting genes to improve growth and yield: AquAdvantage salmon

In 1989, a US company created the genetically modified AquAdvantage salmon, which grows twice as fast as wild salmon (Figure 3.39). This was achieved by introducing a growth hormone gene from a Pacific Chinook salmon into the salmon genome, under the control of a promoter sequence from another fish, the ocean pout. The promoter sequence switches on the growth hormone permanently, rather than only in the spring and summer, the normal growth periods for wild salmon. It would normally take 3 years for salmon to grow big enough for consumption, but the genetically modified salmon reaches the required size in 1.5 years.

This has commercial advantage for the company, but caused concerns that if AquAdvantage salmon escaped into the wild they may out-compete their smaller wild counterparts. AquAdvantage salmon are housed in land-based farms far from streams or the ocean and enclosed by a series of dam barriers.

Headpress/New York Times/Paul Darrow

Figure 3.39 **The genetically modified AquAdvantage salmon is twice the size of non-genetically modified salmon at the same age.**

AquAdvantage eggs are also treated with high pressure to produce triploid eggs (containing three sets of chromosomes rather than the usual two). This makes the salmon sterile (unable to reproduce because their cells cannot achieve homologous pairing of chromosomes in meiosis) and redirects metabolic energy into growth instead of reproduction.

ACTIVITY 3.2

GMOs in science and society

Before genetically modified salmon was permitted to be grown and sold in the US and Canada, the US Food and Drug Administration investigated whether this new GMO met strict safety requirements. This comprehensive analysis was based on scientific evidence and determined that the genetically modified salmon was safe to eat and that the genetic modification was a safe intervention to the fish itself.

In Australia, the CSIRO has an extensive selective breeding program to improve Atlantic salmon. This involves analysing the performance of salmon to select which fish to breed for the next generation. The program aims to select key performance traits such as growth, resistance to disease and carcass quality.

You are policy makers, and have been appointed to a food and safety regulatory board to advise whether genetically modified salmon should be imported and sold in Australia, or if the selective breeding program should be given more funding. Your task is to critically compare and evaluate the AquAdvantage salmon strategy and the CSIRO breeding program and construct an argument to support your opinion. (Remember that when you compare, you look at how they are similar and different.)

Aim

To determine whether the genetically modified salmon should be imported and sold in Australia, or if the selective breeding program should be prioritised by critically analysing reasons for and against both strategies

You will need

- Whiteboard
- Whiteboard markers

What to do

1. In your group, allocate who will argue for AquAdvantage salmon and who will argue for the selective breeding program.
2. Summarise the AquAdvantage or selective breeding program with a paragraph and diagram.
3. Compare and debate AquAdvantage salmon versus the selective breeding program. Aspects to consider include:
   - benefits of genetically modified salmon
   - potential environmental impact
   - impact if genetically modified salmon escaped into the wild
   - containment facility required.
4. After a discussion, evaluate the arguments on both sides to make your own choice.
5. As a personal reflection activity, go through the same process to evaluate Bt crops, Golden Rice and Roundup Ready crops. You may need to do some research on the Internet.

KEY CONCEPTS

- GMOs are used frequently in agriculture. They can be engineered to have increased yield, reduced need for pesticides and increased nutritional value.
- Ethical issues surround the use of GMOs and must be specifically evaluated in each case.

Concept questions 3.8

1 What type of GMO is the fast-growing salmon?
2 GMOs have been controversial since their introduction. What might be some of the benefits and risks of using GMOs?
3 *Bacillus thuringiensis* (Bt) has genes that are toxic to a number of crop pests. How has this information been harnessed by the agricultural community to improve crops?

HOT Challenge

4 Large chemical companies patent the intellectual property associated with a number of GMOs. Eventually these patents expire.
  a Why do companies patent GMOs?
  b What happens when the patents expire?
  c How can chemical companies limit the resistance of the pests to the GMO crops?

BRANCHING OUT

CRISPR-Cas9 reveals we don't know cancer drugs like we thought we did

Cancer is a major cause of death in Australia. Intense medical research aims to discover the genes that cancers depend on to aggressively grow and spread. These discoveries can reveal potentially druggable targets to treat cancer (Figure 3.40). Most cancer drugs target proteins that are essential for cancer cell division and survival.

US scientists were trying to find genes involved in regulating cancer growth. They included a positive control in their experiment. This was a gene called *MELK*, which is important for cancer cell growth. The researchers used CRISPR-Cas9 to disable the genes they were studying. Strikingly, they didn't see any effect of knocking out *MELK* on cancer cell growth.

To double check this effect, the researchers tested a drug developed to inhibit the protein encoded by *MELK*. Even without *MELK*, the drug was still very effective at killing cancer cells.

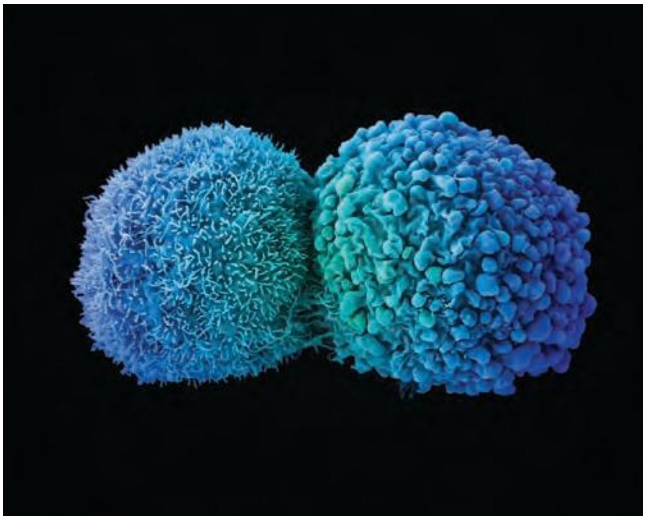

Alamy Stock Photo/Science Photo Library

Figure 3.40 Lung cancer cells. Cancer drugs aim to inhibit cancer cells from growing and spreading.

This perplexing result suggested that the drug interacted with other molecules than that which it was designed for, to kill cancer cells. Puzzled, the researchers proceeded to test other cancer drugs, most of which, like the *MELK* inhibitor, showed effectiveness in preclinical studies and had entered into clinical trials. Each drug gave the same result – when the specific target protein was knocked out using CRISPR-Cas9, the drug still killed cancer cells. These results demonstrated that the mechanism by which researchers thought most cancer drugs worked was incorrect. How is it possible that this new research so strongly contradicted previous findings? Before CRISPR-Cas9, most scientific evidence for the drug targets was collected from experiments that used a technique called RNA interference (RNAi), which silences gene expression by inhibiting mRNA molecules. However, this technique can have off-target effects, altering the expression of other genes that could potentially explain the difference in results.

This research has many implications for cancer drug development. The process of getting a cancer drug to the clinic is long. There are strict and robust tests that a drug needs to pass to show that it is safe and effective. Clinical trials are the last, major part of this long process of tests, involving testing of the compounds in people who have consented. Consider the clinical trials in which drugs from this study were used. These drugs alone were used in 29 clinical trials, with each trial aiming to have up to 1000 volunteers. Unfortunately, many drugs do not make it through a clinical trial for reasons that are often unclear, despite large amounts of promising research before the trial. Perhaps this research might provide some explanation as to why most drugs don't make it through clinical trials.

Questions

1 Describe how applying the CRISPR-Cas9 system for genome editing has contributed to understanding the actions of new drugs to treat cancer.

2 The cancer drugs in this experiment were tested on isolated, cultured cells *in vitro* (in the laboratory) that were genetically modified using the CRISPR-Cas9 system.

  a Why might the findings differ in a human subject?

  b Outline a process to define the drug target; that is, the protein to which the drug binds, beginning in the laboratory and confirming the target in human tissues.

3 Imagine you are a decision maker in Therapeutic Goods Australia (TGA), the body that regulates the pre-market testing, supply and manufacture of drugs in Australia. You have been tasked with updating the guidelines for the assessment of the preclinical research studies of new drugs that are submitted to the TGA for approval for use in cancer.

  a List some new guidelines you might include in the new era of CRISPR.

  b Consider a drug that shows effectiveness in clinical trials for cancer treatment, but which CRISPR studies later show not to work through its previously defined target protein. As a TGA decision maker, what new information would you need to decide whether or not to approve the new drug?

Online Key Concepts
Chapter 3: Summary of key concepts

3 Summary of key concepts

3.1 Genetically modified organisms

KEY CONCEPTS

p. 81

- Genetic engineering refers to the use of living things to make new products or systems by switching genes on or off, removing genes or introducing genes from one species into another.
- Organisms that are altered or produced by genetic engineering techniques are known as genetically modified organisms (GMOs).
- Genetically modified organisms include knock-out organisms, knock-in organisms and transgenic organisms.
- Knock-out organisms have had a gene deleted or its function interrupted.
- Knock-in organisms have had a gene or fragment of DNA inserted into a particular locus in the genome.
- Transgenic organisms have had a gene or fragment of DNA inserted into a non-specific locus.

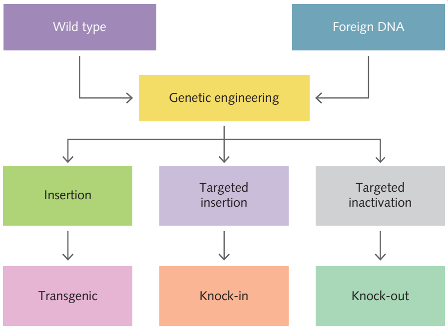


Figure 3.3 Genetically modified organisms include knock-out, knock-in and transgenic organisms

3.2 Enzymes for modifying DNA

KEY CONCEPTS

p. 84

- DNA polymerases catalyse the formation of new DNA molecules from free nucleotides and a template DNA strand.
- The technology that recombines DNA from different sources to modify the DNA sequence is called recombinant DNA technology.
- The cutting tools used are enzymes known as restriction endonucleases, or restriction enzymes.
- Endonucleases can generate blunt or sticky ends.
- DNA ligase is an enzyme used to join different pieces of DNA together.
- Complementary sticky ends help DNA strands bind to each other via hydrogen bonding.

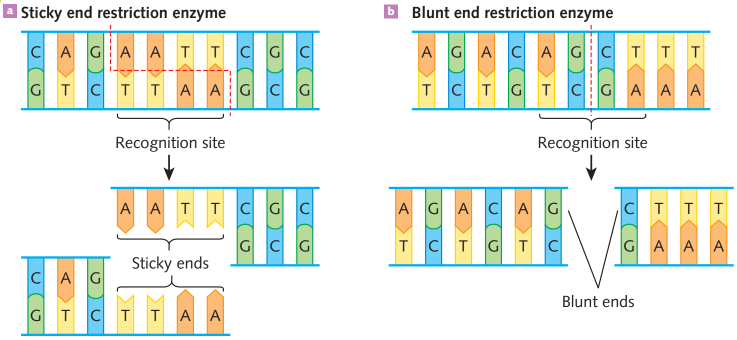


Figure 3.5 a Sticky ends produced by cutting DNA with the endonuclease EcoRI. **b** Blunt ends produced by cutting DNA with the endonuclease AluI

3.3 CRISPR-Cas9

KEY CONCEPTS

p. 87

- The CRISPR system is a bacterial immune defence mechanism against viral infection.
- CRISPR-Cas9 is applied to edit genomes to precisely create knock-out or knock-in genetic modifications.
- Applications of CRISPR-Cas9 technology include editing faulty alleles in disease, improving agriculture and making new mouse strains for scientific research.
- The use of CRISPR-Cas9 technology raises serious bioethical questions.

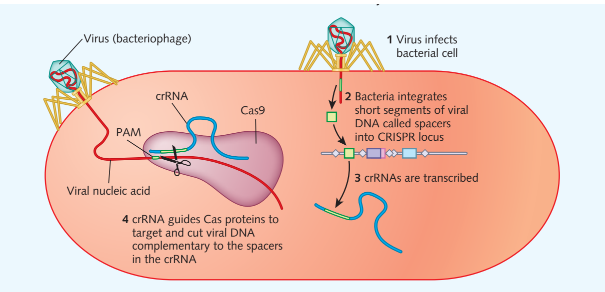


Figure 3.8 The CRISPR-Cas9 system in bacteria and its application to genetically modify DNA. A short molecule of RNA (guide RNA) binds to the target gene and guides the Cas9 enzyme to the gene. the Cas9 enzyme breaks the DNA and the bacterial cell tries to repair the break. This can result in a frameshift mutation – deletion or insertion of a gene – if a donor DNA fragment is incorporated

3.4 Amplifying DNA

KEY CONCEPTS

p. 92

- PCR is a process that amplifies a specific DNA sequence for analysis.
- The sequence of the primers determines the DNA sequence to be amplified.
- The steps involved in PCR are denaturation, annealing and extension. These steps are repeated many times to yield a large number of identical DNA molecules.

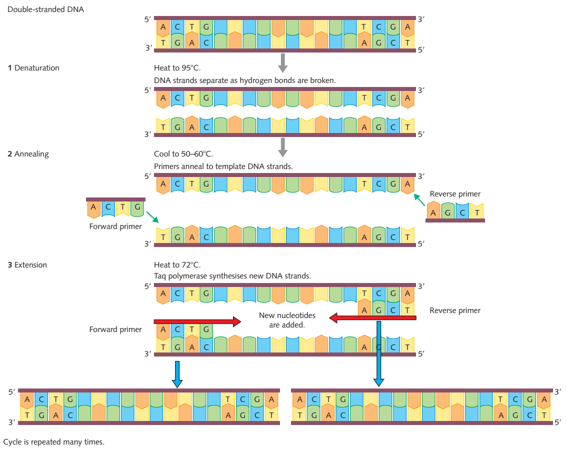


Figure 3.12 Amplifying DNA using PCR

3.5 Gel electrophoresis

KEY CONCEPTS

p. 95

- Gel electrophoresis separates DNA molecules by size.
- Negatively charged DNA travels through a gel matrix towards a positive electrode.
- DNA is visualised with a DNA binding dye.
- The sizes of DNA fragments can be estimated by comparing their movement through the gel with that of fragments known sizes, referred to as molecular size markers.

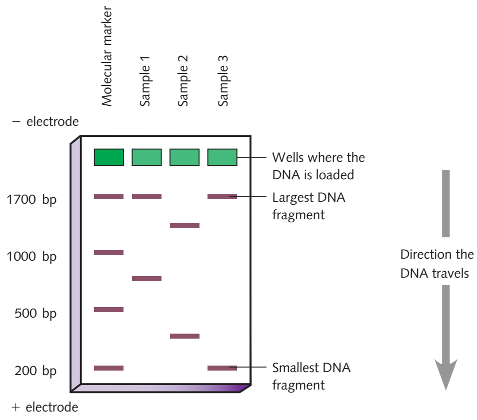


Figure 3.15 Standards are molecular markers of known size that are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel

3.6 DNA profiling

KEY CONCEPTS

p. 98

- DNA profiling is used to solve crimes, determine family relationships and identify human samples.
- By examining STRs in non-coding regions of DNA, a profile can be obtained that can be compared to a database of samples given by family members.

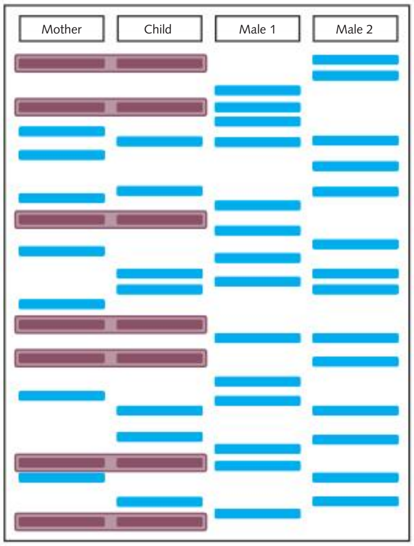


Figure 3.23 This gel electrophoresis run reveals the DNA profiles of the four individuals in case study 2. Primers for seven STR regions were used to generate these DNA profiles

3.7 Recombinant plasmids and human insulin

KEY CONCEPTS

p. 105

- Gene cloning is an alternative to PCR for generating many copies of DNA. It uses bacterial plasmids to produce many copies of a gene.
- A DNA fragment can be inserted into a plasmid and replicated as the bacteria carrying the plasmid divide. The plasmid is then called a recombinant plasmid.
- Recombinant plasmids can be used to produce insulin for use by people with type 1 diabetes.
- Recombinant plasmids usually have an antibiotic resistance gene added that allows them to be selected. Only the plasmid-carrying bacteria can grow in the presence of the antibiotic.

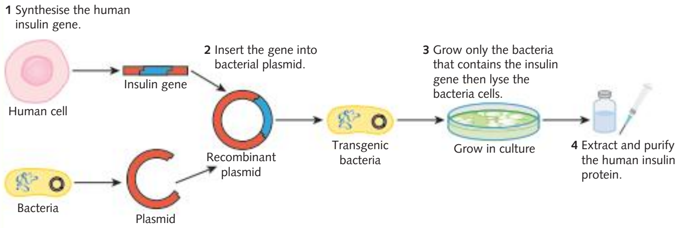


Figure 3.25 The steps involved in producing insulin in bacterial cells

3.8 Genetic engineering in agriculture

KEY CONCEPTS

p. 115

- GMOs are used frequently in agriculture. They can be engineered to have increased yield, reduced need for pesticides and increased nutritional value.
- Ethical issues surround the use of GMOs and must be specifically evaluated in each case.

Figure 3.39 The genetically modified AquAdvantage salmon is twice the size of non-genetically modified salmon at the same age

WB
3.9.1 KEY TERMS
PAGE 79

3 Chapter glossary

agarose gel a gel matrix used for electrophoresis

annealing a process used in the polymerase chain reaction to join separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow

bacteriophage a virus that can infect bacteria and replicate

bioethics the study of ethical issues emerging from advancements in biology

biotechnology the use of living organisms and biological systems and processes for human benefit

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

Cas9 protein an endonuclease that cuts double-stranded DNA at a target location in the genome

CRISPR-Cas9 a bacterial immune defence mechanism in which short RNAs target complementary sequences in viral genomes to guide Cas9 proteins to destroy an invading virus

crRNA (CRISPR RNA) RNA transcribed from the CRISPR locus; guides Cas9 proteins to their complementary sequence in the invading viral DNA genome, targeting it for destruction by Cas9

DNA ligase an enzyme that catalyses the formation of a phosphodiester bond between two pieces of DNA

DNA profiling comparison of individuals based on patterns of non-coding base sequences in the genome

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

frameshift mutation a mutation in DNA caused by the addition or deletion of a nucleotide or nucleotides resulting in a change in the amino acid sequence and protein being made

gel electrophoresis a technique that separates DNA fragments according to their size and charge

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organism (GMO) an organism whose genome has been genetically engineered

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

polymorphism a variation in DNA sequences among individuals

primer a single-stranded DNA molecule that acts as the start of the amplification process

recombinant DNA technology the process of transferring a gene from a cell of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

reporter gene a gene that enables visualisation or quantification of gene expression

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

short tandem repeat (STR) a short non-coding region of DNA of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be used in DNA profiling

single guide RNA RNA that guides the Cas9 protein to the target sequence in a genome for gene editing

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

transformation the process by which the genetic material of an organism is changed by the addition of new genetic material

transgenic (TGO) an organism that has been modified by incorporating a piece of foreign DNA into its genome

vector a vehicle used to transfer DNA sequences from one organism to another

wild type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

WB
3.9.2 EXAM PRACTICE PAGE 82

3 Chapter review

Remembering

1 Match each term in the first column with a description in the second column. Use each item only once.

DNA ligase	Small circular self-replicating DNA molecule
Vector	Sorts DNA molecules by size and charge
Primer	Joins two single-stranded sections of DNA together
Blunt end	Specific site at which restriction enzymes cut DNA
Plasmid	Vehicle to introduce DNA into a host cell
Restriction site	Enzyme that catalyses the synthesis of DNA
Gel electrophoresis	Results from cleavage by a restriction enzyme in the middle of the recognition sequence (restriction site)
DNA polymerase	Synthetic short, single-stranded DNA molecule

2 Recall the features of a plasmid vector.
3 What makes the features of a plasmid vector useful for biotechnology?
4 How could DNA ligate to itself?
5 State why the temperature is lowered to 50–60°C during the annealing phase of PCR.
6 How do biotechnologists use the concept of wild type?
7 In forensic science, what method is used to distinguish one sequence of DNA from another?
8 What is gene cloning used for?

Understanding

9 Consider Figure 3.41. Predict whether the cuts made by restriction enzymes produce sticky or blunt ends. (The lines show the cuts lines in the double-stranded DNA.)

Figure 3.41

10 What would happen if the electrodes on a gel electrophoresis tank were accidentally swapped?
11 For how long would you apply a current to the gel during electrophoresis?
12 Agarose gels can be made with different concentrations of agarose. If increasing the concentration of agarose results in a denser gel matrix, what would be the effect on DNA migration speed?

Applying

13 Predict the minimum band-sharing percentage in the DNA profiles of a mother and her baby.

14 Consider Figure 3.42. Four samples (a, b, c and d) containing DNA fragments of the sizes given below, were accidentally swapped around while being loaded into the gel. Identify which lane corresponds to each sample.
- **a** 200, 250 and 900 bp
- **b** 150, 400 and 600 bp
- **c** 50, 450 and 650 bp
- **d** 100, 100 and 450 bp

15 Predict whether digestion of the human genome by AluI or by EcoRI would result in the larger number of fragments and explain why.

16 The following section of DNA shows a sequence of 120 bases in one strand of DNA. Refer to Table 3.1 on page 84 for restriction sites.

```
ATATGTGTGGATCCGTCTTAGGTTATCGAATTCTAGAGCT
ATGGCCTATTAGCTTCCTGGATCCA ACCTGTATAGAGCTA
CTCGTCAGCTATTGCTACGGGATCCTAGCTGATTGGATTC
```

- **a** How many BamHI and AluI restriction sites are there in the sequence?
- **b** If the sequence was cut by BamHI, how many fragments of DNA would be produced?
- **c** If the sequence was cut by AluI, how many fragments of DNA would be produced?
- **d** If the sequence was cut by both BamHI and AluI, how many fragments would be produced?
- **e** If this piece of DNA was circular and not linear, how many cuts would have been made by BamHI to get the number of fragments stated in part **b**?

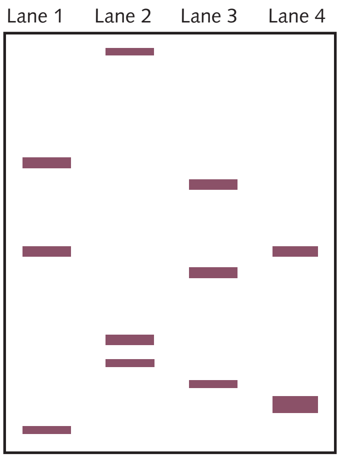


Figure 3.42 Gel electrophoresis

17 What is ethidium bromide used for in gel electrophoresis?

18 It has been said that restriction enzymes form part of the 'immune system' of bacteria. Use the information in this chapter to explain your understanding with reference to viruses.

Analysing

19 When conducting PCR, some unwanted DNA molecules are sometimes present.
- **a** Identify the possible consequences of having an unwanted DNA molecule in the PCR.
- **b** Identify two possible sources of this contamination.
- **c** Suggest what could be done to prevent this contamination.

Evaluating

20 A gene with unknown function, named *Taurin*, has been cloned and used to generate a *Taurin* knock-out mouse strain. In mice, the *Taurin* gene contains three exons and two introns. The knock-out mutation was achieved by inserting a small region of irrelevant DNA into exon 1 of the *Taurin* gene, causing a frameshift mutation that resulted in a premature stop codon and prevented translation of *Taurin* into a protein. The knock-out mouse strain was analysed for any abnormalities by comparing the knock-out mice to wild-type littermate (sibling) control mice. It was noted that the knock-out mice developed tumours characterised by B cells proliferating in an uncontrolled manner (Figure 3.43).
- **a** What is the function of the *Taurin* protein that can be inferred from this observation?
- **b** Why are the knock-out mice compared with wild-type littermate controls?

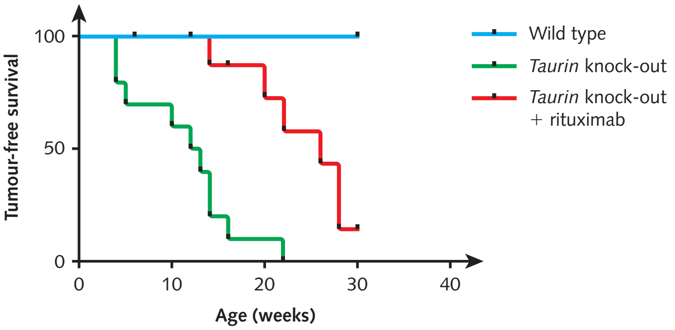


Figure 3.43 Tumour-free survival in Taurin knock-out and wild-type control mice

- **c** Draw diagrams of the *Taurin* gene locus in wild-type mice and the same locus in the knock-out mice.
- **d** How could you use PCR to distinguish between wild-type mice and mice carrying the knock-out mutation? Draw PCR primer binding sites in the diagrams you drew for part **c**.
- **e** The knock-out mice were treated with rituximab, a monoclonal antibody used to deplete B cells. What can the survival curve of the treated mice tell you about your answer to part **a**?
- **f** What important control is missing from the experiment represented in Figure 3.43?

21 Consider Figure 3.44. It shows a developed agarose gel from electrophoresis of a sample taken from a crime site. Decide whether the defendant is guilty or not guilty and justify your reasons.

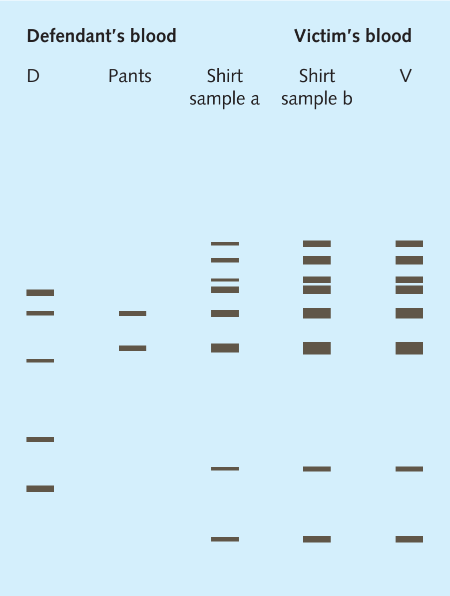
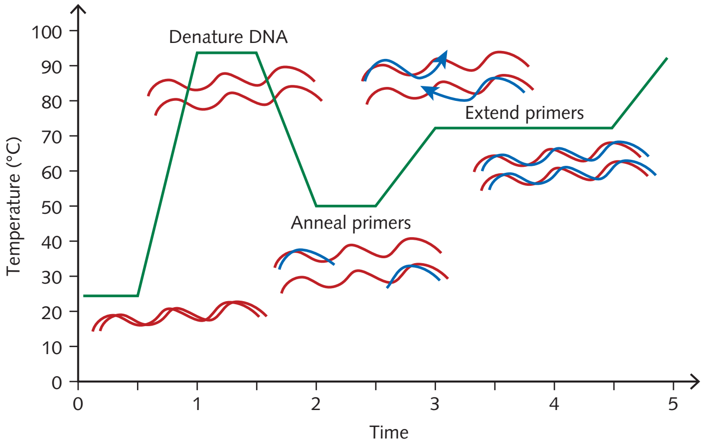


Figure 3.44 An electrophoresis of a sample taken from a crime site



Steve Carr, Terra Nova Genomics, Inc.

Figure 3.45 The polymerase chain reaction

22 Interpret the graph in Figure 3.45 by summarising the activity being undertaken. Make sure you refer to the data in the graph in your analysis.

Creating

23 A new restriction enzyme has been discovered, called StarI. Design a strategy for large-scale production of StarI for use in research laboratories. Draw a flow chart outlining the strategy.

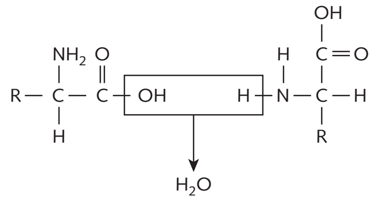
Reflecting

24 Many companies have started labelling their food products 'GM free'. Considering your knowledge of biotechnology techniques and applications, what do you think of the use of this type of labelling for consumers with a non-scientific background?

Unit 3, Area of Study 1 review

Multiple choice

Question 1 ©VCAA 2018 Q3 ADAPTED EASY



The diagram above represents adjacent amino acids being joined together.

The joining of adjacent amino acids

A results in the formation of a dipeptide.
B is a reaction that releases energy.
C is catalysed by the enzyme DNA polymerase.
D is a combustion reaction.

Question 2 ©VCAA 2018 Q2 ADAPTED EASY

The proteome is

A the total set of proteins present within a single organism.
B a complete set of chromosomes found inside a sex cell of an organism.
C the entire set of DNA expressed by an organism at a given time.
D the entire set of tRNA in a cell's cytosome.

Question 3 ©VCAA 2008 Q4 ADAPTED MEDIUM

The genetic code is described as a universal code.

This means that

A each amino acid is only coded by one specific codon.
B some amino acids may be encoded by more than one codon.
C a single nucleotide cannot be part of two adjacent codons.
D in almost all organisms the same DNA triplet is translated to the same amino acid.

Question 4 ©VCAA 2009 EXAM 2 Q17 ADAPTED HARD

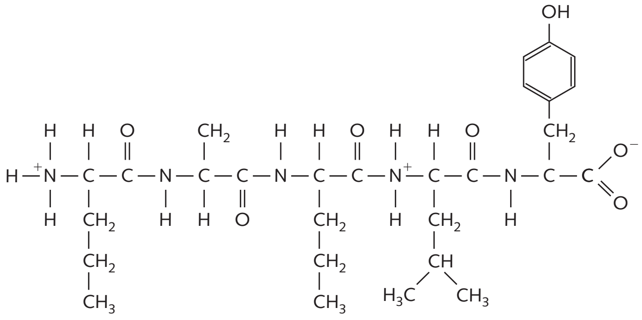
The process of gene expression involves

A transcription, which follows translation.
B four nucleotides on each tRNA molecule, that carry one specific amino acid.
C every cell of the organism that contains a particular gene, all undergoing identical action simultaneously.
D the production of a polypeptide.

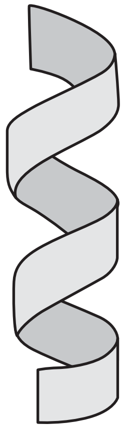
Question 5 ©VCAA 2008 EXAM 1 Q3 ADAPTED MEDIUM

The tertiary structure of a protein is represented by

A



B



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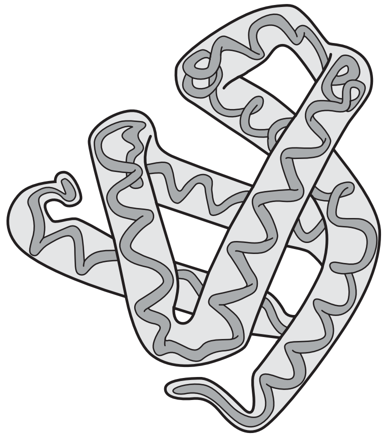

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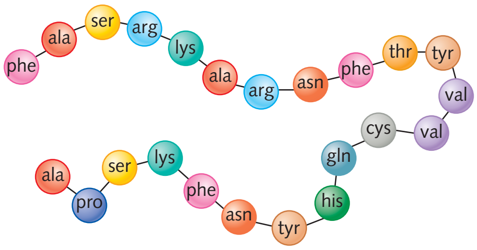


Question 6 ©VCAA 2019 Q38 ADAPTED EASY

DNA ligase

A forms phosphodiester bonds between the two fragments of DNA to join them together.
B cuts DNA molecules at specific nucleotide sequences.
C is an enzyme involved in protein synthesis.
D separates two DNA strands during translation so that a copy can be made.

Question 7 ©VCAA 2017 Q2 ADAPTED MEDIUM

To begin transcription of the five structural genes in the *trp* operon, RNA polymerase needs to bind to the

A promoter gene.
B structural genes.
C regulatory gene.
D operator gene.

Question 8 ©VCAA 2014 Q25 ADAPTED MEDIUM

Genes are often transferred from one species to another using bacterial plasmids. The process can be represented as follows.

bacterial plasmid cut
↓
foreign gene and plasmid mixed
↓
plasmid with inserted foreign gene

These steps are facilitated by enzymes.

Identify the two enzymes required for the first and last steps of the process, from the following table.

	Cuts plasmid	Inserts genes
A	Restriction enzyme	DNA polymerase
B	Restriction endonuclease	DNA ligase
C	DNA ligase	DNA polymerase
D	DNA polymerase	Restriction enzyme

Question 9 ©VCAA 2017 Q34 ADAPTED MEDIUM

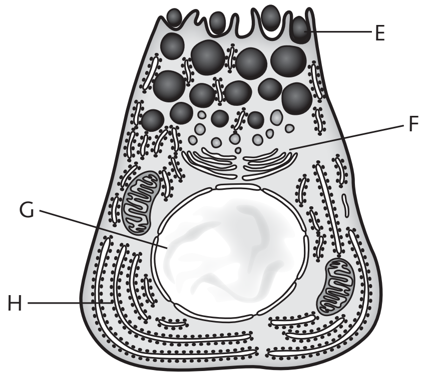
The polymerase chain reaction (PCR) is a process that involves repeated cycles made up of several steps.

During PCR, the

A first step in each cycle is to denature the DNA at a high temperature.
B final step of each cycle involves the use of DNA ligase.
C second step in each cycle is to heat the DNA to a high temperature.
D temperature must be lowered to 37°C before the beginning of each cycle.

Question 10 ©VCAA 2012 EXAM 1 Q7 ADAPTED EASY

Examine the following diagram of a cell in the digestive system, which secretes the hormone leptin, which regulates appetite.

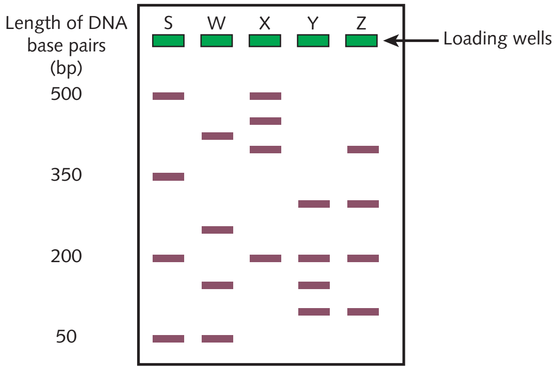


The secretion of proteins from the cell requires organelles operating in which order?

A E, F, G, H
B H, G, E, F
C F, E, H, G
D G, H, F, E

Use the following information to answer Questions 11 and 12.

Four samples of DNA were loaded into four different wells in lanes W, X, Y and Z. A standard ladder (DNA of known lengths) was loaded into the well in lane S. The results of gel electrophoresis are shown below.



Question 11 ©VCAA 2018 Q30 ADAPTED EASY

Which lane represents a sample that was loaded with DNA fragments of four different lengths: 50 bp, 150 bp, 250 bp and 450 bp?

A W
B X
C Y
D Z

Question 12 ©VCAA 2018 Q31 ADAPTED MEDIUM

Which sample lane contains the band that is closest to the positive electrode?

A W
B X
C Y
D Z

Question 13 ©VCAA 2013 Q34 ADAPTED MEDIUM

Insulin is made in commercial quantities by using cultured bacteria that have been transformed with an artificial insulin gene. The steps taken to produce insulin by this genetic engineering are summarised below. The order of the steps has been mixed up.

M Add recombinant plasmids to bacteria and heat to enhance uptake of plasmids

N Devise nucleotide sequence by working backwards from amino acid sequence

O Isolate insulin from human cells and determine its amino acid sequence

P Culture transformed bacteria; harvest and purify the insulin

Q Insert artificial gene into cut plasmids

R Identify successfully transformed bacteria

S Make artificial gene by adding bases in sequence in gene synthesiser

The correct sequence of steps when producing the insulin is:

A P, R, Q, M, O, S, N.
B M, S, N, R, P, Q, O.
C P, R, O, M, Q, N, S.
D O, N, S, Q, M, R, P.

Question 14 ©VCAA 2017 Q40 ADAPTED MEDIUM

Many plant crops are affected by viruses. Some viruses can affect many different crops. Scientists have trialed a spray treatment for one such virus that infects tomato crops. This treatment does not alter the DNA of the tomato plants.

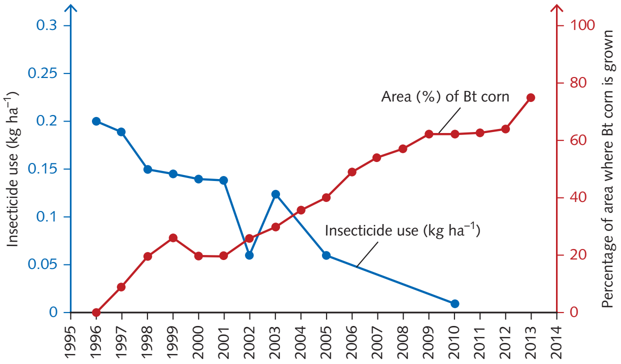
Clay nanoparticles containing double-stranded RNA (dsRNA) are applied to the surface of the growing tomato leaves. The dsRNA released from each of the clay nanoparticles enters the plant cells where it silences a gene from the virus by causing the breakdown of viral RNA.

This technique

A demonstrates dsRNA has a nucleotide sequence complementary to a section of DNA nucleotides in the tomato plants.
B causes dsRNA to stop expression of the viral gene by preventing translation.
C would not be effective on other crop species.
D would result in the tomato being defined as a GMO.

Question 15 ©VCAA 2019 Q37 ADAPTED MEDIUM

Bt corn expresses a protein that acts as an insecticide to protect from insect attack.



American Association for the Advancement of Science

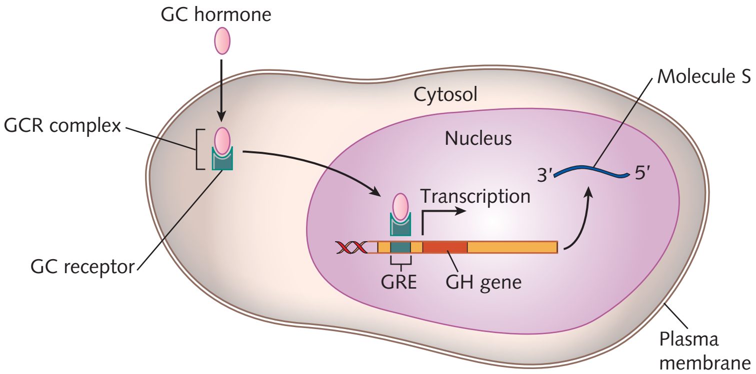
Based on the data in the graph above and your knowledge, what is a benefit of using Bt corn?

A Less insecticide is used with Bt corn crops.
B Bt corn is more expensive to produce than non-Bt corn.
C Negative impacts on ecosystems would be increased.
D More farmers are predicted to plant Bt corn in the future.

Short answer

Question 1 ©VCAA 2015 SECTION B QUESTION 7 ADAPTED EASY

The diagram below shows signal transduction of glucocorticoid (GC) – a hormone in humans. GC binds to a receptor in the cytosol. The glucocorticoid–receptor complex (GCR–complex) then moves into the nucleus and attaches to the DNA, causing transcription to begin.



The glucocorticoid response element (GRE) is the location where the GCR–complex attaches to the DNA. The GRE is located approximately 250 base pairs upstream of the growth hormone (GH) gene. After the GCR–complex has attached to the GRE, an enzyme catalyses the transcription of the gene.

a Name the enzyme that catalyses transcription.

1 mark

b State the role of the transcription product, molecule S.

1 mark

c Describe the processing that molecule S undergoes before it exits the nucleus.

2 marks

d In the human pituitary gland, GC stimulates the production of the human growth hormone protein. However, in the human liver, GC stimulates the production of the enzyme CYP3A4. Explain how the production of distinct proteins in different cell types could occur, given that the genetic sequence is identical in all somatic human cells.

2 marks

e If a rat gene is inserted into the DNA of human pituitary gland cells, these genetically engineered cells can be used to produce rat growth hormone. What characteristic of the genetic code enables a rat protein, such as rat growth hormone, to be made by human cells?

1 mark

Question 2 ©VCAA 2018 SECTION B Q 1 A, B ADAPTED

Trypsin is an enzyme that is released from human pancreatic cells.

Nucleic acids encode instructions for the synthesis of trypsin in a pancreatic cell.

a Outline the steps of translation of trypsin synthesis.

1 mark

b After being synthesised, trypsin is released from pancreas cells via exocytosis.
Copy and complete the table below by naming three different organelles directly associated with the transport of the synthesised trypsin within or from pancreatic cells. State the role of each organelle in this process.

2 marks

Organelle	Role

Question 3 ©VCAA 2009 EXAM 1 SECTION B Q2 ADAPTED

a A section of the template strand of a DNA molecule has the sequence of bases shown.

1 mark

DNA: G T G A C A T T A C T C

Copy the table below and enter the sequence of bases in the corresponding mRNA that is complementary to this DNA.

mRNA												

b The percentage of base G in a molecule of DNA is 20%. What is the percentage of T bases in the same DNA molecule?

1 mark

c Another type of nucleic acid is rRNA.

i Where is rRNA found in a cell?

1 mark

ii Describe the role of rRNA.

1 mark

d The table shows the names of six amino acids together with some of their DNA codes.

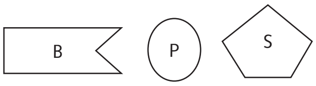
Amino acids	DNA triplet(s)
Cysteine	ACA, ACG
Histidine	GTA, GTG
Aspartic acid	CTA, CTG
Asparagine	TTA, TTG
Leucine	GAA, GAG, GAT, GAC
Methionine	TAC

Use the information in the table and write the order of amino acids coded for by the DNA sequence given in part **a**.

1 mark

e Nucleic acids are made up of nucleotides. Each nucleotide consists of three components – nitrogen base (B), phosphate (P) and sugar (S) – linked together in a particular way.
Draw a diagram to show the way the three components are joined to make a nucleotide.
Use the following symbols in your diagram.

1 mark



Enzymes and the regulation of biochemical pathways

4

By the end of this chapter you will have covered the following material.

Key knowledge

Regulation of biochemical pathways in photosynthesis and cellular respiration

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product pp. 137–141
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration pp. 142–144
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors pp. 145–150

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify independent, dependent and controlled variables in controlled experiments pp. 147–148
- predict possible outcomes pp. 147–148

Plan and conduct investigations

- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 147–148

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 147–148
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 147–148

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 147–148
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 147–148
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 147–148

Analyse and evaluate data and investigation methods

- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 147–148

Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 147–148

Online Chapter Map
Chapter 4 map

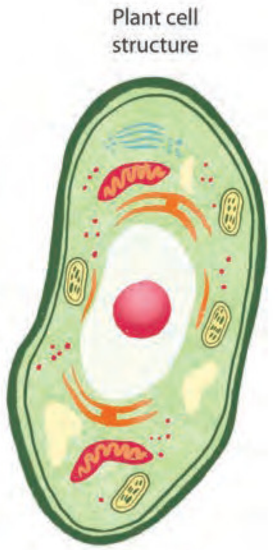
4 Enzymes and the regulation of biochemical pathways

Your body can only carry out all its necessary functions because you have the correct enzymes. Enzymes are proteins, which are encoded in DNA and made by ribosomes.

4.1 Biochemical pathways for cell metabolism

p. 137

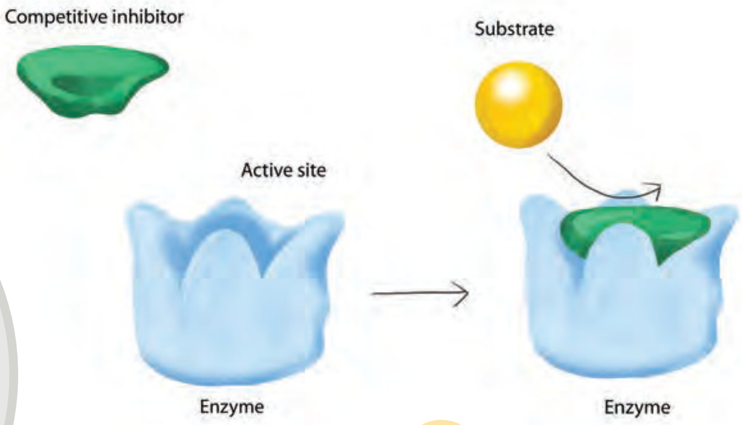
All the chemical reactions that occur in a cell are known as cellular metabolism. Some of these reactions use energy (e.g. photosynthesis) and some release energy (e.g. cellular respiration). These biochemical pathways are regulated in a step-like fashion, with each step controlled by enzymes.



4.3 Photosynthesis and cellular respiration

p. 145

Enzymes are proteins, so enzyme function is affected by environmental factors such as temperature. Heat can denature enzymes, and lower temperatures can slow reactions down. Other molecules can block enzyme activity, which stops or slows down metabolic reactions.

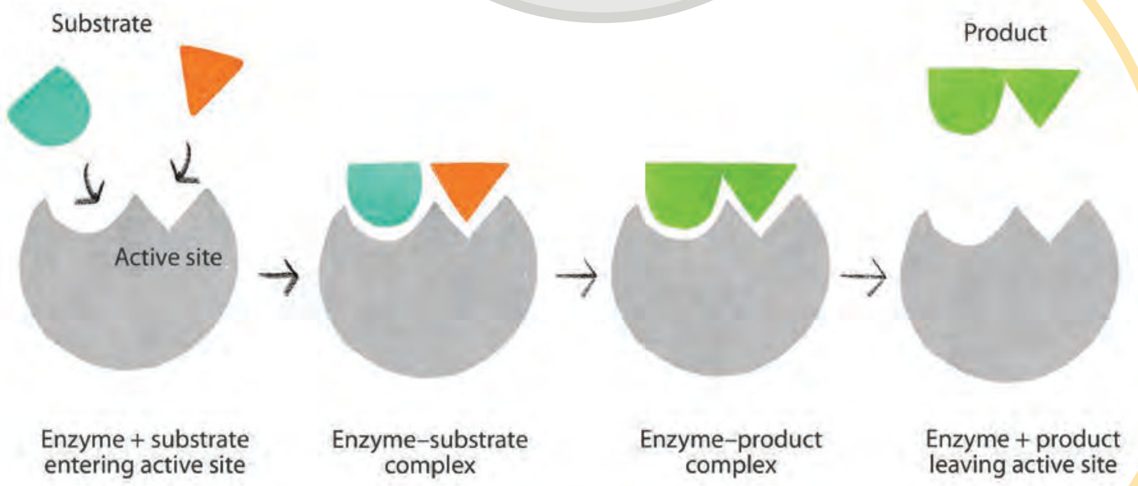


p. 142

4.2

Enzymes: the key to controlling biochemical pathways

Without enzymes, our bodies would function very slowly, if at all. Enzymes speed up chemical reactions by arranging the correct substrate molecules so that they fit together like a lock and key. Different substrates require different enzymes. Once the chemical reaction has occurred, the enzyme's active site is freed so the enzyme can work again.



You have a lot to thank enzymes for. Without them, chemical reactions would occur very slowly, or not at all. Life would not be possible without enzymes.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 4 map (p. 134)

Online Key Terms:
- Chapter 4 flashcards (p. 136)

Weblinks:
- Enzymes, active sites and cofactors (p. 143)
- Enzyme-mediated reaction under different conditions (p. 145)

Online Worksheets:
- Enzymes in biochemical pathways (p. 143)
- Enzyme catalysis (p. 145)

Online Key Concepts:
- Chapter 4: Summary of key concepts (p. 152)

Online Key Terms
Chapter 4 flashcards

Know your key terms

activation energy
active site
adenosine diphosphate (ADP)
adenosine triphosphate (ATP)
aerobic cellular respiration
allosteric site
anabolic reaction
anaerobic cellular respiration
ATP synthase
biochemical pathway
catabolic reaction
catalyst
cellular metabolism
cellular respiration
chlorophyll
chloroplast
coenzyme
cofactor
competitive inhibitor
electron transport chain
endergonic reaction
endothermic reaction
exergonic reaction
exothermic reaction
$FADH_2$
feedback inhibition
glycogen
glycolysis
induced-fit model
lactic acid
loaded
lock-and-key model
NAD^+
NADH
$NADP^+$
NADPH
non-competitive inhibitor
optimum pH
optimum temperature
photosynthesis
reactant
unloaded

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Enzymes are biological catalysts that speed up chemical reactions.
2 Photosynthesis and cellular respiration are biochemical pathways that rely on enzymes to drive each step in their reactions.

WB
REMEMBER
PAGE 85

Your cells use enzymes to power many fundamental reactions in your body. Advances in technology are bringing us closer to using enzymes instead of metal catalysts to provide energy for powering portable devices. Researchers at the University of Oxford in the UK have used two enzymes, one from a bacterium and the other from a fungus, to catalyse reactions in hydrogen fuel cells. The scientists were able to capture the energy from this reaction and use it to power a digital watch. Using enzymes found in nature provides many advantages: the enzymes are biodegradable and cheap and can be a reliable source of power.

In your body, thousands of reactions build up and break down chemicals. The reactions are catalysed by enzymes, and together they are known as cellular metabolism. The rate of cellular metabolism varies among organisms. The rate of cellular metabolism also affects the life span of an organism. For example, the antechinus, a small Australian mammal, has a very short life span partly because it has a very high rate of metabolism (Figure 4.1a). Trees have a lower rate of metabolism and can live for hundreds of years (Figure 4.1b).

All living things on Earth require a source of energy. Energy released in breaking-down metabolic reactions provides cells with the energy needed for efficient functioning and survival of the cells, and ultimately the organism. Enzymes are the key to speeding up these reactions so that they take place at a rate that will sustain life.

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iStock.com/Surkov Dimitri

Figure 4.1a An antechinus marsupial and **b** a eucalyptus tree have different rates of cellular metabolism.

4.1 Biochemical pathways for cell metabolism

Metabolism includes a range of biochemical processes that occur in living organisms. **Cellular metabolism** is the sum of all the chemical reactions taking place in all living cells. It consists of two major types of reactions: catabolic and anabolic reactions.

In **anabolic reactions**, atoms and simple molecules are joined to make more complex molecules. Anabolic reactions require an initial input of energy so they can start to form new chemical bonds. Reactions that require an initial input of energy to get them started are called **endergonic reactions**. Photosynthesis is an anabolic reaction in which the simple inorganic molecules water and carbon dioxide react to produce the complex organic molecule glucose. It is an **endothermic reaction** because light energy is absorbed from the surroundings and used to form the bonds in the glucose molecules.

In **catabolic reactions**, complex molecules are broken down into simpler molecules. Catabolic reactions are spontaneous reactions that do not require an initial input of energy to get them started, so they are called **exergonic reactions**. Cellular respiration is a catabolic exergonic reaction in which glucose, the main initial reactant, is broken down to release the energy stored in the glucose and produce simpler products. Reactions that release energy are called **exothermic**. If oxygen is available and used in the reaction, it is called **aerobic cellular respiration** and the products are water and carbon dioxide. If no oxygen is available or it is not used, it is called **anaerobic cellular respiration**, and the products are **lactic acid** in animal cells, and ethanol and carbon dioxide in plant cells.

WB
4.1.1
ORGANISING
KEY TERMS
PAGE 86

CONNECT
For more information on photosynthesis and cellular respiration, go to Chapter 5.

Note:
Make sure you understand the difference between exergonic and exothermic, and endergonic and endothermic.

Biochemical reactions: pathways with regulated steps

WB
4.1.2
BIOCHEMICAL PATHWAYS AND COENZYMES
PAGE 87

The metabolic reactions that occur in cells do not take place randomly. They are all controlled and regulated to maintain cell functions and to meet the needs of the cell. To achieve this, chemical reactions in cells occur in a series of regulated steps, collectively called **biochemical pathways**, as illustrated in Figure 4.2. These reactions must occur at a rate that allows the cell to function efficiently.

The initial **reactants** are the substrates, the molecules that enter the reaction and are acted upon by enzymes to speed up their conversion into products. At each step, a substrate–enzyme complex is formed, which then separates and releases the new product for that step. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached.

Each step in the pathway is controlled by a specific enzyme (a protein that speeds up the rate of a chemical reaction without being used up or destroyed in the reaction). Enzymes act as catalysts, speeding up reactions by lowering the **activation energy**, which is the energy required for a reaction to start. Without enzymes, the biochemical pathways of the cell, including both cellular respiration and photosynthesis, would occur too slowly to maintain life. Enzymes often require the assistance of other molecules to ensure that reactions can take place and be maintained. Molecules that assist enzymes are called **cofactors** or **coenzymes**.

CONNECT
Activation energy, coenzymes and cofactors are discussed more on page 143.

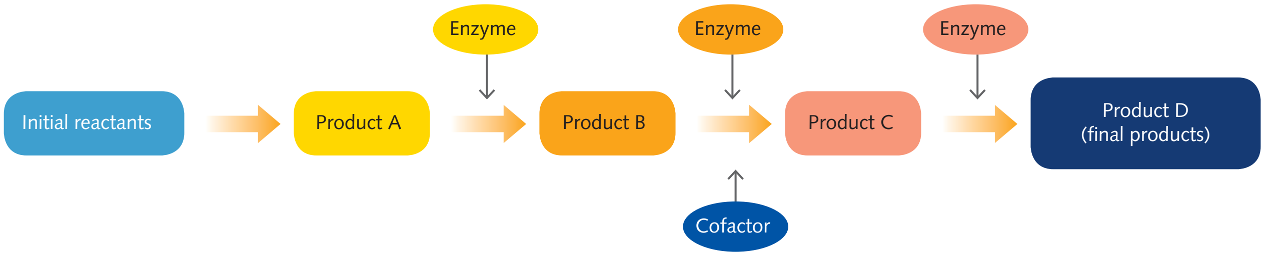


Figure 4.2 A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.

Biochemical pathways can be compared to other systems. A system has inputs, which are processed through a series of steps, and outputs. Cellular metabolism is the sum total of all the biochemical pathways or systems that occur in all living cells. Different cells have different requirements and rates at which they carry out processes. For example, heart muscle cells have a high rate of metabolism to keep the heart beating. The oxygen that is transported in blood is used for aerobic cellular respiration in the cardiac muscle cells to provide enough energy to keep the heart pumping.

Chemical reactions are reversible under certain conditions, and it is important that products are removed from a cell so that they do not accumulate and slow down vital metabolic reactions. To achieve this, cell biochemical reactions go through a series of steps in which the product of one step becomes the reactant for the next step (Figure 4.3, p. 139). In this way, a product from one reaction is continually removed by being the reactant for the next reaction.

EXAM TIP
Make sure that by the end of this chapter you can explain the ways in which metabolic reactions are regulated.

Cells have ways of removing the final product so that a biochemical pathway keeps operating in the right direction. In a plant cell, the final product of photosynthesis is glucose. Glucose, a soluble substance, is converted into the insoluble polysaccharide starch and other substances, which are stored by the plant. Thus, the plant can continue to produce and store more glucose. In cellular respiration, the products of the breakdown, carbon dioxide and water, diffuse from cells and are expelled into the atmosphere by different means.

In some cell biochemical pathways, when the product reaches an adequate or excess amount, the reaction slows down or stops temporarily. This occurs because the product is acting as a reversible inhibitor on one of the enzymes in the reaction (p. 149). If the enzyme is prevented from acting as a **catalyst**, the reaction will no longer occur, so no more product will be formed. Inhibition of the reaction is essential to conserve cell resources and energy by not making excess product. When the amount of product in the cell decreases as it is used up or removed from the cell, the inhibitory effect no longer exists and the biochemical reaction can begin again (Figure 4.3).

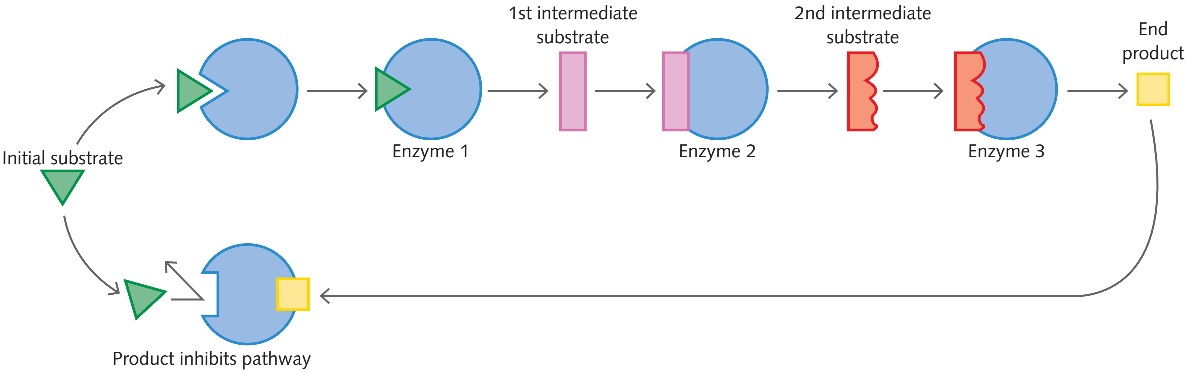


Figure 4.3 A biochemical pathway showing initial reactants, substrate–enzyme interactions, specific enzymes and final product. Excess end product can act to inhibit the reaction temporarily by binding to the enzyme.

KEY CONCEPTS

- Cellular metabolism refers to all the chemical reactions that occur in living cells.
- Anabolic reactions are endothermic, e.g. photosynthesis. Catabolic reactions are exothermic, e.g. cellular respiration.
- Exergonic reactions occur without an initial input of energy; endergonic reactions require an initial input of energy to start.
- Biochemical reactions occur in pathways that involve a series of regulated steps controlled by enzymes.

Concept questions 4.1a

1 Distinguish between the following pairs of energy terms and provide an example of each term.
  a Anabolic and catabolic
  b Endothermic and exothermic
  c Exergonic and endergonic
2 Define 'metabolism'.
3 Explain the statement: 'Endergonic reactions and exergonic reactions are interdependent'.
4 Why do chemical reactions in cells proceed in a series of steps called a biochemical pathway?
5 What is a biological catalyst?

HOT challenge

6 Enzymes often require cofactors or coenzymes to work. What effect will the absence of a specific cofactor or coenzyme have on a catalysed biochemical reaction?

Photosynthesis and cellular respiration

Photosynthesis and cellular respiration are both complex biochemical pathways, consisting of a series of steps from the initial reactants to the final products. Each step is catalysed or sped up by a specific enzyme acting as a catalyst. Coenzymes facilitate these steps. Conditions in the internal environment of cells can affect enzyme function. Factors that affect enzyme function include temperature, pH, substrate concentration and inhibitors.

Photosynthesis

Photosynthesis is the process by which photoautotrophs capture light energy and use it to convert water and carbon dioxide to glucose, water and oxygen. The light energy that is captured is stored as chemical energy in the bonds of the glucose molecule. The glucose can then be used as a structural or storage molecule for the plant, or as a fuel in cellular respiration to provide energy for the plant. Photosynthesis is summarised by the following equations:

$$12H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C_6H_{12}O_6 + 6H_2O$$

OR

$$6H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C_6H_{12}O_6$$

$$\text{Reactants/inputs} \longrightarrow \text{Products/outputs}$$

The process of photosynthesis occurs in **chlorophyll** molecules of plants and some single-celled protists. Photosynthesis is often shown as a simple reaction but it is actually a complex series of reactions, with each step within each reaction being catalysed by a specific enzyme or enzymes.

CONNECT

Photosynthesis will be discussed in detail in Chapter 5.

Cellular respiration

WB 4.1.3 SCIENTIFIC LITERACY PAGE 88

The energy trapped from the Sun and stored in the chemical bonds in glucose during photosynthesis can be released by the process of cellular respiration. Glucose is the main energy source molecule for multicellular organisms. Plants produce their own glucose to power cellular respiration, whereas animals ingest glucose and other complex substances that can be digested into glucose, in their food. As already discussed, the bonds within glucose molecules require energy to form; this energy is released when those bonds are broken. Glucose can be packaged into more dense forms for longer-term energy storage, such as **glycogen** in animals and starch in plants.

Cellular respiration is the process of breaking down glucose, either completely in aerobic cellular respiration using oxygen, or incompletely in anaerobic cellular respiration with no oxygen used. The purpose of this process is to release the energy stored in the bonds of glucose to form the energy-storage molecule **adenosine triphosphate (ATP)** – the 'energy bank' of cells, which can make energy available to cells when it is needed. ATP is the universal energy storage molecule for all living organisms, from bacteria to humans. ATP contains an adenosine molecule, attached to a sugar group (ribose), which is bound to a chain of three phosphate groups. ATP is formed by the addition of the third phosphate group to an **adenosine diphosphate (ADP)** molecule in a reversible reaction catalysed by the enzyme **ATP synthase** according to the following equation:

$$ADP + P_i \xrightleftharpoons{\text{ATP synthase}} ATP$$

where P_i represents an inorganic phosphate group.

The process of aerobic cellular respiration is summarised by the following equation:

$$C_6H_{12}O_6 + 6O_2 + 30 \text{ or } 32\ ADP + 30 \text{ or } 32P_i \longrightarrow 6CO_2 + 6H_2O + 30 \text{ or } 32\ ATP$$

Cellular respiration occurs in the cytosol and mitochondria of all living eukaryotic cells. It occurs in a series of steps, each catalysed by specific enzymes. Cells that carry out anaerobic respiration also do so in a series of steps; however, the process is simpler and results in the incomplete breakdown of glucose. These types of anaerobic respiration will be discussed in more detail in Chapter 5. The following word equations summarise anaerobic respiration in plant and animal cells:

- Plant cells: glucose ⟶ ethanol + carbon dioxide + 2ATP
- Animal cells: glucose ⟶ lactic acid + 2ATP

The energy given off when glucose is broken down is captured and stored in ATP molecules, ready for powering other biochemical pathways. The energy from ATP is used to build complex molecules, in cell division and growth, to maintain cell organisation, to move substances and in many other cell functions and activities (Figure 4.4).

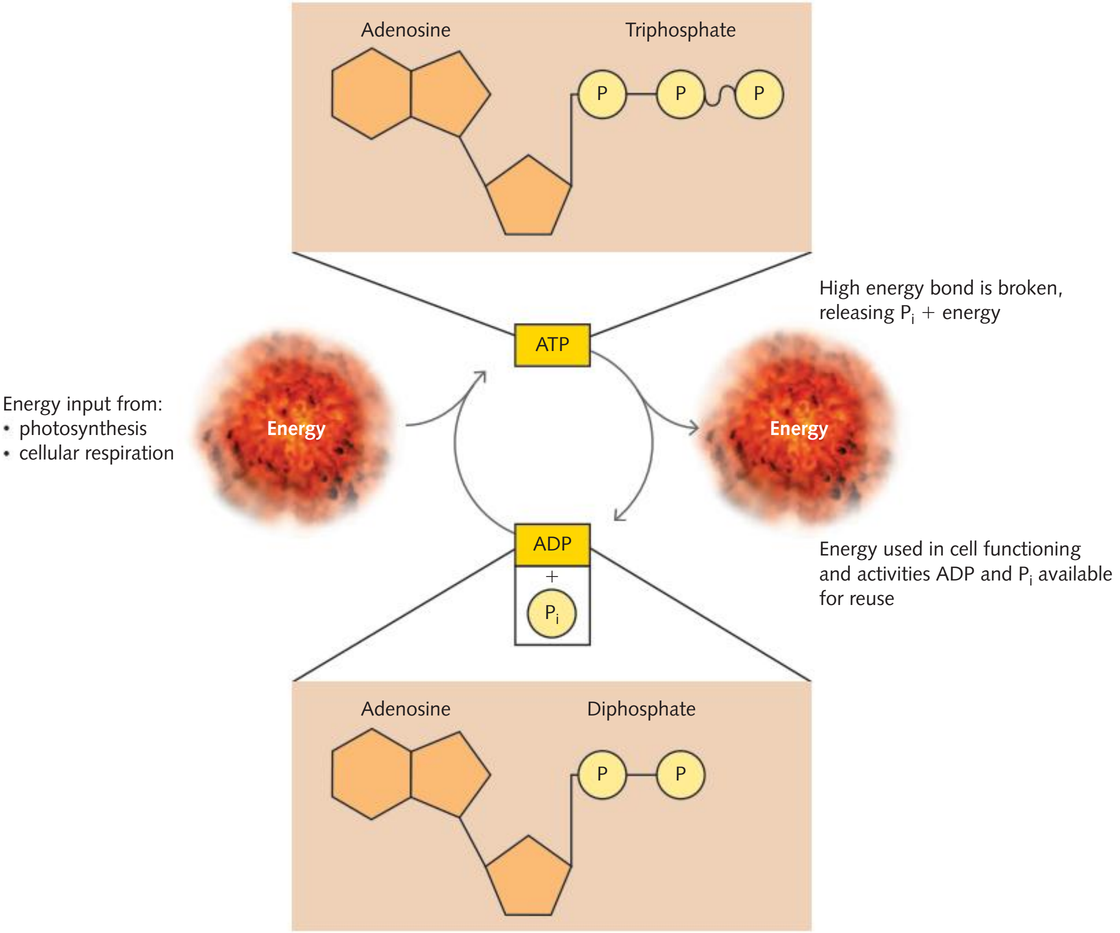


Figure 4.4 **The ATP–ADP cycle. The ATP–ADP system links energy-yielding processes to energy-requiring processes within the cell by conserving, transferring and releasing energy. Both photosynthesis and cellular respiration rely on the ATP–ADP conversions.**

Cells capture the chemical energy released from certain reactions to fuel other reactions that happen simultaneously. Because these reactions do not always occur in the same place within the cell, energy must be transferred between reactions. This transfer is achieved by molecules of ATP, but these molecules never leave the cell to transfer energy to other cells. All cells must independently carry out cellular respiration to produce ATP for their needs. In the process of cellular respiration, much of the energy, up to 60%, is lost as heat energy, which either helps maintain a relatively constant body temperature, or is lost to the environment.

KEY CONCEPTS

» The biochemical pathways involve inputs of initial reactants, formation of enzyme–substrate complexes at each step, and final outputs or products.

» Photosynthesis and cellular respiration are both essential biochemical reactions for life and involve biochemical pathways that are regulated by enzymes in a series of steps.

Concept questions 4.1b

1 What would happen to a biochemical reaction if the final product were not removed?

2 List five features of the ATP–ADP system.

3 Would an endergonic reaction produce ATP or ADP molecules? Explain your answer.

4 Where is the P_i that is used to synthesise ATP sourced from in:

   a animals

   b plants?

5 ATP synthase can be classified as what type of molecule? How do you know?

HOT challenge

6 Cellular respiration is strictly classified as occurring in the presence of oxygen, commonly called aerobic respiration. It produces molecules of ATP. Anaerobic respiration also produces ATP. Anaerobic respiration does not occur in the presence of oxygen. When anaerobic respiration occurs in the cytosol of an animal or plant cell, how many molecules of ATP are produced per molecule of glucose being catabolised? What happens to the rest of the energy that is not released?

4.2 Enzymes: the key to controlling biochemical pathways

It is very busy inside living cells: billions of molecules are randomly moving around, colliding with each other. Most molecules bounce off and continue with their motion but when a specific substrate collides with the correct enzyme at the right orientation, an enzyme–substrate complex is formed, and then dissociates to release the product.

Enzymes are not consumed in the reaction, but are recycled. Enzymes are the ‘workhorses’ of the cell. Without enzymes, the reactions that occur in living organisms would be so slow as to hardly proceed at all, which could result in cell death.

Normally, an enzyme is named by attaching the suffix ‘-ase’ to the name of the substrate on which it acts: for example, proteases act on proteins, and ATPase acts on ATP. However, not all enzymes end in ‘-ase–; for example, Rubisco, an enzyme involved in photosynthesis.

EXAM TIP

If you see a word ending in ‘-ase’ it is probably an enzyme, but be aware that not all enzymes end in ‘-ase’.

Each step in a biochemical pathway is controlled and regulated by a specific enzyme. More than 1000 different reactions can take place in an individual cell at any given time. Enzyme specificity is why enzymes can control each step in a biochemical pathway. There are a multitude of enzymes in living organisms, and each enzyme acts on a specific substrate or small number of substrates.

Each enzyme can also be regulated by:

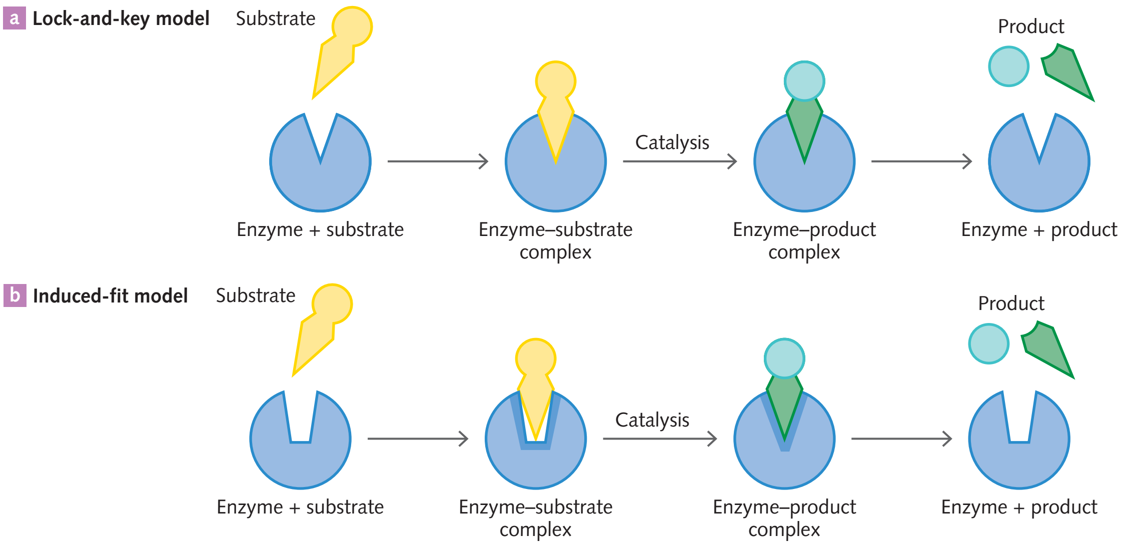
» the availability of its substrate
» cofactors or coenzymes that it needs to be able to function
» other enzymes that physically modify its structure to control its activity
» inhibitors
» environmental factors, such as temperature and pH.

This means that a highly complex series of reactions can be carefully regulated, switched on and off according to the needs of the cell.

Enzyme specificity

WB 4.2.1 SPECIFICITY OF ENZYMES PAGE 90

Most enzymes are large globular proteins. In Chapter 3, you examined the tertiary structure of proteins and learnt how their shape determines their function. The folding of the polypeptide chain in an enzyme into its tertiary structure forms a groove or pocket called its **active site**. The shape of each active site is highly specific for a particular substrate, which must have a compatible shape for binding to occur. This model of enzyme action is known as the **lock-and-key model** (Figure 4.5a).



EXAM TIP
When you discuss the active site, ensure that you describe the active site within an enzyme. There is no active site on a substrate.

Figure 4.5 **Enzyme action. a In the lock-and-key model, the binding of the substrate into the active site of an enzyme mirrors a door's lock-and-key mechanism. The substrate's shape is complementary to the shape of the active site within the enzyme. b In the induced-fit model, the substrate molecule enters the enzyme's active site, causing the enzyme molecule to change shape so that the two molecules fit together more closely.**

The bonds that form between an enzyme and its substrate can also modify the shape of the active site so that the substrate can fit 'snugly' into the active site. This interaction is called the **induced-fit model** of enzyme action (Figure 4.5b). In this situation, the bonds within the substrate molecule are stretched and bent and, as a result, the **activation energy** required to initiate the reaction is dramatically lowered and new product molecules are formed at a faster rate. As the product molecules are not specific to the active site, they no longer bind to the enzyme and are released.

Weblink
Enzymes, active sites and cofactors

Online Worksheet
Enzymes in biochemical pathways

Cofactors and coenzymes

Some enzymes are inactive until they bind with other molecules or ions that change their conformation. This alters the shape and the charge of the enzyme's active site so that it can capture substrate molecules and catalyse reactions more efficiently. Two classes of substance bind to enzymes or to substrates to activate enzymes: cofactors and coenzymes.

Cofactors are inorganic molecules that include metal ions such as magnesium (Mg^{2+}), zinc (Zn^{2+}) and iron (Fe^{2+}).

Coenzymes are non-protein organic substances. They are relatively small molecules compared with enzymes. During these biochemical pathways, coenzymes are reversibly **loaded** and **unloaded** with the groups of atoms they carry. Examples of coenzymes are **NADH** (nicotinamide adenine dinucleotide), **NADPH** (nicotinamide adenine dinucleotide phosphate), **$FADH_2$** (flavin adenine dinucleotide) and ATP. They are essential for many of the steps in photosynthesis and cellular respiration. These molecules can accept electrons and protons and chemical groups such as phosphates during biochemical reactions and transfer them to another reaction in a different step of the process. These coenzymes recycle as they transfer a chemical group from one molecule to the next molecule. In this way, energy is transferred within different stages of the biochemical reactions of photosynthesis and cellular respiration.

The unloaded form of a coenzyme accepts an electron, a proton or a chemical group. It is now loaded because it is storing chemical energy in the bonds between the coenzyme and the chemical group. The energy to drive the reactions in the intermediate steps of the pathways comes from the bonding and releasing of the protons that occur along a concentration gradient. ATP acts as a coenzyme and relies

WB
4.2.2
ENZYMES NEED HELP: COENZYMES AND COFACTORS
PAGE 91

CONNECT
The coenzymes involved in photosynthesis are discussed in more detail in Chapter 5.

EXAM TIP
Make sure you know the loaded and unloaded forms of each coenzyme in Table 4.1.

upon ATP synthase to cycle between ATP and ADP and inorganic phosphate. ATP and other coenzymes are essential to drive reactions of photosynthesis and cellular respiration.

Many intermediate steps in the photosynthesis and cellular respiration biochemical pathways rely on coenzymes. Coenzymes lower the activation energy of each step in the pathway to ensure each reaction is initiated and occurs. For example, in the first stage of cellular respiration, which is called **glycolysis**, glucose is broken down into two pyruvic acid molecules, releasing energy and two hydrogen (H) atoms. The unloaded forms of the coenzymes, **NAD^+** and ADP, pick up the H atoms and energy to produce the loaded coenzyme molecules, NADH and ATP (using another inorganic phosphate ion) (Figure 4.6). When oxygen is present, the two pyruvic acid molecules move into the mitochondria and a series of reactions in the Krebs cycle produces more loaded NADH and another coenzyme, $FADH_2$. All these loaded coenzyme molecules from the two stages drive the reactions that occur in the **electron transport chain**. In this way, coenzymes are loaded and unloaded to move energy, protons, and electrons between reactions in the cell and ultimately generate the necessary ATP required by the cell. (See Table 4.1.)

Photosynthesis involves a series of steps in two major stages. In the first stage, water molecules are split by light energy into oxygen, protons and electrons, and energy is released. They are used to convert the unloaded coenzymes ADP and **$NADP^+$** into the loaded coenzymes, ATP and NADPH. These coenzymes are then used in the reactions of the second stage of carbon fixation to ultimately produce glucose. In this stage, ATP releases energy and NADPH releases H^+ ions to be used in carbon fixation, thus producing ADP and $NADP^+$ that can be recycled and used again in the first stage.

Table 4.1 Loaded and unloaded forms of some coenzymes

Loaded	Unloaded
ATP	ADP
NADH	NAD^+
$FADH_2$	FAD
NADPH	$NADP^+$

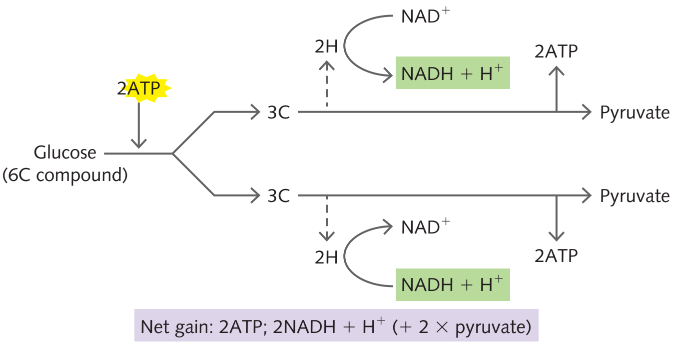


Figure 4.6 The first stage of cellular respiration is glycolysis.

EXAM TIP
Remember that an enzyme's active site is changed during denaturation; it is not the substrate's shape that changes.

KEY CONCEPTS

- Enzymes are biological catalysts that interact with substrate molecules to increase the rate of a reaction.
- Enzymes lower the activation energy required for a biochemical reaction to proceed.
- Substrate molecules fit into the specific active site of an enzyme, according to lock-and-key or induced-fit models.
- Coenzymes may be reversibly loaded and unloaded. They are required for enzyme activation in cellular respiration and photosynthesis.

Concept questions 4.2

1 Define 'catalyst'.
2 What is the role of enzymes in a cell?
3 What happens to an enzyme after it has catalysed a reaction?
4 How do enzymes affect the activation energy of a reaction?
5 Explain what is meant by the induced-fit model of enzyme action. How is this different from the lock-and-key model?

HOT challenge

6 Table 4.1 lists examples of loaded and unloaded coenzyme molecules.
   a For each example, name the particle that has been unloaded to form the unloaded version of the coenzyme.
   b NADPH is a coenzyme involved in photosynthesis. What do you think the source of the H may be in the loaded molecule?

4.3 Photosynthesis and cellular respiration

Enzymes for photosynthesis and cellular respiration function in the intracellular environment of the cell, in both the cytosol and the organelles – the **chloroplasts** for photosynthesis and the mitochondria for cellular respiration. The environment in the cells must provide the conditions necessary for optimal functioning of the enzymes. This will ensure efficient cell functioning and therefore cell survival. Enzymes are sensitive to changes in substrate concentration, temperature and pH and the presence of substances such as inhibitors.

Weblink
Enzyme-mediated reaction under different conditions

Online Worksheet
Enzyme catalysis

WB
4.3.1 EFFECT OF TEMPERATURE PAGE 93

Effect of temperature

The activity of enzymes increases with increasing temperature. This is because molecules gain more energy, move around more quickly, and collide more often. As the number of collisions increases, so does the frequency of substrate molecules entering the active sites of enzyme molecules. Therefore, the rate of the reaction increases as the temperature rises. As temperature continues to increase, enzyme activity reaches a peak at the **optimum temperature** (Figure 4.7). This is the temperature at which the enzyme works at its fastest. However, if the temperature gets too high, the bonds that determine the three-dimensional shape of the enzyme proteins will break. As a result, the protein loses its functional shape. It becomes permanently denatured and the substrate can no longer fit into the active site. The enzyme's activity stops.

At low temperatures, molecular movement slows and so fewer collisions occur between the substrate and enzyme molecules. The rate of the reaction decreases. If the temperature increases again, the molecules begin to move faster as they gain energy. Since the binding in the enzyme protein and its three-dimensional shape have not been altered, the rate of the biochemical reaction will begin to increase.

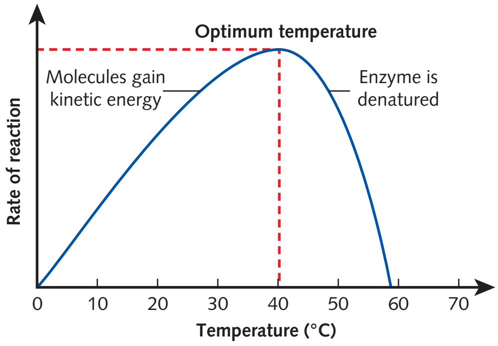


Figure 4.7 The effect of temperature on enzyme activity.

Different enzymes have different optimum temperatures. This reflects the conditions in which the organism is normally found and if it can regulate its body temperature. Enzymes in the human body work best at temperatures of about 37°C, which is the relatively constant core temperature of the body. This means cellular respiration in human cells is optimum at this temperature. The enzymes of other mammals and birds have optimum temperatures that reflect their body temperatures. For example, cattle have an optimum body temperature of 38.5 degrees C and pigs 39 degrees C; birds have higher optimum body temperatures such as 42 degrees C for chickens and ducks.

WB
4.3.2 EFFECT OF CHANGING pH PAGE 96

Effect of changing pH

The pH of the solution surrounding enzymes also affects their structure and the activity of their active site, as well as their interactions with substrate molecules. Each enzyme has an **optimum pH** at which it works fastest (Figure 4.8). Some enzymes can work in a wide range of pH environments, while others are very sensitive and will only work in a narrow pH range. Most enzymes work most effectively around a neutral pH of 7. Like optimum temperature, the optimum pH relates to the environment in which the enzyme works.

A neutral pH of 7 is optimum for both photosynthesis and cellular respiration. Cellular solutions need to be buffered because proteins influence the pH of a solution by donating hydrogen ions or hydroxyl ions. A buffer solution usually contains a weak acid and one of its salts,

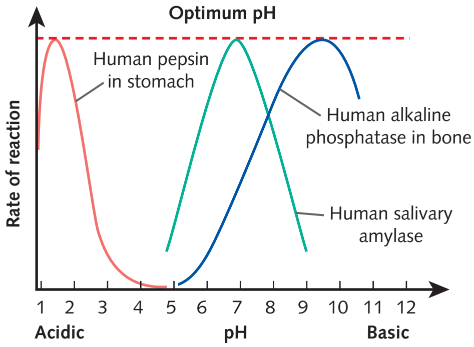


Figure 4.8 The pH range for three different enzymes: pepsin digests proteins in the acidic juices of the stomach; salivary amylase digests carbohydrates in the mouth at a neutral pH; and alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone.

and its pH remains relatively stable while hydrogen ions are released or absorbed during chemical reactions. Changes in pH affect the amino acids making up a protein and therefore the enzymes. If the charges on the amino acids in a protein change, then the bonds that maintain the three-dimensional structure of a protein may be broken. In most cases, if the pH varies, the protein shape is altered so much that the enzyme becomes denatured and can no longer catalyse a reaction. A buffered solution prevents fluctuations in pH while chemical reactions are releasing and taking up hydrogen ions, and this protects the structure of enzymes. Because the chemical reactions in photosynthesis and cellular respiration involve uptake and release of hydrogen ions from the coenzymes, it is important that cells contain buffered solutions so that the enzymes are not affected by changes in pH.

Effect of substrate and enzyme concentration

WB
4.3.3 EFFECT OF SUBSTRATE AND ENZYME CONCENTRATION
PAGE 99

The amount of enzyme present in cells can limit the reaction rate and amount of product formed. With a certain fixed amount of enzyme in the cell, increasing amounts of substrate result in more product being made, until the substrate concentration reaches a point where all the enzyme molecules are working at their maximum capacity (Figure 4.9). This is the saturation point.

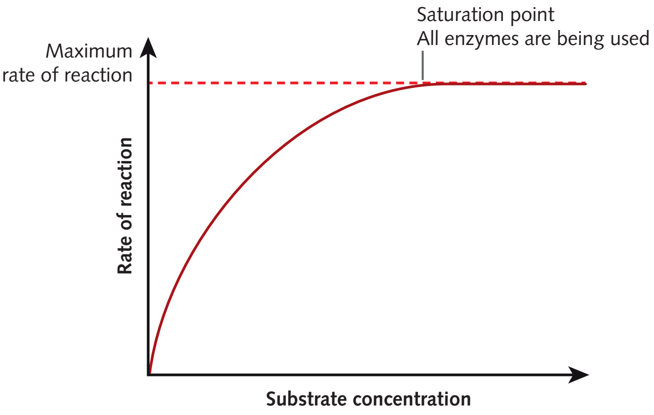


Figure 4.9 **The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At the saturation point, further increases in substrate concentration do not increase the rate of the reaction.**

At higher concentrations of carbon dioxide, photosynthetic cells increase their rate of reaction if adequate light and water are available. However, at a certain point, the enzymes in the photosynthetic cells may reach saturation point. If this happens, the rate of photosynthesis will become constant. For example, during the second stage of photosynthesis, carbon dioxide is the substrate for the enzyme Rubisco, which converts carbon dioxide and ribulose bisphosphate (a five-carbon molecule) to a six-carbon molecule that ultimately splits into two three-carbon molecules that form the building blocks for glucose. As the concentration of carbon dioxide increases, the rate of photosynthesis also increases, until a plateau is reached. The rate of photosynthesis most likely becomes constant because Rubisco is saturated and cannot work any faster.

This also applies to all living cells that undergo cellular respiration. If glucose is available in unlimited supply, enzymes may limit the rate of the reaction if the amount of enzyme available in the respiring cells becomes saturated, as seen in the graph in Figure 4.9.

Enzyme concentrations are regulated in response to the needs of a cell. This regulation is achieved by:

- controlling the expression of the enzyme
- controlling the rate of degradation of the enzyme
- activating the enzyme in response to a stimulus.

Stress-tested by Southern Biological

INVESTIGATION 4.1

Effect of temperature on enzyme activity

Enzymes are proteins and are therefore affected by the same factors that affect all proteins; for example, heat. All enzymes have an optimum temperature at which they work best. The enzyme studied in this investigation is an intracellular enzyme; that is, it speeds up and controls metabolism within cells.

Amylase breaks down starch molecules into separate glucose molecules. Iodine is a stain that turns blue-black in the presence of starch, but stays yellow-brown in the absence of starch. Therefore, the colour of iodine indicates whether starch is present.

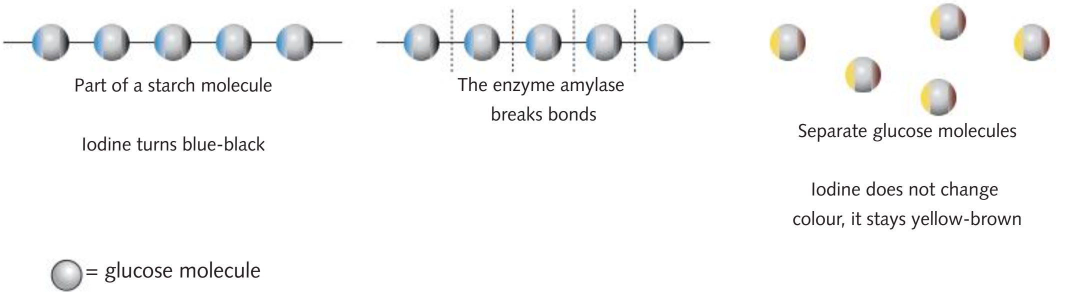


Figure 4.10 **Amylase breaks down starch to glucose.**

Aim

To test the effect of temperature on enzyme function

Materials

- 2% amylase solution
- 5% starch solution
- Iodine solution
- 10 test tubes and test-tube rack
- Large beakers to hold test tubes
- Clock or timer
- Thermometer
- 5 mL pipette
- 10 mL pipette
- Toothpicks
- Spotting tile
- Ice water
- 4 water baths

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Iodine can stain skin and clothing.	Take care to avoid spilling iodine on skin and clothing.
Hot water baths can burn.	Do not touch the sides of the water bath or the water in it.

Method

1. Set up four water baths at 20°C, 40°C, 60°C and 80°C. Put some ice water in a beaker; this is your 0°C water bath.
2. Add 10 mL of starch solution to five test tubes.
3. Add 5 mL of amylase solution to another five test tubes.
4. Place one starch and one amylase test tube into each water bath for 5 minutes so that the enzyme and substrate both reach the desired temperature. You will need to use beakers to hold the test tubes.
5. Place 1 drop of iodine into each well on the spotting tile.
6. Add the amylase to the starch in the 0°C water bath.

7 Start the timer.
8 Every minute, remove a sample of the starch–amylase solution and add it to a drop of iodine on the white tile. Mix with a clean toothpick.
9 Repeat step 8 until the iodine no longer changes colour. This is when there is no starch left.
10 Record your results.
11 Repeat steps 6–10 for each of the temperatures.

Results

1 Copy the results table into your logbook. Record your results in the table.

Results of amylase activity at different temperatures

Temperature (°C)	Time to digest starch (min)
0	
20	
40	
60	
80	

2 Draw a suitable graph to show the time taken for starch to be digested at different temperatures.

Discussion

1 List the dependent and independent variables in this investigation.
2 List three extraneous variables and describe how they were controlled.
3 Explain how the colour produced with iodine indicates the activity of the enzyme. Explain why the colour changes throughout the testing time in some test tubes.
4 Predict the optimum temperature for amylase activity. Justify your answer from the results.
5 Are there any temperatures where amylase does not appear to function? Explain why this might occur.
6 Describe one improvement you could make to this method to show that it is the action of amylase that is causing the breakdown of starch to glucose.

Conclusion

Draw a conclusion on the effect of temperature in determining amylase activity.

Taking it further

1 Modify the above method to design an experiment that investigates the effect of pH on enzyme activity. If possible, carry out the experiment.
2 Fever may cause a person's body temperature to rise above the normal level. Discuss how this could affect cellular activity and in turn the entire body.

Enzyme inhibitors

Enzyme inhibitors change the rate at which a chemical reaction occurs, either slowing it down or stopping it completely.

WB
4.3.4
ENZYME INHIBITORS
PAGE 100

EXAM TIP
Ensure you can label a diagram to show how the attachment of an inhibitor prevents the substrate from binding to the active site of the enzyme.

Non-competitive inhibitors

Non-competitive inhibitors are molecules that bind to a part of the enzyme that is not at the active site, called the **allosteric site** (Figure 4.11b). This alters the structure of the enzyme in such a way that the active site of the enzyme changes shape, and no longer has the complementary shape for the substrate to bind. Some enzymes have two or more active binding sites. These enzymes can move between their active and inactive state when inhibitor or activator molecules bind with them.

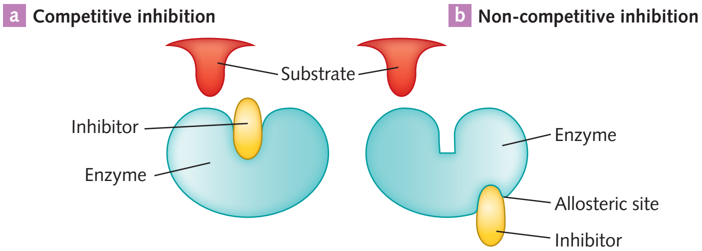


Figure 4.11 a A competitive inhibitor molecule blocks the enzyme's active site. **b** A non-competitive inhibitor binds to the allosteric site, which is not the active site.

The activity of many enzymes is regulated by **feedback inhibition**, in which the output of a process is used to limit the production of more of the product (Figure 4.3). If a large amount of product is present in the cell it will act as an inhibitor by binding to a site on the enzyme other than the active site, thus slowing the rate of reaction. If the product is removed, then inhibition will be reduced, the substrate can enter the active site, and the product will again be formed. This helps cells keep the concentration of products within a certain range.

The importance of non-competitive inhibition can be shown when a cell no longer requires ATP – cellular respiration is switched off to prevent the cell wasting resources and energy. This is also an example of feedback inhibition. ATP acts as a non-competitive inhibitor of another key enzyme, pyruvate kinase, which catalyses the final step in glycolysis. The inhibition of pyruvate kinase stops the cells from breaking down more glucose when a certain threshold of ATP is present in the cell. This prevents the overproduction of ATP and stops unnecessary cell resources and energy from being used.

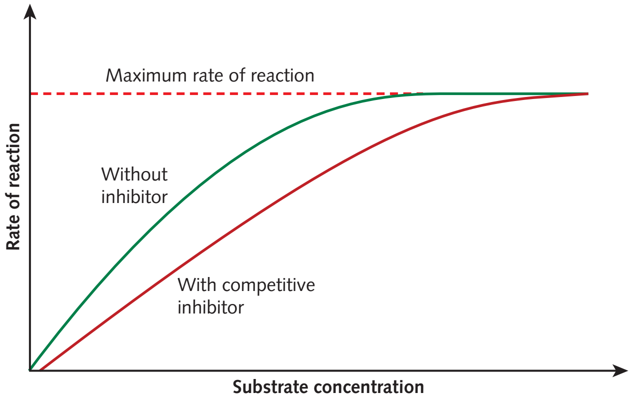


Figure 4.12 The effect on rate of reaction with and without a competitive inhibitor. Addition of a competitive inhibitor results in fewer enzyme–substrate complexes forming and lowers the overall rate of a biochemical reaction. If there is plenty of substrate (towards the right of the horizontal axis), the inhibitor is effectively outnumbered, the substrate gains entry to all the active sites of the enzyme molecules and the maximum rate of reaction is reached.

Competitive inhibitors

Competitive inhibitors compete directly with the substrate for space in the active site and prevent the substrate from binding (Figure 4.11a), either lowering the rate (Figure 4.12) or stopping the reaction from taking place. The shape of the inhibitor molecule is complementary to the shape of the active site of the enzyme, so it can bind in place of the substrate.

Cyanide is a competitive inhibitor of a key enzyme, cytochrome c oxidase, which is involved in the electron transport chain – the last stage of aerobic cellular respiration. When cyanide binds to the key substrate, cytochrome c, cells can no longer produce the full complement of ATP. Therefore, cells need to switch to anaerobic respiration. This results in a build-up of lactic acid in the blood, which can be fatal.

Herbicides used on plants block the action of key enzymes involved in the biochemical pathways of the two stages of photosynthesis. Most of these inhibiting molecules block the transfer of electrons from one molecule to the next; without electron transfer, plants cannot produce glucose.

CONNECT

The Krebs cycle is discussed in detail in Chapter 5.

The second stage of cellular respiration, called the Krebs cycle, can be controlled by the enzyme fumerase, which acts as a competitive inhibitor on intermediate molecules. If the concentration of products starts to build up, this pathway can be slowed down; if the concentration of the products is low, the process can be sped up (Figure 4.13).

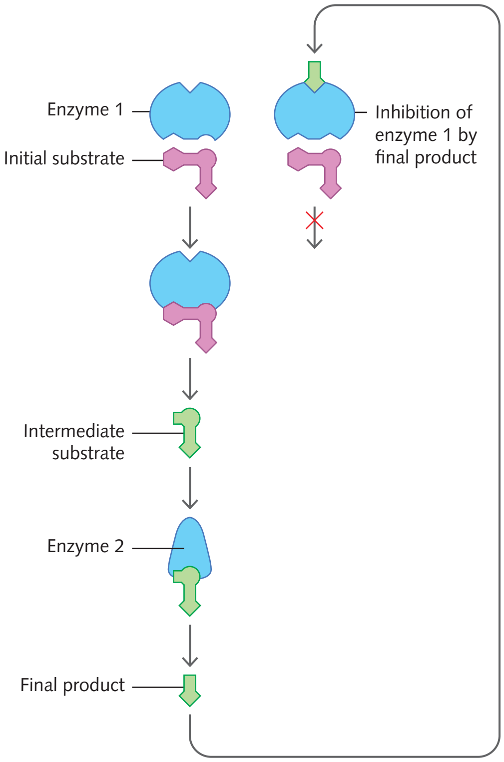


Figure 4.13 An example of end product inhibition

KEY CONCEPTS

- Enzymes are sensitive to temperature. Lower temperatures reduce activity; higher temperatures can denature enzymes, making them permanently inactive.
- Enzymes are sensitive to pH, which will affect rates of biochemical reactions.
- The relative concentrations of enzyme and substrate can affect the rate of a biochemical reaction.
- Temperature, pH and concentration of inputs can affect the rate of biochemical reactions in photosynthesis and cellular respiration.
- A non-competitive inhibitor alters an enzyme's activity by changing its conformation without binding to its active site.
- A competitive inhibitor blocks the active site of an enzyme.
- The actions of inhibitors can be reversible or irreversible and can block key components of both cellular respiration and photosynthesis.

Concept questions 4.3

1 How can the amount of product produced in a reaction affect an enzyme's activity?

2 Distinguish between a non-competitive inhibitor and a competitive inhibitor.

3 A human protease works best at 37°C.

  a What would happen to the enzyme's activity at very low temperatures?

  b How does this differ from the activity of the enzyme at very high temperatures?

  c What has happened to the active site in both cases?

4 Explain what happens to the protein structure of an enzyme that becomes denatured.

HOT challenge

5 Explain, using a diagram, how the saturation point of an enzyme can be reached. Make sure you discuss any contributing factors and what part the substrate plays.

BRANCHING OUT

Drug design

Scientists are continually striving to discover new drugs to treat diseases. By looking closely at the molecular structure of a target protein (or an enzyme), scientists can design a molecule that can bind to the target and interfere with its normal function. Much like fitting a jigsaw piece into the complementary gap in a growing puzzle, a carefully designed drug can fit the exact shape of a target protein and block its action. This is called rational drug design. Rational drug design was used when an Australian team of scientists at CSIRO and Monash University, led by Dr Peter Colman, developed a drug called Relenza® to prevent the influenza virus from infecting human lung cells.

A drug is any biological substance that is taken for non-dietary needs. This substance can occur naturally or be synthetic (man-made). When drugs are introduced into an organism, they produce a characteristic action or effect that alters some bodily function. This can include relieving symptoms of a disease, even curing it in some circumstances.

Some drugs, such as vaccines, may prevent a particular disease from occurring. Many students confuse drugs with antibodies. A drug may have a similar mode of action to an antibody, in how they inhibit the activity of a pathogen, but drugs and antibodies are not the same. A vaccine can be considered a drug that induces the immune system to produce antibodies and memory cells against specific pathogens.

Drugs work by interacting with receptor molecules on target cells in our bodies. This interaction leads to a change in the target cells' activities that is usually beneficial to our health. This may involve either stimulating or inhibiting certain activities of the cell.

This is much the same for naturally occurring chemical messengers within the body, such as hormones. In many ways, a drug is a chemical messenger but, unlike hormones, drugs are synthesised outside the body. For example, insulin is a hormone that is normally synthesised in the body in response to high blood glucose levels. People with type 1 diabetes do not produce insulin – but they can administer synthetic insulin, a form of insulin that could be considered a drug.

The mechanisms and principles of how cells detect and respond to signals apply in much the same way, except the stimulus and messenger molecule are the drug itself.

Drugs can have side effects. Many of these occur when the drugs interact with receptors that are not on target cells, or inhibit the normal activity of the cell, producing unfavourable reactions. One of the challenges in discovering new drugs is to find those that are specific to the target cells, and do not disrupt the normal functioning of the host.

Questions

1 How is the specificity of a drug determined?

2 How is the study of enzymes helping the evolution of drugs to treat human diseases?

3 Explain the advantages of using rational drug design over conventional methods of designing drugs.

Online key concepts
Chapter 4: Summary of key concepts

4 Summary of key concepts

4.1 Biochemical pathways for cell metabolism

KEY CONCEPTS

p. 137

- Cellular metabolism refers to all the chemical reactions that occur in living cells.
- Anabolic reactions are endothermic, e.g. photosynthesis. Catabolic reactions are exothermic, e.g. cellular respiration.
- Exergonic reactions occur without an initial input of energy; endergonic reactions require an initial input of energy to start.
- Biochemical reactions occur in pathways that involve a series of regulated steps controlled by enzymes.
- The biochemical pathways involve inputs of initial reactants, formation of enzyme–substrate complexes at each step, and final outputs or products.
- Photosynthesis and cellular respiration are both essential biochemical reactions for life and involve biochemical pathways that are regulated by enzymes in a series of steps.

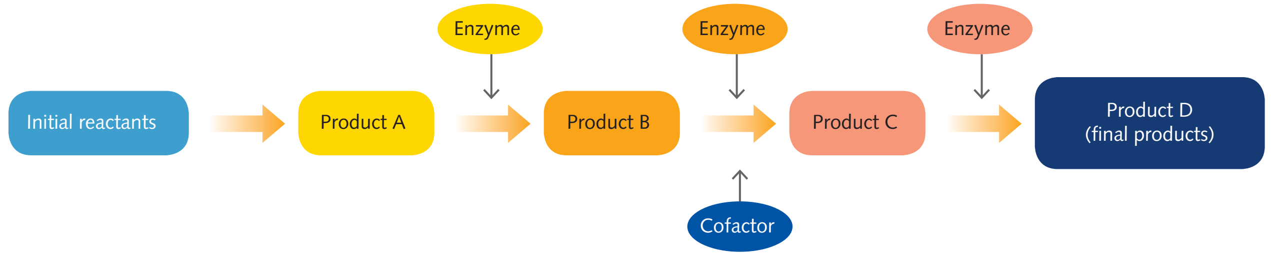


Figure 4.2 A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.

4.2 Enzymes: the key to controlling biochemical pathways

KEY CONCEPTS

p. 142

- Enzymes are biological catalysts that interact with substrate molecules to increase the rate of a reaction.
- Enzymes lower the activation energy required for a biochemical reaction to proceed.
- Substrate molecules fit into the specific active site of an enzyme, according to lock-and-key or induced-fit models.
- Coenzymes may be reversibly loaded and unloaded. They are required for enzyme activation in cellular respiration and photosynthesis.

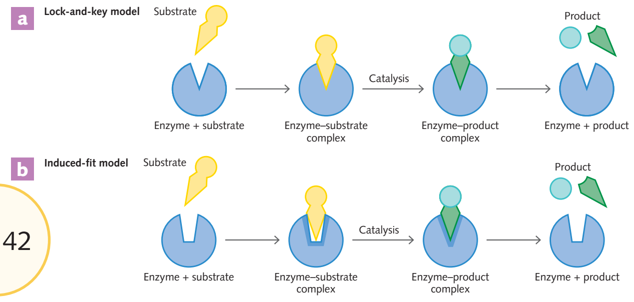


Figure 4.5 Enzyme action. **a** In the lock-and-key model, the binding of the substrate into the active site of an enzyme mirrors a door's lock-and-key mechanism. The substrate's shape is complementary to the shape of the active site within the enzyme. **b** In the induced-fit model, the substrate molecule enters the enzyme's active site, causing the enzyme molecule to change shape so that the two molecules fit together more closely.

4.3 Photosynthesis and cellular respiration

KEY CONCEPTS

p. 145

- » Enzymes are sensitive to temperature. Lower temperatures reduce activity; higher temperatures can denature enzymes, making them permanently inactive.
- » Enzymes are sensitive to pH, which will affect rates of biochemical reactions.
- » The relative concentrations of enzyme and substrate can affect the rate of a biochemical reaction.
- » Temperature, pH and concentration of inputs can affect the rate of biochemical reactions in photosynthesis and cellular respiration.
- » A non-competitive inhibitor alters an enzyme's activity by changing its conformation without binding to its active site.
- » A competitive inhibitor blocks the active site of an enzyme.
- » The actions of inhibitors can be reversible or irreversible and can block key components of both cellular respiration and photosynthesis.

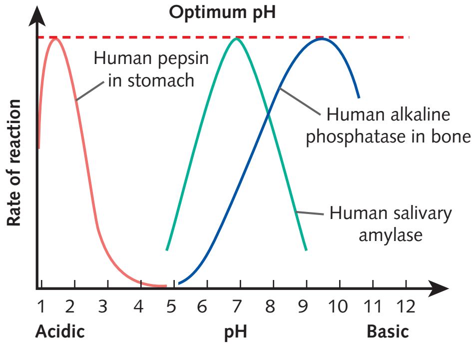


Figure 4.8 The pH range for three different enzymes: pepsin digests proteins in the acidic juices of the stomach; salivary amylase digests carbohydrates in the mouth at a neutral pH; and alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone.

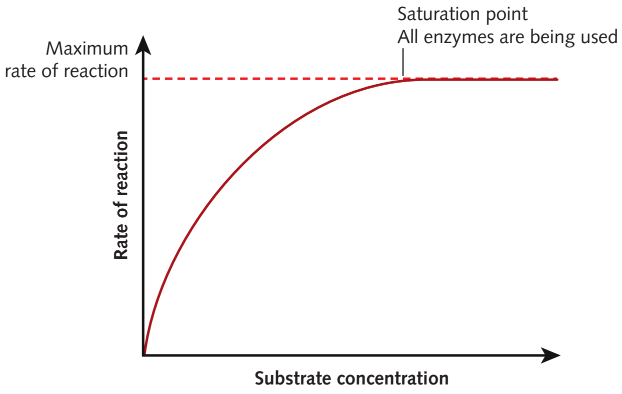


Figure 4.9 The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At the saturation point, further increases in substrate concentration do not increase the rate of the reaction.

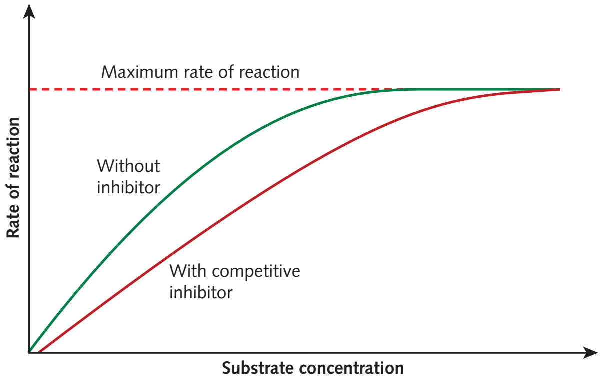


Figure 4.12 The effect on rate of reaction with and without a competitive inhibitor. Addition of a competitive inhibitor results in fewer enzyme–substrate complexes forming and lowers the overall rate of a biochemical reaction. If there is plenty of substrate (towards the right of the horizontal axis), the inhibitor is effectively outnumbered, the substrate gains entry to all the active sites of the enzyme molecules and the maximum rate of reaction is reached.

WB
4.4.1
KEY TERMS
PAGE 102

4 Chapter glossary

activation energy the energy required to initiate a reaction

active site the place on the surface of an enzyme molecule where substrate molecules attach

adenosine diphosphate (ADP) a low-energy compound made of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound made of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell

allosteric site a binding site on a protein (usually an enzyme), that is not part of the active site; binding of a specific molecule at this site results in a change in activity of the protein

anabolic reaction a reaction in which atoms and small molecules are joined together to make larger molecules

anaerobic cellular respiration cellular respiration in the absence of oxygen

ATP synthase an enzyme that provides energy for the cell through synthesis of ATP

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to a final product

catabolic reaction a reaction in which larger molecules are broken down into smaller molecules

catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change

cellular metabolism the sum of metabolic reactions in a cell

cellular respiration a process occurring in all living cells where large molecules are broken down to release energy

chlorophyll the green pigment in plant chloroplasts; it absorbs light energy, making it available for photosynthesis

chloroplast a membrane-bound organelle containing chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates

coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction

cofactor a molecule that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction

competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity

electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the addition of phosphate to ADP to yield ATP

endergonic reaction a chemical reaction that requires the input of energy for it to proceed

endothermic reaction a reaction that absorbs energy from its surroundings

exergonic reaction a spontaneous reaction that releases energy

exothermic reaction a chemical reaction that releases energy, usually in the form of heat or light

$FADH_2$ the loaded form of flavin adenine dinucleotide, a coenzyme that acts in both cellular respiration and photosynthesis

feedback inhibition a control mechanism used by cells in which an enzyme's activity is stopped or reduced by the product

glycogen an energy-storage polysaccharide in animals that is made of many connected glucose molecules

glycolysis an energy-yielding process occurring in the cytosol of cells in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

induced-fit model a model of enzyme action that explains that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

lactic acid a product of anaerobic cellular respiration in animals

loaded carry protons, electrons or chemical groups that are needed for anabolic reactions to occur

lock-and-key model a model of enzyme action that suggests that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

NAD^+ the unloaded form of the nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

NADH the loaded form of nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

$NADP^+$ the unloaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

NADPH the loaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

optimum pH the pH at which an enzyme works fastest

optimum temperature the temperature at which an enzyme works fastest

photosynthesis the anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose

reactant the inputs of a chemical reaction that are required to form products or outputs

substrate a substance on which an enzyme acts; a reactant for an enzyme-controlled reaction

unloaded can accept protons, electrons or chemical groups that are released from catabolic reactions

4.4.2
EXAM PRACTICE
PAGE 103

4 Chapter review

Remembering

1 Identify each of the following as an anabolic or a catabolic process. Justify your choice.
  - a Protein synthesis
  - b Digestion
  - c DNA synthesis
  - d Photosynthesis
  - e Cellular respiration

2 What is added to ADP to produce ATP? What is the significance of this in maintaining a cell's energy supply?

3 Enzymes are responsible for the production of sperm and male sex hormones in the testicles of human males. Some of these enzymes have an optimal temperature of about 33°C, which is about 4°C lower than body temperature. If this temperature is increased or lowered, sperm and testosterone production is adversely affected.
  - a Why would an increase in temperature affect sperm production?
  - b Draw a graph to show reaction rate of the enzyme responsible for sperm production against temperature.

Understanding

4 Amylase, pepsin, trypsin and lipase are human enzymes. For each type of enzyme, find out:
  - a the general substrate
  - b where it is most active in the human body
  - d the optimum temperature
  - e the optimum pH.

5 DNA ligases catalyse the formation of a phosphodiester bond between single strands of DNA. DNA ligases are present in all living organisms. In eukaryotic cells, the cofactor is ATP. ATP is broken to down to AMP (adenosine monophosphate). From your knowledge of the formation of ATP, how might the formation of AMP occur in this process?

6 Why are DNA ligases mostly found in the nucleus of a cell?

7 What is the difference between a loaded coenzyme and an unloaded coenzyme?

8 The activation energy of a reaction is important. If the activation energy is not reached, then the reaction does not proceed. How do enzymes aid in that process?

9 The following equation is sometimes used to summarise enzyme action. Use your own knowledge to interpret what this equation means.

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

Applying

10 The naturally occurring enzyme polyphenol oxidase causes many cut fruits to brown quickly when exposed to air. Rubbing freshly cut fruit with lemon juice can prevent the brown discoloration. Explain why this happens.

11 Cyanide binds to the enzyme cytochrome oxidase, preventing it from transferring electrons to the final acceptor molecule, oxygen, in aerobic cellular respiration.
  - a Where in the cell would cyanide target this enzyme?
  - b Explain why cyanide is such a fast-acting poison that results in the death of the organism.

12 The pH of human blood and body fluids (excluding gastric juices) is 6.8–7.0. Explain why maintaining this pH is important.

13 During a heart attack, blood flowing to the heart muscle is interrupted by a blockage of a coronary artery. How would you expect the metabolism in the heart muscle to change?

14 After a heart attack, people often have small amounts of lactate in the blood, which comes from the damaged heart muscle. Suggest an explanation for this observation.

15 The rate of photosynthesis is directly related to temperature, which affects the enzymes involved. Discuss the effect of temperature on the rate of photosynthesis in a plant exposed on a hilltop from sunrise to sunset during the course of a hot summer's day.

Analysing

16 The graph in Figure 4.14 demonstrates the progress of a reaction with and without a catalyst. The black arrows depict the amount of activation energy required to start the reaction.

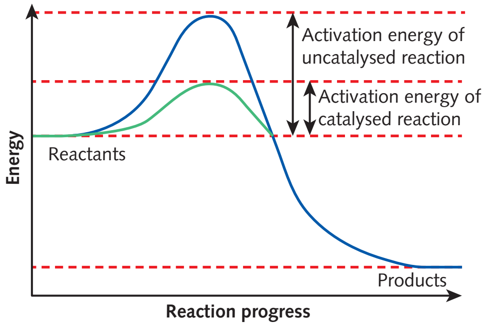
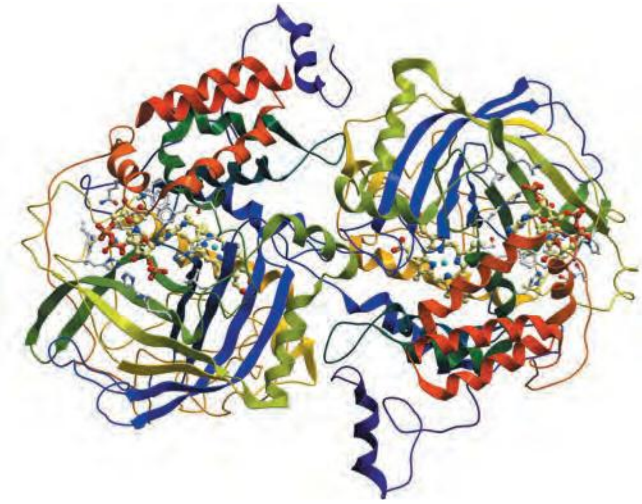


Figure 4.14 The progress of a reaction with and without a catalyst

a Which reaction has the lower activation energy?
b Is this reaction endothermic or exothermic? How can you tell?

17 Figure 4.15 shows a computer model of the enzyme catalase. Catalase degrades hydrogen peroxide in the cell before hydrogen peroxide can do any damage. When degraded by catalase, hydrogen peroxide produces free oxygen and water. Catalase is found in high quantities in the liver.

Draw a flow chart for this reaction that demonstrates the substrate, the enzyme–substrate complex and the products.



Alamy Stock Photo/Leonid Andronov

Figure 4.15 A computer model of the enzyme catalase

18 You are given two test tubes (A and B) containing two types of yeast cells that are the same in every way except that one can only carry out aerobic respiration and the other one can only carry out anaerobic respiration. The yeast in tube A grows rapidly, whereas the yeast in tube B grows slowly. Which tube contains the cells that can carry out only aerobic respiration? Justify your choice. Devise an experiment to support your choice.

Evaluating

19 Organisms such as the bacterium *Thermophilus* can thrive in hot springs at about 80°C. Use resource materials to find out why some enzymes are more heat stable than others.

20 Figure 4.5 (p. 143) demonstrates two models for enzyme catalysis. Copy and complete the following table by stating 'yes' or 'no'.

	Lock and key	Induced fit
One substrate only		
Requires enzyme and substrate		
Exact fit only active site		
Active site changes to help substrate fit		

Creating

21 Investigate the use of a commercial enzyme and record your findings under the following headings.

- Source of enzyme
- Properties or action of enzyme
- Industrial or commercial applications

Some tradenames of commercial enzymes are Neutrase, Lipolase, Lactozyme and Termamyl.

22 Design a simple investigation to find out whether catalase is present in a particular bacterium. You have the following reagents and equipment:

- dilute hydrogen peroxide
- bacterial isolate
- light microscope
- microscope slide plus coverslip
- dropper.

If catalase is present, the hydrogen peroxide will degrade to free oxygen and water.

a Write a word equation to describe the possible reaction you are investigating.

b How will you measure any reaction activity? (What will you look for and how will you measure it?)

c What do you expect to happen if catalase is not present?

d What is the independent variable in this investigation?

e What is the dependent variable?

f Write a hypothesis for the investigation.

5 Biochemical pathways: photosynthesis and cellular respiration

By the end of this chapter you will have covered the following material.

Key knowledge

Photosynthesis as an example of biochemical pathways

- inputs, outputs and locations of the light dependent and light independent stages of photosynthesis in C_3 plants (details of biochemical pathway mechanisms are not required) pp. 163–169
- the role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis pp. 166–168
- the factors that affect the rate of photosynthesis: light availability, water availability, temperature and carbon dioxide concentration pp. 169–170

Cellular respiration as an example of biochemical pathways

- the main inputs, outputs and locations of glycolysis, Krebs Cycle and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required) pp. 172–181
- the location, inputs and the difference in outputs of anaerobic fermentation in animals and yeasts pp. 181–183
- the factors that affect the rate of cellular respiration: temperature, glucose availability and oxygen concentration pp. 184–185

Biotechnological applications of biochemical pathways

- potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields pp. 185–186
- uses and applications of anaerobic fermentation of biomass for biofuel production pp. 187–188

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation pp. 170–171
- formulate hypotheses to focus investigation pp. 170–171
- predict possible outcomes pp. 177–181

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation pp. 170–171, 177–181
- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 177–181
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 170–171, 177–181

»

»

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 170–171, 177–181
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 170–171, 177–181
- demonstrate ethical conduct when undertaking and reporting investigations pp. 170–171, 177–181

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 170–171, 177–181
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 170–171, 177–181
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 170–171, 177–181
- plot graphs involving two variables that show linear and non-linear relationships pp. 170–171, 177–181

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 177–181
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 177–181
- identify outliers, and contradictory or provisional data pp. 177–181
- repeat experiments to ensure findings are robust pp. 177–181
- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors pp. 170–171, 177–181

Construct evidence-based arguments and draw conclusions

- distinguish between opinion, anecdote and evidence, and scientific and non-scientific ideas pp. 177–181
- evaluate data to determine the degree to which the evidence supports the aim of the investigation, and make recommendations, as appropriate, for modifying or extending the investigation pp. 177–181
- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 177–181
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 170–171, 177–181
- identify, describe and explain the limitations of conclusions, including identification of further evidence required pp. 177–181
- discuss the implications of research findings and proposals pp. 177–181

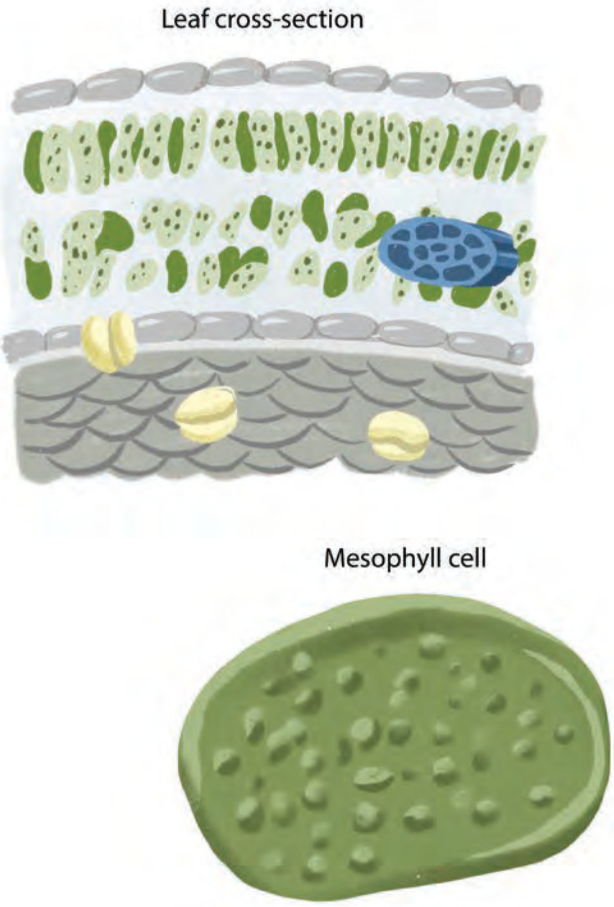
Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 170–171, 177–181
- discuss relevant biological information, ideas, concept, theories and models and the connections between them pp. 170–171, 177–181

Online Chapter Map
Chapter 5 map

5 Biochemical pathways: photosynthesis and cellular respiration

Two of the major biological biochemical pathways are photosynthesis and cellular respiration. Photosynthesis uses CO_2 and produces O_2. Cellular respiration uses O_2 and produces CO_2. Thus, they form nature's perfect recycling system.



p. 163

5.1 Photosynthesis as a biochemical pathway

Photosynthesis occurs within the chloroplasts of plant cells. Each step of this biochemical pathway is controlled by enzymes. Some steps require light and occur in the granum. Other steps do not require light and occur in the stroma. For these steps to occur at an optimal rate, intensity of light, temperature and concentration of CO_2 are all important.

p. 172

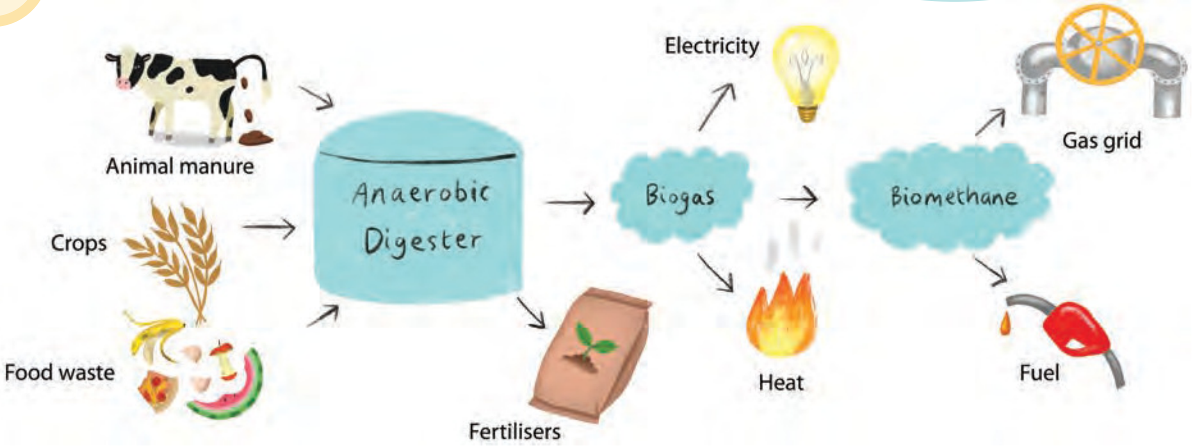
5.2 Cellular respiration as a biochemical pathway

Within the mitochondria of all living cells, cellular respiration breaks down glucose to release the energy stored within the bonds. When oxygen is present, large amounts of ATP are produced. When oxygen is absent, small amounts of ATP are produced. Each step in this biochemical pathway is controlled by enzymes, which work best at optimal temperatures and glucose and O_2 concentrations.

p. 185

5.3 Biotechnological applications of biochemical pathways

Scientists use biotechnologies to manipulate photosynthesis and cellular respiration to make plants more efficient and improve crop production. CRISPR is used to remove, add or change sequences of DNA within a plant genome to increase productivity. Anaerobic cellular respiration of biomass produces bioethanol which can be used to produce biogas and biodiesel and can be used to replace fossil fuels for transport and to produce electricity.



Does biotechnology hold the key to our future? Governments and scientists are pinning their hopes on being able to feed and clothe billions of people through DNA manipulation. Are humans interfering with nature and producing future problems? What are your thoughts?

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 5 map (p. 160)

Online Key Terms:
- Chapter 5 flashcards (p. 162)

Weblinks:
- Two stages of photosynthesis (p. 169)
- Cellular respiration (p. 175)
- Ecological Justice Hub (p. 189)

Online Worksheets:
- Two stages of photosynthesis (p. 169)
- Cellular respiration (p. 175)

Video:
- Photosynthesis and cellular respiration? (p.184)

Online Key Concepts:
- Chapter 5: Summary of key concepts (p. 190)

Online Key Terms
Chapter 5 flashcards

Know your key terms

acetyl CoA
aerobe
alcoholic fermentation
anaerobe
autotroph
biofuel
biomass
bundle sheath cells
C_3 plant
C_4 plant
Calvin–Benson cycle
CAM (crassulacean acid metabolism) plant
carbon fixation
chemoautotroph
cristae
cytochrome
grana
heterotroph
Krebs cycle
lactic acid fermentation
light-dependent stage
light-independent stage
limiting factor
matrix
mesophyll cells
mitochondrion
photoautotroph
photorespiration
pigment
pyruvate
stroma
thylakoid membrane

Remember

WB
REMEMBER
PAGE 105

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Photosynthetic autotrophs use the Sun's energy to synthesise organic compounds.
2 ATP is the main energy carrier in a cell.
3 Chloroplasts are organelles that are the site of photosynthesis.
4 The outputs of photosynthesis are the inputs for aerobic cellular respiration.
5 Cellular respiration produces ATP.

The 'biosphere' is the collective term for all life forms on Earth. Nearly all life on Earth obtains energy directly or indirectly from the Sun. Solar radiation is transformed into other types of energy that flow through the biosphere as organisms live, grow and reproduce. Much of the energy is lost as heat energy because no chemical process is 100% efficient at converting one form of energy to another.

Living things can be grouped according to how they obtain their organic molecules. **Autotrophs** can manufacture their own complex organic molecules from simple inorganic molecules taken in from their surroundings, using an external energy source. **Heterotrophs** obtain their organic molecules by feeding on other organisms and their products, which they then digest into simpler substances.

Autotrophs that capture solar energy are called **photoautotrophs**. They contain **pigments** that capture light energy and use carbon dioxide as the sole source of carbon to produce organic molecules, such as glucose, in photosynthesis. Autotrophs include all plants, photosynthetic protists, and photosynthetic bacteria (called cyanobacteria). However, not all autotrophs rely on solar energy as their primary energy source. Many bacteria, called **chemoautotrophs**, still convert one or more carbon-containing molecules (usually carbon dioxide or methane) and nutrients into organic matter. They obtain their energy from inorganic chemical reactions (oxidation), using various inorganic materials, including hydrogen sulfide, hydrogen gas and iron compounds, as their primary energy source.

Heterotrophic organisms ingest complex organic molecules, such as carbohydrates, lipids and proteins (Figure 5.1). All animals, fungi, some protists and most bacteria are heterotrophs. Both autotrophs and heterotrophs use organic molecules to fuel metabolic reactions. The organic molecules are broken down to release the stored energy in their chemical bonds in the process of cellular respiration. Thus, the energy stored is released for building structures and efficient functioning of the cell.

Alamy Stock Photo/Luca Motz

Figure 5.1 Cycling energy in the biosphere: autotrophs capture solar energy to build cell structure (photosynthesis), which is eaten by heterotrophs to provide them with energy, which is released during cellular respiration.

5.1 Photosynthesis as a biochemical pathway

Plants use either the C_3 or C_4 carbon fixation pathway in photosynthesis. **C_3 plants** are more common than **C_4 plants**. As you learned in Chapter 4, photosynthesis produces complex organic molecules from inorganic molecules, using light as an energy source. Light is captured by chlorophyll and converted into chemical energy in the bonds of glucose (a six-carbon, or 6C, molecule). Photosynthesis occurs as a series of steps in a biochemical pathway, each catalysed by specific enzymes, which take place in specialised membrane-bound organelles called chloroplasts.

WB

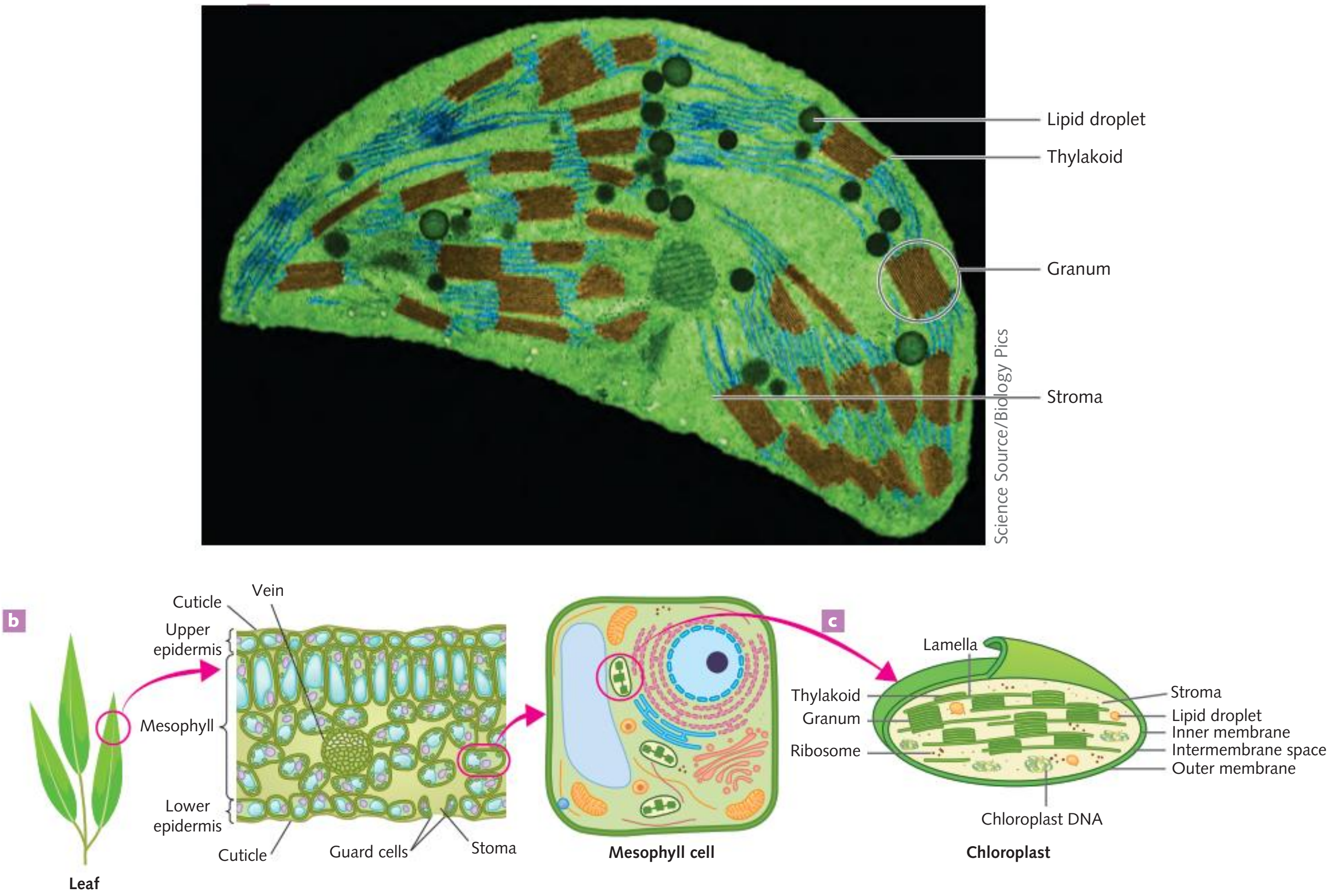
5.1.1
STRUCTURE AND FUNCTION OF CHLOROPLASTS
PAGE 106

Chloroplast structure

Chloroplasts (Figure 5.2) are organelles in eukaryotic cells of green plants and some protists and are the sites of photosynthesis. Chloroplasts have an outer and an inner membrane. Enclosed by the inner membrane is the **stroma**, a gel-like matrix that is rich in enzymes. Suspended in the stroma is a third membrane system called the **thylakoid membranes**. Thylakoids are flat, sac-like structures grouped together into stacks called **grana** (singular: granum).

EXAM TIP

Not all plant cells contain chloroplasts. Root cells and phloem are some examples of plant cells that do not contain chloroplasts.



Science Source/Biology Pics

Figure 5.2 a A cross-section of a leaf chloroplast taken with a transmission electron microscope (TEM) **b** A leaf cross-section showing the location of mesophyll cells that contain chloroplasts. **c** A generalised diagram of a chloroplast showing the main components. Note the presence of both DNA and ribosomes.

EXAM TIP
VCAA also accepts the alternative equation for photosynthesis shown on page 140.

Stages of photosynthesis

Photosynthesis is summarised by the following word equation:

$$\text{Water} + \text{Carbon dioxide} \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} \text{Oxygen} + \text{Glucose} + \text{Water}$$

Reactants/inputs Products/outputs

Photosynthesis consists of many reactions divided into two distinct stages: the **light-dependent stage** and the **light-independent stage**. Each stage takes place in specific sites within the chloroplast. The first stage (the light-dependent stage) requires light energy to be absorbed.

Light-dependent stage

The light-dependent reactions occur on the thylakoid membranes of the chloroplast grana, where the chlorophyll is located. When a chlorophyll molecule absorbs light energy, it uses it to produce ATP. Water molecules are split in photolysis, producing protons (H^+), electrons and oxygen gas. Water is an input and oxygen is a by-product of this stage of photosynthesis.

The energised electrons are transferred and relayed through many molecules in an electron transport chain and are eventually conveyed to an acceptor molecule. This acceptor molecule is the coenzyme $NADP^+$ (nicotinamide adenine dinucleotide phosphate), which accepts a proton (H^+) and a pair of electrons and becomes NADPH. Another coenzyme is formed when ADP and an inorganic phosphate ion (P_i) form ATP in a reaction catalysed by ATPase. Both $NADP^+$ and ADP + P_i are inputs and NADPH and ATP are outputs of the light-dependent stage (Figure 5.3). The two coenzymes, NADPH and ATP, are used in the next stage of photosynthesis, the light-independent stage.

A simplified summary equation of the light-dependent stage is:

$$\underbrace{\text{Water} + \text{NADP}^+ + \text{ADP} + \text{P}_i}_{\text{Inputs}} \xrightarrow[\text{Chlorophyll}]{\text{Light}} \underbrace{\text{oxygen} + \text{NADPH} + \text{ATP}}_{\text{Outputs}}$$

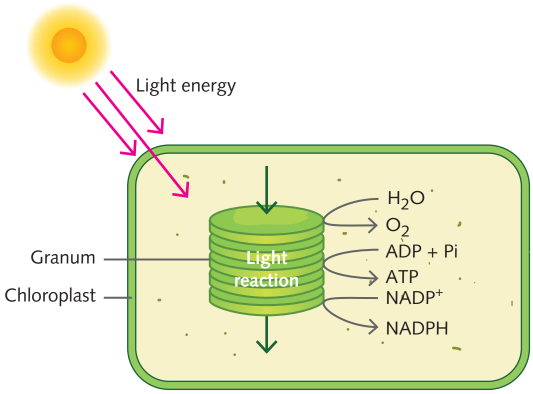


Figure 5.3 Light-dependent reactions occur in the thylakoids of the grana. Light energy is absorbed by chlorophyll pigments and splits water molecules. Electrons move along the electron transport chain. ATP is generated from ADP and P_i, in a reaction catalysed by the enzyme ATPase. $NADP^+$ picks up an H^+ ion to become NADPH. The products of the light-dependent reactions (ATP and NADPH) are used for carbon fixation to produce glucose in the light-independent stage.

Note:

Chloroplasts contain their own genetic material (DNA and RNA) and ribosomes that are independent of those of the nucleus and cytoplasm. Chloroplasts have several characteristics in common with prokaryotic cells, which has led biologists to propose the endosymbiotic theory. This suggests that chloroplasts evolved from ancient prokaryotic cells that were taken up by endocytosis by a much larger primitive nucleated host cell and subsequently established a symbiotic relationship within the eukaryotic cell.

KEY CONCEPTS

- Autotrophs are organisms that can manufacture their own complex organic molecules from simple inorganic molecules, using an energy source. They include photoautotrophs and chemoautotrophs.
- Heterotrophs obtain their complex organic substances by consuming other organisms and their products.
- Photosynthesis is the process by which photoautotrophs produce glucose from carbon dioxide, water and light energy.
- Photosynthesis occurs in chloroplasts and has a light-dependent and a light-independent stage.
- The light-dependent stage of photosynthesis occurs in the thylakoids that make up the grana in a chloroplast.
- In the light-dependent stage of photosynthesis, chlorophyll absorbs light energy to split water, releasing hydrogen ions (protons) into the electron transport chain, electrons and the by-product oxygen.
- Water, ADP, P_i and $NADP^+$ are inputs for the light-dependent stage.
- ATP, NADPH and oxygen gas are outputs of the light-dependent stage.
- ATP and NADPH become inputs for the next stage of photosynthesis, the light-independent stage.

Concept questions 5.1a

1 Distinguish between a photoautotroph and a chemoautotroph.
2 Recall the general overall equation for photosynthesis.
3 Describe the structural features of chloroplasts.
4 Describe the role of chlorophyll in photosynthesis.
5 What is the water used for in the light-dependent stage of photosynthesis?

HOT challenge

6 Produce a table that summarises the light-dependent stage of photosynthesis in terms of:
   - location
   - inputs (requirements)
   - outputs (products).

(In your table, highlight the products or outputs of the light-dependent reaction that are used as inputs in the light-independent reaction of photosynthesis.)

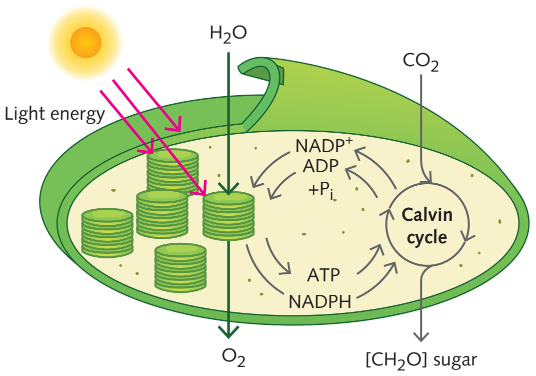


Figure 5.4 The close relationship between the light-dependent stage and the light independent Calvin–Benson cycle.

Light-independent stage

The light-independent reactions occur in the stroma of chloroplasts. In these reactions, glucose molecules are produced from carbon dioxide in a biochemical pathway called the **Calvin–Benson cycle** (also known as the Calvin cycle) (Figure 5.4). This reaction requires a supply of carbon dioxide gas, hydrogen ions and chemical energy in the form of ATP. The loaded acceptor molecule NADPH is the source of hydrogen ions, and ATP molecules provide the chemical energy for the conversion of carbon dioxide to glucose molecules. Both the coenzymes NADPH and ATP, which were formed in the light-dependent stage, are used in this light-independent stage and are recycled to the grana as $NADP^+$, ADP and P_i to be used again in the light-dependent stage.

Water is an output of the light-independent stage. The water is produced by rearrangement of the oxygen atoms in carbon dioxide. Half of the oxygen atoms from the carbon dioxide are incorporated into the carbohydrate, the other half into water. Therefore, this water is different from the water consumed as an input in the light-dependent stage. For this reason, water is represented on both sides of the photosynthesis equation (p. 164).

The following equation is a simplified summary of the light-independent stage.

$$\underbrace{\text{Carbon dioxide} + \text{NADPH} + \text{ATP}}_{\text{Inputs}} \rightarrow \underbrace{\text{glucose} + \text{water} + \text{NADP}^+ + \text{ADP} + \text{P}_i}_{\text{Outputs}}$$

EXAM TIP
Make sure you remember the inputs, outputs and locations of the light-dependent and light-independent stages of photosynthesis in C_3 plants. You do not need to know the details of the chemical pathways.

The role of rubisco in carbon fixation and adaptations of c_4 and CAM plants to maximise its efficiency for photosynthesis

The first step of the Calvin–Benson cycle involves attaching carbon from carbon dioxide to a five-carbon molecule called ribulose bisphosphate (RuBP). This step is called **carbon fixation** and is catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (commonly known as **Rubisco**) – the most abundant enzyme on Earth. After several more steps that involve coenzymes ATP and NADPH, 3C molecules are formed, two of which join to make the 6C molecule glucose. Glucose is a vitally important output of photosynthesis because it is the key building block of the plant's carbohydrates: sucrose, starch and cellulose and because some of the glucose will be broken down in cellular respiration to release the chemical energy stored in the bonds of the glucose molecule to provide the energy for cell functioning.

All plants use the enzyme Rubisco to catalyse reactions that produce organic carbon compounds out of inorganic carbon dioxide. Rubisco might be the most plentiful enzyme on Earth but it is highly inefficient. In C_3 plants, Rubisco fixes carbon by joining it to a five-carbon sugar. Then it cuts the new six-carbon sugar chain into two identical three-carbon molecules, and hence the name C_3 plants as the 3C molecules are the first product after carbon fixation. However, both carbon dioxide and oxygen can bind to Rubisco's active site. The binding of oxygen is called **photorespiration** (not related to cellular respiration) and is affected by two key factors-environmental temperature and substrate concentration.

(I) Temperature: At low temperatures, CO_2 is more likely to bind to Rubisco than oxygen, so the rate of photorespiration in C_3 plants is low and the rate of photosynthesis is not affected by photorespiration. However, as the temperature increases, more O_2 and less CO_2 will bind to Rubisco, so the rate of photorespiration increases and photosynthetic efficiency decreases. Up to 25% of the Rubisco can bind with oxygen reducing photosynthetic efficiency and resulting in lowered glucose production. Another factor that will affect the photosynthetic cells is the production of a waste product (ammonia) from photorespiration which is toxic and must be broken down by the cells using cell energy from cellular respiration, further reducing their glucose supply.

WB

5.1.2 PHOTOSYNTHESIS IN C_3, C_4 AND CAM PLANTS AND THE ROLE OF RUBISCO PAGE 107

(II) Substrate concentration: During the daytime when light is available, the stomata of plants will usually open allowing CO_2 to enter and oxygen and water vapour to diffuse out. However, in hot, dry, and/or windy conditions, the stomata will close during the day to conserve water. This causes the oxygen produced in the light-dependent stage to build up inside and around the photosynthesising mesophyll cells and CO_2 levels to drop. This greater concentration of oxygen results in increased photorespiration and less Rubisco available for photosynthesis. This means C_3 plants can survive in a wide range of temperatures and are found in environments ranging from temperate to tropical however the optimal environment is temperate. They represent 83% of the world's total flora and include all trees, cereals such as rice and wheat, and the majority of fruits, vegetables and nuts.

Overall, photorespiration is an unwanted process for plants as it reduces photosynthetic efficiency. To reduce photorespiration which occurs at a higher rate in hot, dry conditions, some plants have evolved adaptations to increase the chance of Rubisco binding with CO_2 and less chance of binding with oxygen. These plants, called C_4 and CAM plants, have increased photosynthetic rates and improved plant health.

In C_4 plants, carbon fixation occurs in two steps. CO_2 is first fixed in the mesophyll cells into an additional biochemical pathway by an enzyme called PEP carboxylase, which has a high affinity for CO_2. It is joined to a 3C molecule to form a 4C molecule known as malate, hence the name C_4 plants. Malate moves out of the mesophyll cells into specially adapted **bundle sheath cells**. Here the carbon dioxide is liberated and then fixed by Rubisco in the Calvin cycle. In this way the carbon dioxide gradient stays low in mesophyll cells so that it will continue to diffuse in from the outside, even when the stomata are almost closed. This partitioning also means that C_4 plants move the Calvin cycle into an area with a high carbon dioxide concentration. Why is this important? The enzyme Rubisco will spend more of its time fixing carbon dioxide in photosynthesis than fixing oxygen in photorespiration. In C_3 plants, photorespiration increases as the temperature rises, so carbon fixation by Rubisco slows. This does not happen in C_4 plants as the concentration of CO_2 remains high in the bundle sheath cells, so they are more competitive in high temperatures. C_4 plants are found mainly in grassland and hotter areas and make up only 3% of the world's total flora. C_4 plants include maize (corn), switchgrass, some weed species, and sugar cane, which is renowned for its high glucose production.

CAM (crassulacean acid metabolism) plants grow in very hot, dry areas and have closed stomata during the day and open stomata at night. This again is a four-carbon molecule, but CAM plants differ from C_4 plants. CAM plants do not transport malate away from the mesophyll cells but store it during the night and then liberate carbon dioxide from these molecules and use it in the Calvin cycle during the day. This use of malate requires four more ATP molecules than the C_3 pathway, so these plants tend to grow more slowly than other plants. They lose up to 95% less water than C_3 plants as they only open their stomata at night. This reduces the exposure of Rubisco to oxygen, so there is less photorespiration and more Rubisco binds to CO_2. This results in an increased photosynthesis rate. CAM plants are found in deserts (and occasionally in tropical areas), so this adaptation is very useful. Interestingly, CAM plants can swap to the C_3 pathway if there is a period of rainfall, giving them a sudden growth spurt. They can also keep their stomata closed all night and day during drought conditions and exist by fixing carbon dioxide that is released from respiration reactions within the plant. CAM plants make up about 10% of the world's total flora. Pineapples, jade plants, cacti, and some orchids are examples of CAM plants. The C_4 and CAM plants have adapted through evolution and have very low photorespiration rates. This has been achieved by reducing the exposure of the Rubisco enzyme to oxygen, therefore increasing its binding with carbon dioxide and increasing photosynthetic efficiency when compared to C_3 plants.

KEY CONCEPTS

- In the light-independent stage of photosynthesis, coenzymes ATP and NADPH from the light-dependent stage provide the chemical energy and protons to fix carbon in the Calvin–Benson cycle.
- Inputs of the light-independent stage are carbon dioxide, NADPH and ATP.
- Outputs of the light-independent stage are glucose, water, $NADP^+$, ADP and P_i.
- C_4 and CAM plants have adapted photosynthetic pathways that enable them to survive in hot and dry environments.

Concept questions 5.1b

1. Where does the Calvin–Benson cycle take place?
2. What is the purpose of the Calvin–Benson cycle?
3. Why is the Calvin–Benson cycle known as the light-independent stage of photosynthesis?
4. Why does the Calvin–Benson cycle need ATP and NADPH and where do they come from?
5. Can the light-independent stage of photosynthesis occur during daylight even though it is referred to as the 'dark reaction'?
6. Compare the photosynthetic pathways of C_3, C_4 and CAM plants. How are their respective pathways an adaptation to their environment?

HOT challenge

7. Glucose is termed a C_6 molecule, yet in C_3 plants, photosynthesis makes the energy-rich C_6 organic molecule glucose. What do C_6 and C_3 refer to and what does C_3 plants mean?

Inputs and outputs of photosynthesis

WB 5.1.3 INPUTS AND OUTPUTS OF PHOTOSYNTHESIS PART A PAGE 109

The two stages of photosynthesis require certain inputs (water and carbon dioxide) and produce outputs (oxygen, glucose and water). Coenzymes cycle between the two stages in loaded and unloaded forms. For the number of coenzymes cycling between the two stages, 12 cycles of light-dependent reactions must occur for every six cycles of light-independent reactions. These inputs and outputs are presented in Tables 5.1 and 5.2.

Table 5.1 Summary of inputs and outputs for the light-dependent stage of photosynthesis, which occurs in the chloroplast thylakoid (for 12 cycles of light-dependent reactions)

Inputs		Outputs	
Molecule	Total number	Molecule	Total number
H_2O	12	O_2	6
$NADP^+$	12	NADPH	12
ADP P_i	12 12	ATP	12

Table 5.2 Summary of inputs and outputs for the light-independent stage of photosynthesis, which occurs in the chloroplast stroma (for six cycles of light-independent reactions)

Inputs		Outputs	
Molecule	Total number	Molecule	Total number
CO_2	6	H_2O	6
NADPH	12	$NADP^+$	12
ATP	12	ADP P_i	12 12
		Glucose	1

Comparing the inputs and outputs for both stages of photosynthesis reinforces the fact that some components are recycled between the two stages. NADPH and ATP formed during the light-dependent stage are used during the light-independent stage. The NADP^+, ADP and inorganic phosphate (P_i) produced during the light-independent stage are returned as inputs to the light-dependent stage. Because these components are recycled, they are not shown in the photosynthesis equation on page 164.

Factors affecting rate of photosynthesis

The main factors affecting photosynthesis are environmental – light, carbon dioxide and temperature – although other factors, such as the presence of inhibitors and changes in pH, can also change the rate of photosynthesis. This means that the factors become limiting.

WB
5.1.4
FACTORS THAT AFFECT THE RATE OF PHOTOSYNTHESIS
PAGE 112

Light

In the light-dependent phase, photons (the basic units of light) are needed to excite the chlorophyll molecules. These then split water molecules, freeing electrons to enter the electron transport system. Therefore, the amount of light available is an important factor that limits the rate of the first reaction in photosynthesis. The rate of photosynthesis increases as light intensity increases, but eventually a maximum point is reached and adding more light does not increase the rate of photosynthesis. This point is called the light saturation point (Figure 5.5) and happens when more ATP and NADPH are produced in the light-dependent stage than can be used in the light-independent stage.

Weblink
Two stages of photosynthesis

Online Worksheet
Two stages of photosynthesis

White light is a mixture of all the different wavelengths of the visible spectrum. The wavelengths of white light available to a plant affect its rate of photosynthesis. Light energy of various wavelengths is absorbed by different pigments within the thylakoid membranes of chloroplasts. These pigments include chlorophylls (green), carotenoids (orange) and xanthophylls (yellow). Chlorophylls absorb blue and red wavelengths and reflect green wavelengths, which is why plants appear green. All green protists and plants have chlorophylls as their major photosynthetic pigments. Figure 5.6 shows that there are three main pigments involved in photosynthesis – chlorophyll a, chlorophyll b and β-carotene. Each absorbs light in a specific range of wavelengths. Chlorophyll a and chlorophyll b are the main photosynthetic pigments that absorb light in the violet, blue and red wavelengths of white light.

WB
5.1.5
INPUTS AND OUTPUTS OF PHOTOSYNTHESIS PART B
PAGE 115

Carbon dioxide

The main **limiting factor** in photosynthesis is usually the availability of carbon dioxide. Ambient carbon dioxide concentrations are relatively low, at 0.04% of Earth's atmosphere. During the light-independent stage, carbon dioxide is the substrate for the enzyme Rubisco, which converts it to an unstable six-carbon compound that splits into two three-carbon compounds, which then form glucose. As the concentration of carbon dioxide increases, the rate of photosynthesis increases, provided there are enough active sites on the

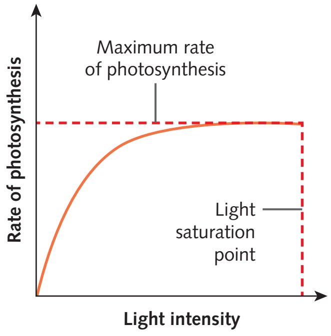


Figure 5.5 The effect of light intensity on the rate of photosynthesis: as light intensity increases, photosynthetic rate increases up to a maximum rate – this level of light is known as the light saturation point.

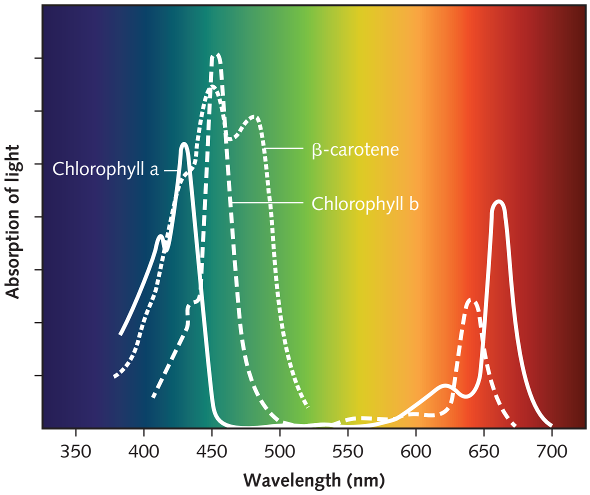


Figure 5.6 Absorption of light at different wavelengths for the various pigments found within chloroplasts of plants used in photosynthesis. Optimal absorption of light occurs at different wavelengths for different pigments.

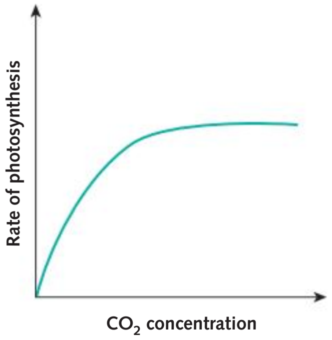


Figure 5.7 The effect of carbon dioxide concentration on the rate of photosynthesis: as CO$_2$ concentration increases, photosynthetic rate increases up to a maximum rate.

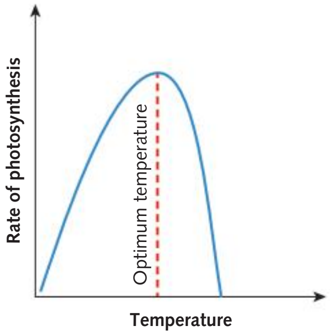


Figure 5.8 The effect of temperature on the rate of photosynthesis: as temperature increases, photosynthetic rate increases up to the optimum temperature. Above this, photosynthetic rate decreases because of denaturation of enzymes.

enzyme available to catalyse the reactions (p. 142), and there is enough ATP and NADPH from the light-dependent stage to fuel the light-independent reactions. Increasing the carbon dioxide concentration raises the light saturation point because then the light-independent reactions can use more ATP and NADPH and the rate of photosynthesis can increase overall (Figure 5.7).

Temperature

The third main factor that influences the rate of photosynthesis is temperature. We know from collision theory that for chemical reactions to occur, molecules must collide with one another. At low temperatures, the enzymes for photosynthesis and the substrate molecules move slowly, and so few collisions occur between the molecules, and the rate of photosynthesis is low. As the temperature increases, the molecules have more kinetic energy so they move faster and collide and interact more frequently, increasing the rate of photosynthesis. However, as with all proteins, above a certain optimum temperature the enzymes become denatured and the photosynthetic reactions stop (Figure 5.8).

Developed by Southern Biological

INVESTIGATION 5.1

Effect of light wavelength on photosynthesis

In this investigation, you will use algal balls to test whether the wavelength of light affects their photosynthetic rate. Algal balls consist of many algal cells trapped in a jelly-like substance called sodium alginate, which immobilises the algae but allows them to keep photosynthesising.

Aim

To test the effect of light wavelength on photosynthesis

Materials

- 60 algal balls
- 4 × 7 mL empty dram vials
- Light source
- 40 mL (approx.) hydrogen carbonate indicator
- Plastic pipette
- Set of pH standards or colour chart
- Red, purple and green cellophane
- Strainer
- Spoon
- Disposable gloves (optional)

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Some algae pose an environmental hazard.	Know and follow all regulatory guidelines for the disposal of laboratory wastes.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

1 Separate the algal balls from the surrounding liquid using the strainer. To do this, pour the algal balls into a strainer over a small beaker.
2 Use the spoon to place an equal number of balls into each dram vial.
3 Using a plastic pipette, fill all the vials with the hydrogen carbonate indicator. Make sure the caps are secured.
4 Keep one vial to act as your control.
5 Copy the results table into your logbook and complete column A by comparing the colour of the hydrogen carbonate indicator to the set of standard references shown in Figure 5.10. (You can use a colour chart if standards are not available.)
6 Place each vial approximately 10 cm away from your light source (Figure 5.9). Make sure the vials do not get hot.
7 Wrap a different piece of coloured cellophane around the other three vials.
8 After 40 minutes complete column B of the results table by comparing the colour of the hydrogen carbonate indicator to the set of standard references shown in Figure 5.10. (You can use a colour chart if standards are not available.)
9 Complete the final column of the results table by subtracting the amount in column A from the amount in column B.

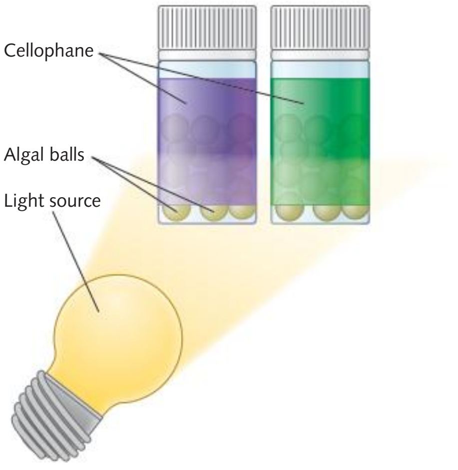


Figure 5.9 The experimental set-up

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Figure 5.10 Standard reference colours for hydrogen carbonate indicator

Results

Results table

Cellophane colour	Colour of solution before exposure (A)	Colour of solution after exposure (B)	Colour change (B – A)
None (control)			
Red			
Purple			
Green			

Discussion

1 Carbon dioxide dissolved in water forms carbonic acid. Hydrogen carbonate indicator is used to measure the acidity of a system. The pH of the system is low (yellow) when there is a lot of dissolved CO_2. As CO_2 is removed, the pH rises and the colour becomes purple. Use this information to construct a bar graph of CO_2 changes as a function of wavelength.
2 Why do we include a control in the experiment? What does this control represent in term of light wavelength?
3 Describe the process that is happening in the vials with regards to photosynthesis and respiration.

Taking it further

Light availability is another factor that affects photosynthesis. Design an experiment to test how distance from light effects the rate of photosynthesis.

KEY CONCEPTS

- A limiting factor is a factor that limits the rate of a reaction.
- The limiting factors in photosynthesis are light intensity, carbon dioxide concentration and temperature.

Concept questions 5.1c

1 List three limiting factors on the rate of photosynthesis.
2 In the context of enzyme activity, explain why the rate of photosynthesis levels off at high carbon dioxide concentrations.
3 At low light intensities, the rate of increase in photosynthesis is linear. What happens to the rate at very high light intensities?
4 What occurs at the light saturation point of photosynthesis? Identify this point on the graph in Figure 5.5.
5 Explain why the rate of photosynthesis varies as the temperature varies.

HOT challenge

6 Would the concentration of oxygen in the ambient environment of a C_3 plant ever be considered a limiting factor? Why or why not?

WB 5.2.1 CELLULAR RESPIRATION PAGE 117

5.2 Cellular respiration as a biochemical pathway

As discussed in Chapter 4, the complex series of reactions involved in the process of cellular respiration provides the ATP required by all living organisms (Figure 5.11). Most organisms use glucose as the primary source of energy to drive anabolic cellular reactions and for cellular activities and functions. During cellular respiration, the chemical bonds in glucose are broken, resulting in the release of energy. The energy is used to convert ADP and P_i into ATP, where the energy is temporarily stored, even for a fraction of a second, before it is released for cell use.

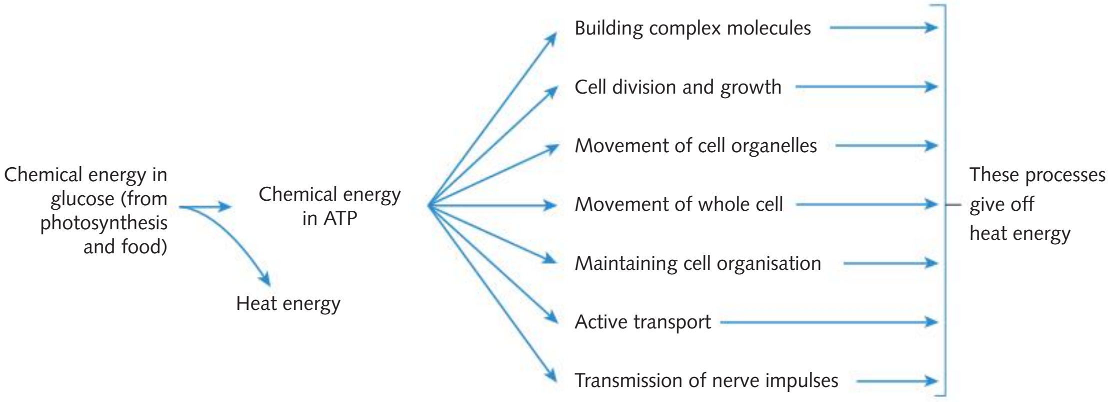


Figure 5.11 Uses of energy in the cell

Most animals, plants, protists and fungi are called **aerobes** because they require oxygen for aerobic cellular respiration. Bacteria and some other micro-organisms are **anaerobes** because they do not use oxygen and instead carry out anaerobic respiration.

For all organisms, the oxidation of glucose to supply the cell with energy, regardless of whether oxygen is used or not, starts with the biochemical pathway glycolysis.

Glycolysis occurs in the cytosol

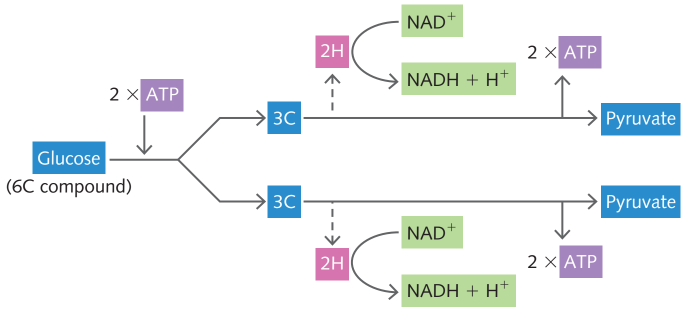


Figure 5.12 Glycolysis is the first stage of cellular respiration. Glucose is broken down into pyruvate, and coenzymes ATP and NADH are produced.

The biochemical pathway of glycolysis occurs in the cytosol of the cytoplasm. Glycolysis consists of 10 reactions, each step controlled by a specific enzyme. The initial reactant is the six-carbon compound glucose, which is broken down into two molecules of a three-carbon compound, **pyruvate** (or pyruvic acid). During this process, two unloaded acceptor molecules, NAD^+, are loaded with hydrogen to form two loaded NADH molecules, and a net of two ATP molecules are formed from ADP and P_i, using the energy released in the exergonic reactions splitting the glucose into two molecules (Figure 5.12). This process occurs at a fast rate and the ATP molecules are available for immediate use by the cell if required. This ATP production may be adequate for the needs of certain micro-organisms, or plant and animal cells under certain conditions, but it is not adequate for most multicellular organisms. The fact that nearly all organisms carry out glycolysis, either as their sole source of energy or as the first step in more elaborate pathways to gain sufficient ATP for their needs, points to glycolysis being one of the earliest reactions to produce energy for the cell.

The by-product of glycolysis, pyruvate, is used in the further reactions of aerobic or anaerobic cellular respiration. This reduces the concentration of pyruvate, which is toxic to cells if it accumulates. What occurs to break down pyruvate after glycolysis differs between prokaryotic and eukaryotic cells and depends on whether oxygen is present or absent.

A simplified summary equation of glycolysis is:

$$\underbrace{\text{Glucose} + NAD^+ + ADP + P_i}_{\text{Inputs}} \rightarrow \underbrace{\text{pyruvate} + NADH + ATP}_{\text{Outputs}}$$

Table 5.3 summarises the inputs and outputs of glycolysis.

Table 5.3 Summary of inputs and outputs of glycolysis

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Glucose	1	Pyruvate	2
ATP	2	ADP P_i	2 2
ADP P_i	4 4	ATP	4
NAD^+	2	NADH	2

Note that 2 ATP molecules are used to initiate glycolysis and 4 ATP molecules per glucose molecule are produced. Overall, there is a net output of 2 ATP molecules for each glucose molecule.

KEY CONCEPTS

- The purpose of cellular respiration is to release the energy stored in the bonds of glucose to produce ATP, which can provide energy to fuel cellular reactions.
- Glycolysis occurs in the cytosol. It involves the breakdown of glucose to two pyruvate molecules, two ATP molecules and two loaded acceptor molecules, NADH.
- ATP is available for immediate use by the cell. Pyruvate is processed in subsequent reactions that differ between organisms and depend on the availability of oxygen.

Concept questions 5.2a

1 Write the chemical word equation for glycolysis.
2 What is the initial substrate in the glycolysis pathway? What is the final product?
3 Where does glycolysis take place in all cells?
4 Glycolysis is an anaerobic process. What does this mean?
5 Glycolysis produces four molecules of ATP. Why is the calculated net number of molecules produced by glycolysis stated as 2?

HOT challenge

6 Glycolysis, the process of synthesising ATP, requires which of the following as an input: a six-carbon sugar, two three-carbon sugars, NADH or oxygen?

Cellular respiration using oxygen

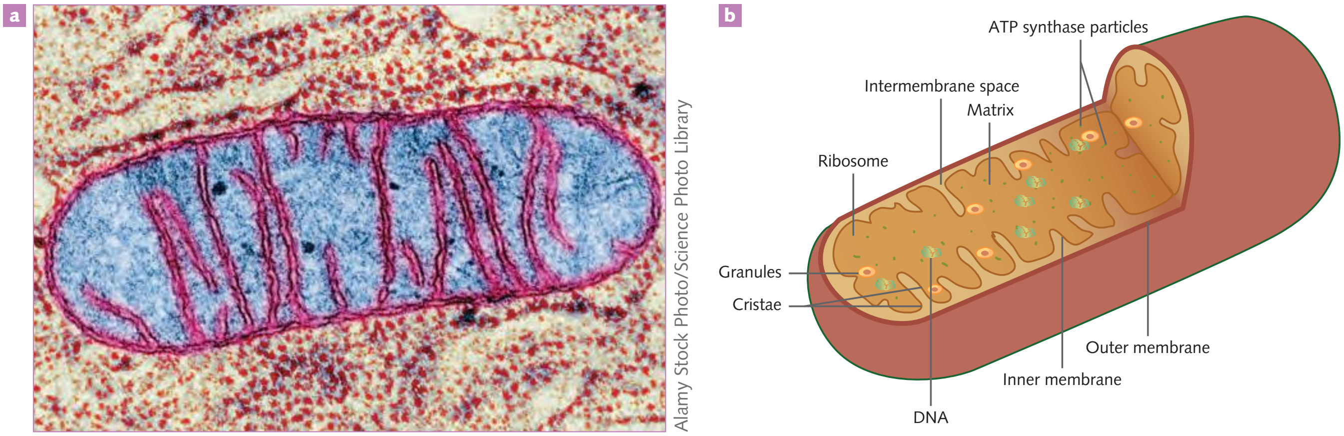
WB 5.2.2 CELLULAR RESPIRATION USING OXYGEN PAGE 118

In eukaryotic cells that have oxygen available, the two molecules of pyruvate per glucose molecule formed in glycolysis enter organelles called mitochondria (singular: **mitochondrion**). The pyruvate molecules are the initial substrate in a series of reactions that use oxygen to produce a much larger amount of ATP than in glycolysis. Mitochondria are often described as the 'energy powerhouses' of the cell because inside them many ATP molecules are produced in two further biochemical pathways called the **Krebs cycle** (or citric acid cycle) and the **electron transport chain**.

EXAM TIP
Remember that the total number of ATP molecules produced per molecule of glucose is through the additive combination of the processes of glycolysis, Krebs cycle and electron transport chain.

Structure of mitochondria

Mitochondria are small organelles that are found throughout the cytosol of eukaryotic cells. They are attached to the cytoskeleton. Each mitochondrion consists of an outer smooth membrane and a highly folded inner membrane. The folds in the inner membrane, called **cristae**, protrude into the inner space of the mitochondrion, which is filled with a protein-rich fluid, the **matrix**. The space between the outer and inner membranes is also filled with fluid and is called the intermembrane space (Figure 5.13).



Alamy Stock Photo/Science Photo Library

Figure 5.13 a An electron micrograph of a mitochondrion and b a diagram of part of a mitochondrion. Note the presence of mitochondrial DNA and ribosomes.

Mitochondria, like chloroplasts, have their own genetic material – mitochondrial DNA (mtDNA) and RNA – and ribosomes. Like chloroplasts, they are also capable of division independently of the cell's nucleus, so they make copies of themselves and have many characteristics of prokaryotic cells. This evidence points to their origins as independent organisms.

Krebs cycle occurs in mitochondrial matrix

The two three-carbon pyruvate molecules formed in glycolysis are transported into the mitochondrial matrix. Pyruvate undergoes an intermediate reaction that results in the formation of two molecules of the loaded coenzyme **acetyl CoA** (a two-carbon molecule), which are then broken down. The final products from the pyruvate that entered the Krebs cycle are carbon dioxide, ATP formed from the energy released in the reactions, and two types of loaded acceptor molecules, NADH and $FADH_2$.

The following is a simplified summary equation of the Krebs cycle:

$$\underbrace{\text{Pyruvate} + \text{ADP} + P_i + \text{NAD}^+ + \text{FAD}}_{\text{Inputs}} \longrightarrow \underbrace{\text{carbon dioxide} + \text{ATP} + \text{NADH} + \text{FADH}_2}_{\text{Outputs}}$$

Table 5.4 summarises the inputs and outputs of the Krebs cycle.

Table 5.4 Summary of inputs and outputs for the Krebs cycle, which occurs in the mitochondrial matrix. Values are per glucose molecule

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Pyruvate	2	CO_2	6
NAD^+	6	NADH	6
FAD	2	$FADH_2$	2
ADP P_i	2 2	ATP	2

Electron transport chain occurs in mitochondrial cristae

The loaded acceptor molecules produced in the Krebs cycle (NADH and $FADH_2$) enter the electron transport chain, which takes place in the inner mitochondrial membrane on the cristae. NADH and $FADH_2$ are coenzymes that contribute to the production of ATP and water in the electron transport chain. With the addition of the loaded coenzymes, electrons are transferred through a series of enzymes and compounds called **cytochromes** that are embedded within the inner mitochondrial membrane. NADH and $FADH_2$ donate the H^+ to become NAD^+ and FAD to be recycled and used again. H^+ is taken up by oxygen, which is an electron acceptor, to form the by-product, water. The energy released during this electron transport chain is used to drive the production of ADP and P_i into ATP, catalysed by the enzyme ATP synthase. Some research indicates that 3 H^+ are required to produce each ATP molecule, so in the electron transport chain, there is a net ATP production of 26 or 28 ATP per glucose molecule depending on the type of cell (more produced in heart, liver, and kidney cells). Note: there is variation between the theoretical and actual yields of ATP.

EXAM TIP

The specific details of the glycolysis, the Krebs cycle and the electron transport chain aren't required by VCAA. But you must know the inputs, outputs and locations of each process.

The following is a simplified summary equation of the electron transport chain:

$$\underbrace{\text{Oxygen} + \text{ADP} + P_i + \text{NADH} + \text{FADH}_2}_{\text{Inputs}} \rightarrow \underbrace{\text{water} + \text{ATP} + \text{NAD}^+ + \text{FAD}}_{\text{Outputs}}$$

Table 5.5 summarises the inputs and outputs of the electron transport chain. Table 5.6 summarises the inputs and outputs of aerobic respiration.

Table 5.5 Summary of inputs and outputs of the electron transport chain (values are per glucose molecule)

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
O_2	6	H_2O	6
ADP P_i	26 or 28 26 or 28	ATP	26 or 28
NADH	10	NAD^+	10
$FADH_2$	2	FAD	2

Weblink
Cellular respiration

Online Worksheet
Cellular respiration

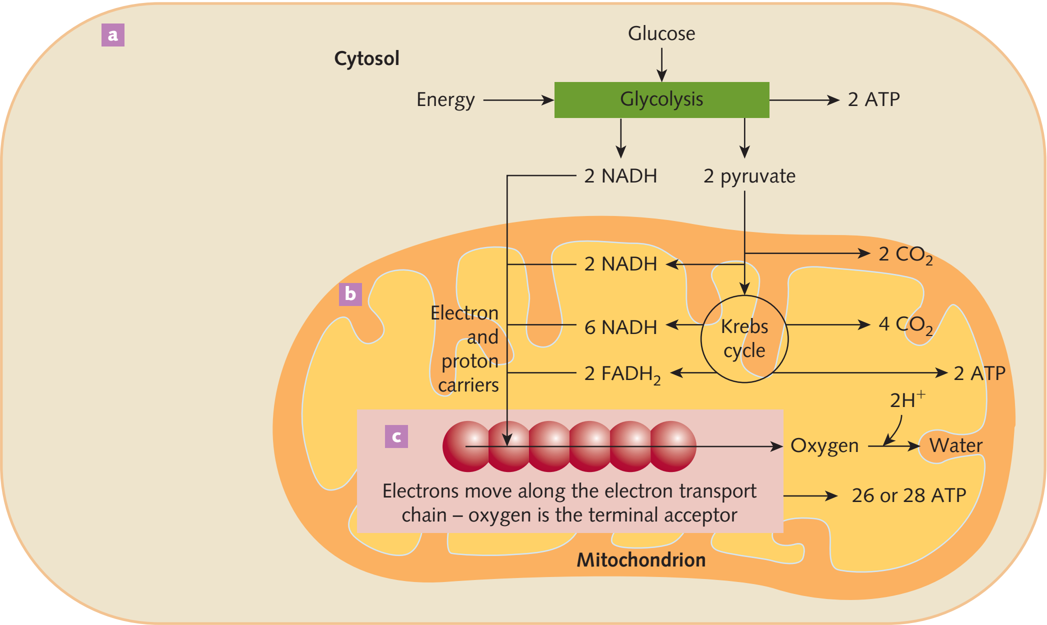


Figure 5.14 a Glycolysis, the first stage of cellular respiration, occurs in the cytosol. In glycolysis, glucose breaks down to pyruvate. **b** Pyruvate enters the mitochondria. In the presence of oxygen, the Krebs cycle and **c** electron transport chain occur.

Table 5.6 Total inputs and outputs for cellular respiration in the presence of oxygen (aerobic respiration)

Process	Location	Inputs	Outputs
Glycolysis	Cytoplasm	1 glucose 2 ADP + 2 P_i 2 NAD^+	2 pyruvate molecules 2 ATP 2 NADH
Krebs cycle	Mitochondrial matrix	2 pyruvate molecules 2 ADP + 2 P_i 6 NAD^+ 2 FAD	4 CO_2 2 ATP 6 NADH 2 $FADH_2$
Electron transport chain	Mitochondrial inner membrane, cristae	Oxygen 8 NADH 2 $FADH_2$ 26 or 28 ADP and 26 or 28 P_i	Water 8 NAD^+ 2 FAD 26 or 28 ATP
Total			30 or 32 ATP per glucose

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INVESTIGATION 5.2

Investigating aerobic and anaerobic respiration

Aim

To investigate aerobic and anaerobic respiration

Time requirement

45 minutes

Materials

- Glucose solution 5 mL
- Janus Green B oxygen indicator solution (5 mL)
- 80–100 Yeast balls
- Distilled water 5 mL
- 2 Glucose test strips
- 2 Plastic pipettes
- 2 Glass beakers (for 37°C water baths)
- Plastic spoon
- Timer or stopwatch
- Marker
- Paper towel
- Stirring rods
- 2 Syringes (10–20 mL)
- 2 Syringe stands
- 2 Metal washers
- Strainer (optional)

What are the risks in this investigation?	How can you manage these risks to stay safe?
Janus Green B may cause eye irritation.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end the end of the investigation.
Yeast can cause an allergic reaction in sensitive people.	Wash hands after use. Do not eat in class, due to the possibility of contamination. Be aware of any allergies.
Disposable gloves may pose allergy risk	Use a type of glove that removes allergy risk and is suitable to chemicals being used.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

Determining the initial glucose concentration of the solution:

1 Use a plastic pipette to add two drops of Janus Green B oxygen indicator to the glucose solution. Swirl gently to mix.
2 Collect a glucose test strip and dip the yellow square tab at the tip into the glucose solution for 2–3 seconds.
3 To remove excess liquid, pat the test strip dry with a paper towel. Leave the strip to rest for three minute.
4 To determine the initial concentration of glucose in milligrams per decilitres, compare the colour of the test strip with the colour chart on the packaging.
5 Copy the results tables into your logbook. Record the initial glucose concentration in Results table 1 for the respirometer containing yeast ball + glucose solution.
6 Repeat steps 1–4 with distilled water (in place of glucose solution) to determine the glucose concentration of the control. Record the results in the table.

Assembling the respirometers

1 Remove the white plunger from the clear chamber of a syringe.
2 Place a metal washer over the base of the syringe stand to enable the respirometer to remain submerged in a water bath during data collection (Figure 5.15).

Figure 5.15 Place a washer over the base of the syringe.

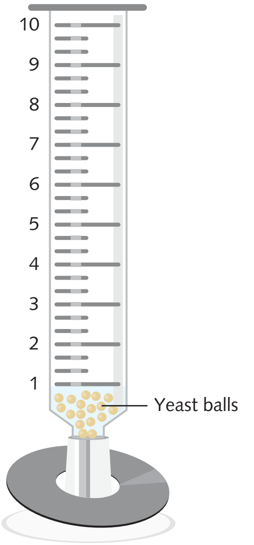

Figure 5.16 **Add yeast balls to the 1 mL mark of the syringe**

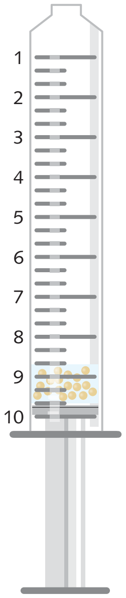

Figure 5.17 **The inverted respirometer**

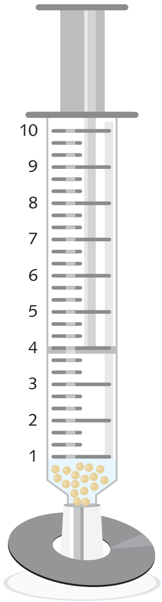

Figure 5.18

3 Carefully place the clear syringe chamber on the syringe stand. Then, use a plastic spoon to fill the syringe chamber with yeast balls to the 1.0 mL mark (Figure 5.16). Do not transfer excess water into the chamber as you add the yeast balls; use a strainer if necessary.
4 Carefully insert the white plunger into the syringe but only enough to connect the two pieces (approximately 2 mm). Remove the respirometer (clear syringe chamber + white plunger) from the base of the syringe stand and invert it (Figure 5.17).
5 Dislodge the yeast balls from the tip of the syringe by lightly tapping the clear chamber.
6 Gradually compress the plunger on the inverted respirometer to the 1 mL mark. Make sure no yeast balls are expelled through the syringe.
7 Place the tip of the syringe in the solution and slowly draw up (aspirate) 3 mL of glucose + oxygen indicator solution until the solution reaches the 4.0 mL mark on the syringe. The total volume of the yeast balls and oxygen indicator solution is now 4 mL.
8 Place the respirometer back onto the syringe stand with the washer. Do not depress the white plunger.
9 Repeat steps 6–13 to prepare the control respirometer, drawing liquid from the gluclose and Janus Green B solution. Label this respirometer E for experiment.

Data collection from the respirometer system

1 Collect two glass beakers containing 200 mL of warm (approximately 37°C).
2 Label one beaker ‘Yeast balls + glucose’ and the other ‘Yeast balls + water’ (control).
3 Carefully submerge each respirometer into the appropriately labelled beaker.
4 Record the starting volume for each respirometer as 4 mL.
5 Using the graduated volume markings on the syringe chamber, record the volume of each syringe, and the indicator solution colour every minute for 30 minutes. Record this information in Results tables 1 and 2, along with any other observations you make.
6 To calculate the change in gas volume at each minute interval, calculate the difference between the initial reading (4 mL) and the final reading on the syringe.
7 When the plunger reaches the 10 mL mark, stop collecting data and remove the respirometers from their water baths.
8 To disassemble the respirometers, cover them with a paper towel and carefully remove the white plunger from the clear chamber containing yeast balls.
9 To determine the final concentration of glucose within each respirometer, dip the end of a glucose test strip into the solution in each syringe and compare the colour of the test strips with the colour chart on the packaging. Record the final glucose concentration levels in your results tables.

10 Share your individual experiment results with the class to generate a class data set and use the class data to calculate the mean volume for each time interval in the glucose respirometer. Record the results in the 'Class mean CO_2 volume' column in the results tables.

Results

Copy the results tables into your logbook. Complete the results tables and graph your results.

Results table 1 Glucose respirometer results

Water bath temperature (°C) ____________________
Initial colour of solution ____________________
Final colour of solution ____________________
Initial glucose concentration (mg/dL) ____________________
Final glucose concentration (mg/dL) ____________________

Time (min)	Reading on pipette (mL)	–	Initial reading	=	CO_2 volume (your group)	Colour of indicator solution	Observations	Class mean CO_2 volume
0		–		=				
1		–		=				
2				=				
3		–		=				
4		–		=				
5		–		=				
6		–		=				
7		–		=				
8		–		=				
9		–		=				
10		–		=				
11		–		=				
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22		–		=				
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24		–		=				
25		–		=				
26		–		=				
27		–		=				
28		–		=				
29		–		=				
30								

Results table 2 Non-glucose respirometer results

Water bath temperature (°C) ____________________
Initial colour of solution ____________________
Final colour of solution ____________________
Initial glucose concentration (mg/dL) ____________________
Final glucose concentration (mg/dL) ____________________

Time (min)	Reading on pipette (mL)	–	Initial reading	=	CO_2 volume (your group)	Colour of indicator solution	Observations
0		–		=			
1		–		=			
2		–		=			
3		–		=			
4		–		=			
5		–		=			
6		–		=			
7		–		=			
8		–		=			
9		–		=			
10		–		=			
11		–		=			
12		–		=			
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21		–		=			
22		–		=			
23		–		=			
24		–		=			
25		–		=			
26		–		=			
27		–		=			
28		–		=			
29		–		=			
30							

Discussion

1 Were the results of the investigation consistent among all groups? If not, what are some possible reasons for the variation in results? Were there any experimental errors or uncontrolled variables?
2 Explain why the average value of results from several investigations is more reliable than a single set of test results.
3 How does this investigation design address the suggestion that carbon dioxide released did not require the presence of carbohydrate?
4 Explain why the indicator solution in the respirator changed colour.
5 Provide two pieces of evidence from the investigation that illustrate that glucose was fermented by yeast balls during cellular respiration.
6 Yeast cells serve as a model organism for cellular processes occurring in other eukaryotic cells, such as those of humans. Explain why the cells of our body normally do not carry out fermentation, yet we give off carbon dioxide when we exhale.

Taking it further

Following the same method as this experiment, is it possible that carbon dioxide can be released from sugar without the presence of yeast? Design an additional controlled experiment that would allow you to test this idea.

KEY CONCEPTS

- » Pyruvate from glycolysis enters the mitochondria where it enters the Krebs cycle.
- » In the Krebs cycle, pyruvate is completely broken down, releasing carbon dioxide as a by-product and producing ATP and the loaded coenzymes NADH and $FADH_2$.
- » On the cristae of the mitochondria, a complex series of steps involving cytochrome enzymes transfers the electrons to the oxygen, which reacts with H^+ from the coenzymes to produce water, in the electron transport chain.
- » The net output of ATP from aerobic cellular respiration is 30 or 32 ATP per glucose molecule.

Concept questions 5.2b

1 What is the role of oxygen in aerobic cellular respiration?
2 What is the source of the by-product carbon dioxide in aerobic cellular respiration?
3 How many net molecules of ATP are produced in one cycle of aerobic cellular respiration? In practice, the number is somewhat less. Suggest a reason for this.
4 Where does acetyl CoA come from and what is its function?
5 What molecule is the terminal electron acceptor at the end of the electron transport chain?

HOT challenge

6 Water is produced at the end of the electron transport chain. It is an output of cellular respiration. What are the source and form of the oxygen and of the hydrogen that are combined to make water?

Cellular respiration without oxygen

WB
5.2.3
CELLULAR RESPIRATION WITHOUT OXYGEN
PAGE 119

The first energy-releasing pathways evolved around 3.8 billion years ago when there was not much free oxygen in the atmosphere. The process was essentially anaerobic because it could be completed in the absence of oxygen. Many bacteria and protists still live in places where oxygen is absent or not always available, and they produce ATP by anaerobic pathways. Such organisms have evolved biochemical pathways that allow glycolysis to continue in the cytosol by using molecules other than oxygen as the final electron acceptor. Prokaryotes have evolved many different anaerobic pathways.

As you have seen, oxygen is required for aerobic respiration in eukaryotic cells. If oxygen is absent, the Krebs cycle and the electron transport chain in the mitochondria shut down. The cell then relies entirely on glycolysis to maintain a supply of ATP for its energy needs. However, without the Krebs cycle and electron transport chain to remove them, the products of glycolysis build up in the cytosol. These products are pyruvate and the loaded coenzyme NADH. The problem is that NADH must be continually unloaded, forming NAD^+, to sustain glycolysis. Eukaryotic and some prokaryotic cells have solved the problem by reacting NADH with pyruvate in the processes of **alcoholic fermentation** or **lactic acid fermentation**.

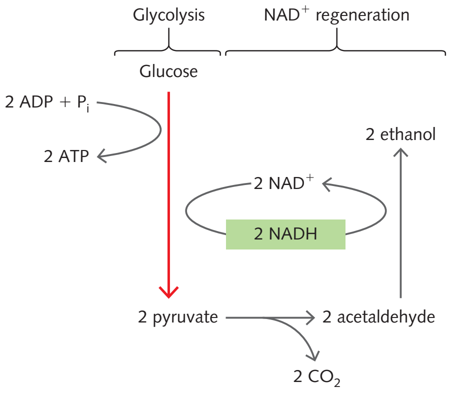


Figure 5.19 Alcoholic fermentation involves the breakdown of pyruvate generated from glycolysis into carbon dioxide and acetaldehyde, which accepts protons from NADH to form ethanol (alcohol).

Alcoholic fermentation

Many micro-organisms, including yeast and some bacteria, carry out alcoholic fermentation (Figure 5.19). The step producing ATP is still glycolysis, but a second step converts the potentially toxic pyruvate together with NADH molecules into carbon dioxide and ethanol. In the second stage, no additional ATP is made, so the net output remains at two ATP molecules per glucose molecule produced in glycolysis. The equation for alcoholic fermentation is:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow \text{ethanol} + \text{carbon dioxide} + 2\text{ATP}$$

Humans make use of these metabolic waste products in the production of wine, beer and bread, using anaerobic cellular respiration by yeast (Figure 5.20). However, plants cannot use ethanol. It cannot be reconverted into carbohydrates, nor can it be broken down in the presence of oxygen. Ethanol is an alcohol, which is toxic to cells and cannot be allowed to accumulate. Many plants (or parts of plants) can respire anaerobically for a short time, such as germinating seeds and roots living in water-logged soil, where there is little oxygen. However, before the concentration of ethanol reaches a certain level, the plants must revert to aerobic respiration; otherwise, the plants will be poisoned by the ethanol.

Yeast uses anaerobic respiration, but respiration is better under aerobic conditions. If too little oxygen is present, the ethanol concentration rises so much that the yeast cells are killed. When making beer and wine, it is important to not let conditions become too anaerobic. Industrial fermentation microbiologists work to develop new strains of yeast that tolerate high concentrations of ethanol.

Shutterstock.com/Marcel Bakker

Figure 5.20 Plants and yeast produce ethanol and carbon dioxide in the anaerobic respiration process of alcoholic fermentation. This reaction has been used by industry to produce bread and wine. In bread, the carbon dioxide makes the bread rise while the ethanol evaporates during baking.

Lactic acid fermentation

Lactic acid, or lactate, is the end product of anaerobic cellular respiration in animal cells. In this process, glycolysis produces 2 ATP molecules per glucose molecule, followed by the breakdown of the pyruvate to produce lactic acid (Figure 5.21). Animal muscle cells respire anaerobically when they need a short, rapid burst of energy or if not enough oxygen is available. Lactic acid fermentation cannot be sustained for very long because the build-up of toxic lactic acid will result in cell death.

After strenuous exercise, muscle soreness and cramps may develop. In 1929, Archibald Hill proposed that these symptoms were the result of lactic acid building up in the muscle tissue. However, the latest theory gaining widespread acceptance in the scientific world suggests that these symptoms are a result of an increase in extracellular potassium ion concentration, which leads to the observed muscle fatigue cramps.

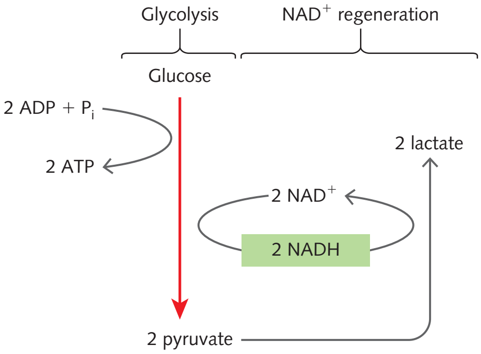


Figure 5.21 Lactic acid fermentation occurs in muscle cells. In the process, NADH is converted to NAD^+, allowing glycolysis to continue.

Comparing aerobic and anaerobic cellular respiration

Aerobic cellular respiration involves the complete breakdown of glucose to release enough energy to produce 30/32 ATP molecules per glucose molecule. In anaerobic cellular respiration, glucose is only partly broken down and enough energy is released to form only 2 ATP molecules per glucose molecule. The remainder of the energy is still trapped in the bonds of the complex molecules of ethanol or lactic acid. In animals, the lactic acid molecules can be converted back into pyruvate, which can then be broken down into carbon dioxide and water in the Krebs cycle and electron transport chain (Figure 5.22). Table 5.7 summarises anaerobic and aerobic cellular respiration.

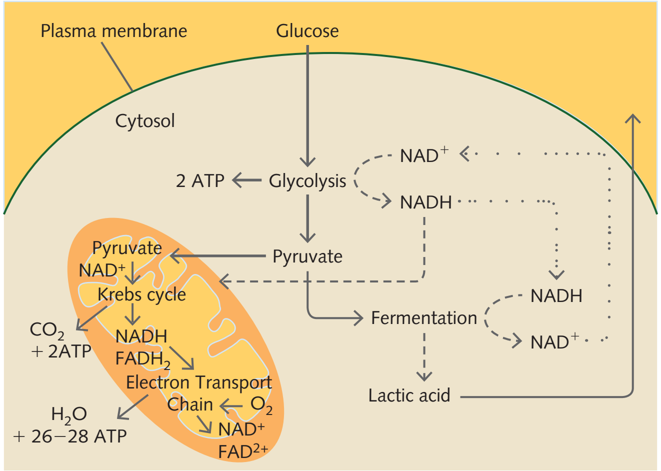


Figure 5.22 A summary of aerobic and anaerobic respiration in an animal cell

Table 5.7 A summary of anaerobic and aerobic cellular respiration

	Anaerobic	Aerobic
Reactants	Glucose	Glucose/oxygen
Amount of ATP produced per glucose molecule	2 ATP	(Varies according to cell type) 30 or 32 ATP
Location	Cytosol	Cytosol (glycolysis), mitochondrial matrix (Krebs cycle) and cristae of mitochondria (electron transport chain)
Stages	Glycolysis and conversion	Glycolysis, Krebs cycle, electron transport chain
Products	Animals: lactic acid Yeast: ethanol and CO_2	CO_2 and H_2O
Speed of reaction	Faster for short burst of energy	Slower for longer, sustained energy supply

Putting photosynthesis and aerobic cellular respiration together

Photosynthesis and aerobic cellular respiration are closely related and interdependent – that is, the outputs of one are the inputs of the other (Figure 5.23). In plants and other autotrophs, the two processes occur in the same cells when both chloroplasts and mitochondria are present. But many cells in green plants, such as root cells, do not have chloroplasts. These cells and those of heterotrophs depend on the products of photosynthesis to carry out cellular respiration. Thus, there is a dependency between autotrophs and heterotrophs.

EXAM TIP

Cellular respiration occurs in all living cells all the time. Photosynthesis occurs in living cells of plants and autotrophs in the presence of light energy.

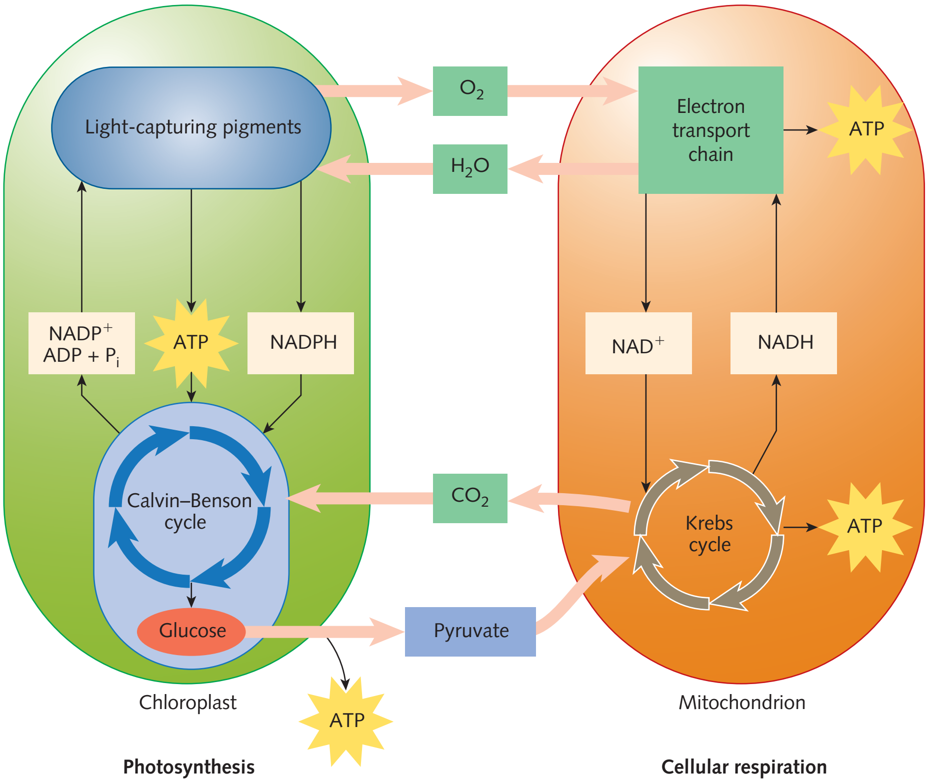


Figure 5.23 **Photosynthesis uses the products of cellular respiration and cellular respiration uses the products of photosynthesis.**

Factors affecting rate of cellular respiration

The rate of cellular respiration is influenced by both environmental and physiological factors: temperature and the concentrations of glucose and oxygen. As with all biochemical reactions, as the concentrations of substrates increase, the rate of cellular respiration increases up to a saturation point, at which time other factors limit the reaction rate. These other factors may include the amount of enzyme available, the pH of the intracellular environment, and the presence of cofactors, coenzymes, and competitive and non-competitive inhibitors of the enzymes.

Yeast is an accessible organism for observing the effect of various factors on the rate of cellular respiration. When mixed with a glucose and water solution, yeast undergoes anaerobic cellular respiration, producing carbon dioxide that forms foam. Several independent variables can be altered to observe the effect they have on anaerobic cellular respiration, including temperature, concentration of glucose solution, and the availability of oxygen. The amount of foam produced can be measured as the dependent variable.

WB
5.2.4
PUTTING PHOTOSYNTHESIS AND AEROBIC CELLULAR RESPIRATION TOGETHER
PAGE 120

video
Photosynthesis and cellular respiration?

Temperature

The rate of cellular respiration increases as temperature increases because of increased molecular movement and frequency of collisions between the substrate and enzyme molecules. At a certain high temperature, the enzymes involved begin to denature and the rate of cellular respiration slows down and almost stops. Organisms reach the upper limit of their tolerance range and cells and tissues start to shut down.

At lower temperatures, the kinetic energy of all the molecules involved is lower and this affects the speed of their reaction. When it is cold, plant growth rates decrease and some animals go into hibernation to minimise their energy requirements.

Glucose concentration

Cellular respiration depends on an ongoing supply of glucose so cells must constantly replenish their glucose stores. Photosynthetic cells can produce their own glucose, while other non-photosynthetic plant cells rely on transport of glucose to them. In multicellular organisms, glucose can be stored in specialised cells and released and transported, as required, to the rest of the tissues. Plants store glucose as starch grains in the cells of roots, stems and even leaves. Animals accumulate and store glucose as glycogen in liver and muscle cells. The need for glucose must be communicated between the cells that demand it and the cells that store it. If glucose stores become depleted, the body draws on alternative sources to meet the energy needs of its cells such as lipids or proteins.

Oxygen Availability

The availability of oxygen affects the type of cellular respiration carried out by the cells, whether aerobic or anaerobic. During anaerobic respiration, the ATP is supplied only from glycolysis, which generates a much smaller amount due to incomplete glucose breakdown than from aerobic respiration (two ATP compared to 30/32 ATP per glucose molecule).

However, ATP production by anaerobic respiration is faster than that of the more complex set of reactions involved in aerobic respiration. Consequently, if the demand for ATP outweighs the supply from aerobic respiration, eukaryotic cells shift to anaerobic respiration. This occurs in the muscle cells of athletes, such as sprinters, when they perform brief bursts of strenuous exercise. The switch can only be temporary because the accumulation of lactic acid in cells can denature enzymes and inhibit metabolism. It must be removed to restore normal cell functioning, which takes time to achieve.

KEY CONCEPTS

- Eukaryotes have two anaerobic cellular respiration pathways for producing ATP from glucose in the absence of oxygen.
- Yeasts, plants and some bacteria carry out alcoholic fermentation; some animal cells carry out lactic acid fermentation.
- The products of anaerobic respiration are toxic at high concentrations and so anaerobic respiration is only a short-term solution for obtaining chemical energy.
- The rate of cellular respiration is affected by temperature, glucose concentration and oxygen availability as well as other factors.
- Photosynthesis and cellular respiration are linked – the inputs of one are the outputs of the other.

Concept questions 5.2c

1 List two differences between aerobic respiration and fermentation.

2 Compare the products of anaerobic respiration with those of aerobic respiration in:

a animal cells

b plant cells.

3 Describe how cellular respiration is altered when:

a temperature is very high or very low

b oxygen levels are very high or very low

c glucose levels are very high or very low.

4 Draw a simple schematic diagram to show how photosynthesis and cellular respiration are linked.

5 Complete aerobic respiration of one glucose molecule releases enough energy to make 30 or 32 ATP molecules. In anaerobic respiration, one glucose molecule releases enough energy to create 2 ATP. What has happened to the remaining energy in the glucose molecule in anaerobic respiration?

HOT Challenge

6 In eukaryotic cells, if oxygen is not present, aerobic respiration will not proceed. The products of glycolysis are converted to other outputs through a further anaerobic process and the other outputs collect in the cell. What are the other possible outputs and what effects can they have on the cell? Is this further anaerobic process reversible?

5.3 Biotechnological applications of biochemical pathways

The world's population is estimated to reach about 9.7 billion by 2050, which will affect the world's ability to produce enough food. In addition, the challenges of climate change, and increases in pests and diseases to crops already under threat, may contribute to lower crop production rates in Australia and around the world. Lower crop production rates are expected to have negative impacts on the amount of food available for people across the world. It is hoped that development of new technologies, many of which use biotechnology and genetics, may help to meet the increasing demand to feed the world's growing population.

Using CRISPR-Cas9 to improve photosynthesis

WB
5.3.1
APPLICATIONS OF CRISPR-CAS9 TECHNOLOGIES
PAGE 121

CONNECT
CRISPR is discussed in detail in Chapter 3.

The CRISPR-Cas9 gene-editing tool is a technology that has enormous potential to enhance crop yields. CRISPR (clustered regularly interspaced short palindromic repeats) is a process mediated by the protein endonuclease enzyme, Cas-9. Many scientists are researching and experimenting to determine how the CRISPR-Cas9 technology can be used to enhance photosynthesis in plants to increase crop yields. The CRISPR-Cas9 protein acts as an accurate 'cut and paste' tool for geneticists so they can introduce specific DNA sequences into plant genomes that code for more efficient photosynthetic traits. CRISPR is efficient, precise in its cutting site, cuts double-stranded DNA, and is easy to use. Possible applications of CRISPR to increase yield include enhancing light availability (by altering plant architecture), increasing light capture and energy conservation. CRISPR can be used to directly modify a trait, by targeting the exact genes or regulatory genes to be modified. These techniques are cheaper and faster than both traditional cross-breeding methods and other transgenic methods.

Tobacco (*Nicotiana* species) is used widely for this research because tobacco plants reproduce quickly, are easy to grow and can be engineered easily by modern transgenic methods. Once the transfer of certain genes can be accomplished in tobacco, scientists are hoping to transfer these genes to crop plants such as soybeans, potatoes, maize, rice and wheat in order to increase their biomass, which in turn could increase the amount of food available for the world's growing population.

Applications so far include engineered plant pigments that use a broader spectrum of light wavelengths from the Sun's radiation, allowing plants to absorb sunlight more effectively. Too much light can damage a plant so, under extreme light conditions, plants have adapted a mechanism to 'switch off' the process of photosynthesis. However, the mechanism to then 'switch on' photosynthesis is slow. Scientists have used CRISPR to engineer a process to speed up this transition and to allow plants to maintain a more consistent rate of photosynthesis. This has achieved enhanced production in tobacco plants and scientists are hoping this can be transferred directly to crop-yielding plants.

Scientists are also looking to enhance the efficiency of Rubisco, one of the key enzymes involved in driving photosynthesis. Rubisco is a protein that binds to carbon dioxide during photosynthesis. However, oxygen and carbon dioxide molecules compete for the same binding site on Rubisco and approximately 20% of the protein is susceptible to oxygen binding in place of carbon dioxide (this process is known as photo*respiration*). In addition, when Rubisco binds to oxygen, a toxic compound is produced that must be recycled, and this costs the plant further energy and resources that could otherwise be used for growth. Scientists aim to make Rubisco more efficient at binding to carbon dioxide to increase productivity. Scientists who have engineered an alternative pathway for photorespiration to decrease this wasted energy and enhance photosynthesis estimate that the savings in resources for plant growth could be as high as 40%.

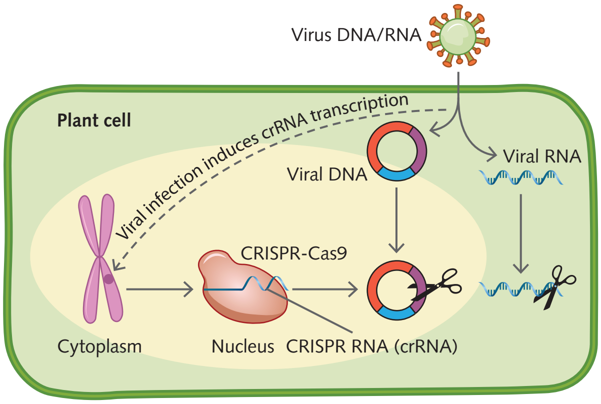


Figure 5.24 Using the CRISPR-Cas9 system to defend against viral pathogens. Coding sequences for Cas9 and the CRISPR RNA (crRNA) are integrated into the plant genome, and their transcription is induced by infection. The crRNA targets the CRISPR-Cas9 complex to cut the DNA or RNA of the virus, conferring resistance to the invading virus.

Other areas of potential applications for the CRISPR-Cas9 system include increasing the yield of crops under particular biotic and abiotic stresses by providing disease-resistant genes (Figure 5.24); and to improve tolerance in crops growing in drought and salinity-affected areas. Rice, barley, wheat and maize are staple food crops for more than half of the world's population, and scientists hope that CRISPR-enhanced crops can significantly produce greater yields of biomass in these crops by conferring resistance against external stresses.

CRISPR is also being researched to improve fibre quality in cotton production. Other potential uses for CRISPR technology include protein seed storage in soybean, virus resistance in tomatoes, starch yield in potato crops, disease resistance in citrus fruit, and pest resistance in grapes.

Other potential applications include introducing novel genes into plants to produce new 'behaviours' to enhance their efficiency.

Artificial DNA sequences, including regulatory and transcriptional elements, can be inserted into plant genomes so that the plant can undertake processes such as nitrogen fixation, a protein production method that is exclusive to legumes. If a plant can fix nitrogen, then it can maintain growth and biomass in nitrogen-deficient soils, and globally could reduce the need for inorganic fertilisers, in turn reducing pollution from fertiliser run-off. Other novel areas of interest include using CRISPR to reverse the effects from hundreds of years of inbreeding in species such as maize and rice, to counter the reduced genetic variation in these species. Such historical reduction in genetic variation has had a negative impact on the plant's ability to survive under specific environmental stresses. CRISPR could be used in crops to manipulate specific traits that are under the control of only one gene in the wild species, so they would be controlled by a system of polygenes to increase genetic variation, so that in times of stress these plants have alternative pathways to keep thriving and producing high yields.

Genome editing using CRISPR will no doubt be a powerful tool for specific crop improvement within agriculture. Not only is the technique simple and efficient, it also provides the precise ability to edit regulatory and structural genes to enhance yield, resistance to pests, increase quality of biomass and increase the ability of crops to improve photosynthetic yield under stressful conditions.

Biomass for biofuel production

WB
5.3.2
ANAEROBIC FERMENTATION OF BIOMASS FOR BIOFUEL PRODUCTION
PAGE 122

In the summer of 2019–2020, Australia experienced a series of bushfires that killed 75 people and an estimated 3 billion animals, burned more than 11 million hectares of land, and destroyed almost 2000 homes. There has been much discussion about climate change and its contribution to this and other natural disasters. British naturalist Sir David Attenborough says it is time for governments across the globe to address this issue. He has called on countries such as China to take action, in the hope that other countries will follow and make carbon emissions reduction a priority. Protests against inaction on climate change have increased in recent years and people are thinking about how to reduce their carbon footprint.

One way to reduce the use of fossil fuels is to harness the energy in plant and animal **biomass** to produce **biofuels**. Anaerobic fermentation using biomass is often referred to as 'anaerobic digestion' because it uses micro-organisms to decompose plant and animal waste products in the absence of oxygen (like detrivores in a food web), and as a measure of organic waste management. Anaerobic digestion occurs naturally in particular ecosystems, such as deep lakes and ocean basin residues, due to their lack of oxygen.

There are different ways of producing biofuels however many are made using fermentation involving the breakdown of plant starch and sugar from biomass from already existing products from forestry, agriculture, and food manufacturing. Examples of such biomass are sugarcane, corn, waste wood, and paper. The fermentation process which involves enzymes results in the production of ethanol and carbon dioxide. The ethanol is collected and refined to make biogas (methane) and other biofuels such as biodiesel (made from animal fats and vegetable oils) and liquid biofuel (bioethanol). Bioethanol is frequently used as motor fuel or as an additive in gasoline. As well as a transportation fuel, it can be used as a fuel for power generation, feedstock in the chemical industry, fuel for fuel cells, in the cosmetics industry, and in manufacturing processes.

One of the waste products of such decomposition and purification is methane gas, a flammable organic gas produced by bacteria, which can be collected and used as a fuel source. This fuel resource is known as biogas and has been produced since 1859 in India and the 1870s in England. In modern times, communities in developing nations have used biofuel from fermentation 'generators' on a household scale. Vegetable waste and animal manure is used in these fermenters to produce methane gas for heat and light production. Fermenters are often made from relatively inexpensive plastics and pipe and can be kept safely in trenches beside rural homes. The ability of various livestock animals to produce such biogas varies. For example, in warm climates, 1 kg of cattle dung can produce 40 L of biogas in a day, whereas 1 kg of chicken droppings can produce up to 70 L.

Biogas consists of only 50–75% methane compared to natural gas, which consists of 80–90% methane. However, in biogas, all the carbon in the methane being burned to produce carbon dioxide has already been drawn down from the atmosphere as the original plant photosynthesised. On the other hand, burning of natural gas extracts carbon that has been stored for millions of years and adds this to the carbon already existing in the modern atmosphere. Biogas production, therefore, is a carbon neutral process, where the entire path from plant to livestock to manure to decomposition is considered a closed system. Biogas-generated methane burns readily in oxygen to produce carbon dioxide and water vapour but leaves no carbon footprint.

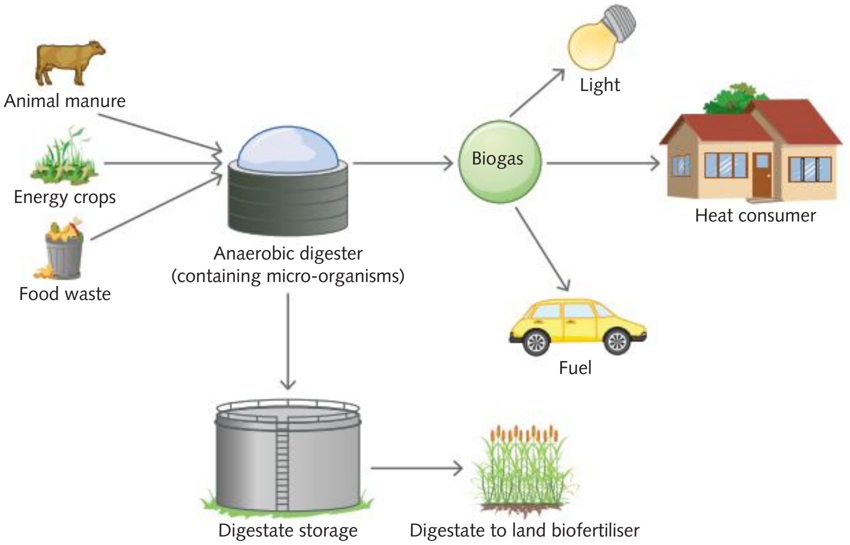


Figure 5.25 Biogas can be converted to electricity. Organic material is broken down (digested) to produce biogas, which is used to produce heat, electricity and fuel for cars. Residue is used as fertiliser.

Applications of biogas on a larger scale are being developed, particularly in Germany (Figure 5.25). Rural farmers may sustain heat for cooking and gas lamps for light from their biogas generator while engineers in Germany are using biogas in two ways to generate electricity:

- methane fuel cells, which are costly to build and require clarified gas
- conventional combustion engines to drive electrical generators.

Conversion of biogas to electricity is becoming a standard technology and an ethical, sustainable and cost-effective method to produce energy from waste products. Advantages of biofuels over traditional fossil fuels include:

1. Biomass is readily available and can be used immediately, unlike the long period of time for formation of fossil fuels.
2. Biomass is renewable as it can be readily collected from plants and animals or collected from left over wastes from agriculture and industry, such as farm straw, picked corn stalks, and even tip waste.
3. Biofuels are often carbon neutral as there is no net output of carbon dioxide into the atmosphere compared to the large output from combustion of fossil fuels.

KEY CONCEPTS

- The CRISPR-Cas9 protein acts as a 'cut and paste' tool for geneticists so they can introduce specific DNA sequences into plant genomes; for example, to code for more efficient photosynthetic traits.
- Possibilities of CRISPR-Cas9 include increasing crop yield by enhancing light availability (by altering plant architecture), increasing light capture and energy conservation.
- Anaerobic fermentation of biomass by micro-organisms produces ethanol which is converted into biogas, which is used as a fuel for transport and electricity.
- Biogas production is a carbon neutral process, where the entire path from plant to livestock to manure to decomposition is considered a closed system.

Concept questions 5.3

1. List some potential uses for CRISPR biotechnology tools in growing more efficient food crops. Distinguish what aspects of the plants would be targeted.
2. CRISPR-Cas9 protein is a natural 'cut and paste' tool. Where does it come from?
3. The increased growth of biomass crops to produce biofuels in the Amazon rainforest in Brazil has been shown to be both a great leap forward and a disaster. Research the pros and cons of this newer cropping program.
4. Australia has some of the most nutrient-deficient soils in the world, yet produces more than $75 billion worth of food through cropping, grazing and fishing. Australia only requires currently $25 billion worth of food to feed its own citizens. The surplus food production is mostly exported to other countries. In Australia, why might a decreased need for fertilisers to supplement the deficient soils engineered through the use of CRISPR be a positive step for crop production?
5. Rubisco is the most abundant enzyme on Earth, but can easily be inhibited.
   a. What are the main issues with the Rubisco process in plant photosynthetic pathways?
   b. How might biotechnical engineering help to increase plant photosynthesis rates by 40%?

HOT challenge

6 In Australia, cotton cropping is controversial because it requires huge amounts of water and pesticides. How has the process of CRISPR reduced some of these concerns, and what might be the future for cotton in Australia?

BRANCHING OUT

A closer look at global issues with local solutions

Stuart Muir Wilson is the project coordinator at the Ecological Justice Hub in Brunswick, Victoria (Figure 5.26). Stuart is an architect, bioenergy expert and humanitarian who has worked on ecological catastrophes for more than 11 years in many countries, including Australia, Germany, India, Nepal and Mexico and countries in Eastern Africa.

His projects have included building 'tiny homes' and running a community garden that provides fresh vegetables to homeless people. The aim of Ecological Justice is to bring about social and environmental justice through projects, education and training to serve marginalised and vulnerable people.

Projects at the Hub include courses for the community such as mushroom growing, woodwork and timber recycling, zero waste harvesting, cooking and composting demonstrations. Many of the projects aim to reduce global warming through community awareness and to provide ideas, education and support to help regenerate environmental recovery.

The Hub also has a biogas reactor designed and built by Stuart (Figure 5.27). It uses biomass in the form of compost to produce methane that is then converted into electricity and heat for neighbouring dwellings. The bioreactor works by anaerobically fermenting biomass waste and captures methane and carbon dioxide. The carbon dioxide is filtered out and the methane gas is refined so it can be used as a source of electricity. The bioreactor provides a closed loop solution that links the carbon footprint from waste to electricity. This means that one of its main benefits to communities would be less reliance on fossil fuels for electricity needs.

The wider implications for anaerobic fermentation producing biogas could mean farmers and industry are offered a cost-effective waste disposal option that doesn't require the use of expensive and unnecessary landfill. Governments could offer farmers and industry subsidies to take up an opportunity to use a bioreactor. Considering their low cost and relative simplicity, and that humans produce a lot of organic waste, biogas generators could well be the key local solution to global warming.

Stuart Muir Wilson

Figure 5.26 Stuart Muir Wilson is project coordinator at the Ecological Justice Hub in Brunswick, Victoria.

Stuart Muir Wilson

Figure 5.27 The portable bioreactor at the Ecological Justice Hub in Brunswick produces electricity from biomass waste products, supplying electricity without the use of fossil fuels.

Weblink
Ecological Justice Hub

Question

1 Discuss the approach that Stuart is taking to the complex issue of environmental justice in terms of consequences-based bioethics.

2 Identify any social and economic factors relevant to the work of the Ecological Justice Hub.

Online Key Concepts
Chapter 5: Summary
of key concept

5 Summary of key concepts

5.1 Photosynthesis as a biochemical pathway

KEY CONCEPTS

p. 163

- Autotrophs are organisms that can manufacture their own complex organic molecules from simple inorganic molecules, using an energy source. They include photoautotrophs and chemoautotrophs.
- Heterotrophs obtain their complex organic substances by consuming other organisms and their products.
- Photosynthesis is the process by which photoautotrophs produce glucose from carbon dioxide, water and light energy.
- Photosynthesis occurs in chloroplasts and has a light-dependent and a light-independent stage.
- The light-dependent stage of photosynthesis occurs in the thylakoids that make up the grana in a chloroplast.
- In the light-dependent stage of photosynthesis, chlorophyll absorbs light energy to split water, releasing hydrogen ions (protons) into the electron transport chain, electrons and the by-product oxygen.
- Water, ADP, P_i and $NADP^+$ are inputs for the light-dependent stage.
- ATP, NADPH and oxygen gas are outputs of the light-dependent stage.
- In the light-independent stage of photosynthesis, coenzymes ATP and NADPH from the light-dependent stage provide the chemical energy and protons to fix carbon in the Calvin–Benson cycle.
- Inputs of the light-independent stage are carbon dioxide, NADPH and ATP.
- Outputs of the light-independent stage are glucose, water, $NADP^+$, ADP, and P_i.
- C_4 and CAM plants have adapted photosynthetic pathways that enable them to survive in hot and dry environments.
- A limiting factor is a factor that limits the rate of a reaction.
- The limiting factors in photosynthesis are light intensity, carbon dioxide concentration and temperature.

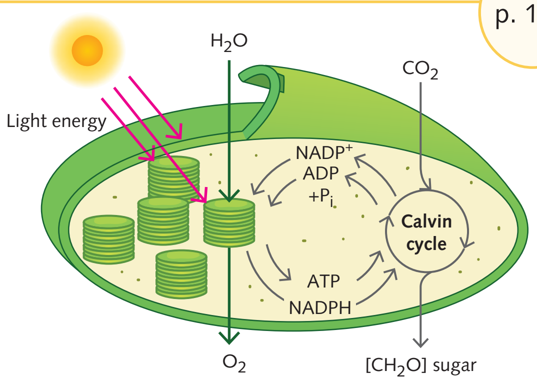


Figure 5.4 The close relationship between the light-dependent stage and the light independent Calvin–Benson cycle.

5.2 Cellular respiration as a biochemical pathway

KEY CONCEPTS

p. 172

- The purpose of cellular respiration is to release the energy stored in the bonds of glucose to produce ATP, which can provide energy to fuel cellular reactions.
- Glycolysis occurs in the cytosol. It involves the breakdown of glucose to two pyruvate molecules, two ATP molecules and two loaded acceptor molecules, NADH.
- ATP is available for immediate use by the cell. Pyruvate is processed in subsequent reactions that differ between organisms and depend on the availability of oxygen.
- Pyruvate enters the mitochondria where it is broken down.
- In the Krebs cycle, pyruvate is completely broken down, releasing carbon dioxide as a by-product and producing ATP and the loaded coenzymes NADH and $FADH_2$.
- On the cristae of the mitochondria, a complex series of steps involving cytochrome enzymes transfers the electrons to the oxygen, which reacts with H^+ from the coenzymes to produce water, in the electron transport chain.
- The net output of ATP from aerobic cellular respiration is 30/32 ATP per glucose molecule.
- Eukaryotes have two anaerobic cellular respiration pathways for producing ATP from glucose in absence of oxygen.
- Yeasts, plants and some bacteria carry out alcoholic fermentation; some animal cells carry out lactic acid fermentation.
- The products of anaerobic respiration are toxic at high concentrations and so anaerobic respiration is only a short-term solution for obtaining chemical energy.
- The rate of cellular respiration is affected by temperature, glucose and oxygen availability as well as other factors.
- Photosynthesis and cellular respiration are linked – the inputs of one are the outputs of the other.

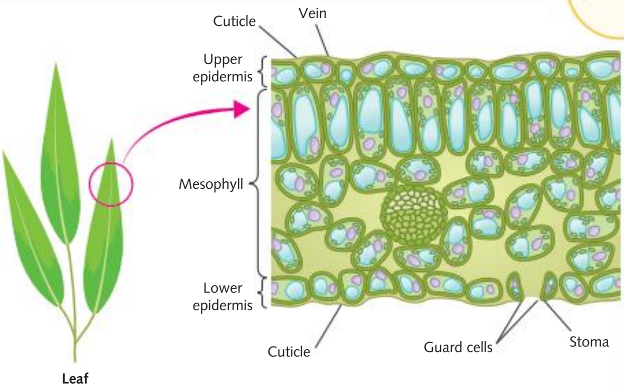


Figure 5.2 b A leaf cross-section showing the location of mesophyll cells that contain chloroplasts.

5.3 Biotechnological applications of biochemical pathways

KEY CONCEPTS

p. 185

- The CRISPR-Cas9 protein acts as a ‘cut and paste’ tool for geneticists so they can introduce specific DNA sequences into plant genomes that code for more efficient photosynthetic traits.
- Possibilities include increasing crop yield by enhancing light availability (by altering plant architecture), increasing light capture and energy conservation.
- Anaerobic respiration of biomass produces ethanol that is converted into biofuels such as biogas, which is used as a fuel for transport and electricity.
- Biogas production is a carbon neutral process, where the entire path from plant to livestock to manure to decomposition is considered a closed system.

Stuart Muir Wilson

Figure 5.27 The portable bioreactor at the Ecological Justice Hub in Brunswick produces electricity from biomass waste products, supplying electricity without the use of fossil fuels.

WB
5.4.1
KEY TERMS
PAGE 124

5 Chapter glossary

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

aerobe an organism that requires oxygen to survive and reproduce

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

anaerobe an organism that does not require oxygen to survive and reproduce

autotroph an organism that makes its own food from inorganic substances, using light (through photosynthesis) or chemical energy (through chemosynthesis); includes green plants, algae and certain bacteria

biofuel a fuel that has used biomass as its original source

biomass the total dry weight of organic material

bundle sheath cells photosynthetic cells arranged tightly packed around the vein of a leaf, so deeper in leaf tissue compared to the mesophyll cells

C_3 plant a plant that directly uses CO_2 as an input for the first stage of the Calvin cycle of photosynthesis

C_4 plant a plant that uses CO_2 to make a C_4 storage compound which moves from the mesophyll cells to the bundle sheath cells and a lower O_2 concentration, thereby reducing photorespiration

Calvin–Benson cycle a biochemical pathway in which sugar molecules are produced using carbon dioxide

CAM (crassulacean acid metabolism) plant a plant that shuts its stomata during the day and fixes carbon during the night when its stomata are open; an adaptation to hot dry environments and to reduce photorespiration and increase photosynthesis

carbon fixation the conversion of atmospheric carbon from carbon dioxide into carbohydrates in the stroma of chloroplasts in eukaryotic cells

chemoautotroph an organism that makes its own food from inorganic substances, using chemicals as the primary energy source

cristae the folding of the inner mitochondrial membrane into the matrix, thus increasing the total surface area of the inner membrane

cytochrome a family of membrane-bound proteins that carry out electron transport; located in the mitochondrial inner membrane and in chloroplast thylakoid membrane

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

heterotroph an organism that cannot make its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl CoA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll to split water molecules to produce oxygen, hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions, carbon dioxide, hydrogen ions and ATP produce carbohydrate

limiting factor the factor that limits the rate of a reaction

matrix a gel-like fluid in mitochondria, where the Krebs cycle (citric acid cycle) of cellular respiration takes place

mesophyll cells the photosynthetic cells of two types, palisade and spongy, that occupy the inside of the leaf between the two outer epidermal layers

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

photoautotroph an organism that makes its own food from inorganic substances, using light as its primary energy source

photorespiration the process in which plants bind with oxygen instead of carbon dioxide in high oxygen concentrations

pigment a molecule that absorbs certain wavelengths of light and reflects all others

pyruvate a three-carbon molecule that is the end product of glycolysis

stroma the jelly-like, semifluid interior of a chloroplast

thylakoid membrane the interconnected, folded membrane within a chloroplast

WB
5.4.2
EXAM PRACTICE
PAGE 126

5 Chapter review

Remembering

1 Why is chlorophyll seen as green?

2 When do plants respire?

3 What are the reactants in aerobic cellular respiration?

Understanding

4 Figure 5.28 shows a generalised cross-section of a leaf.

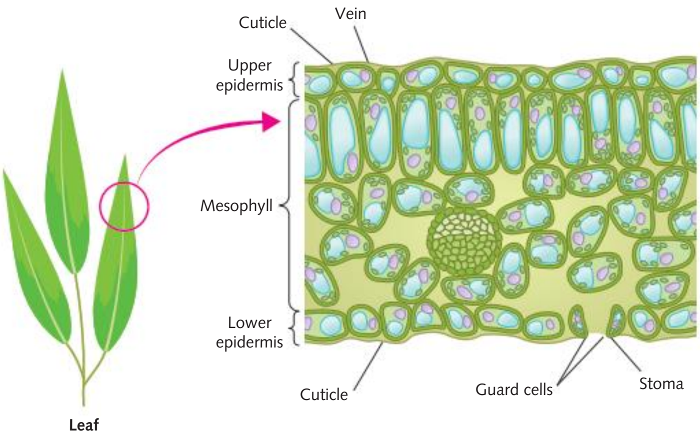


Figure 5.28 A cross-section of a leaf

- a State the photosynthetic equation.
- b What structures bring water to the leaf?
- c What structures engage in gaseous exchange? What gases are exchanged with the atmosphere?
- d Roots usually do not have chloroplasts in their cells. Why are they vital structures for photosynthesis?

5 What are the reactants in photosynthesis? What are the products in photosynthesis?

6 Which molecule absorbs the energy required to power photosynthesis?

Applying

7 Why are plants the ultimate recyclers when it comes to photosynthesis and respiration?

8 What effect does increasing the temperature of the intracellular environment of plant cells have on photosynthesis?

Analysing

9 In an investigation into photosynthesis, a student added sodium hydrogen carbonate to the water. Explain why.

10 An investigation was made into photosynthesis of the pondweed *Elodea*. How would you account for the following results?

- » Experiment A produced 81 bubbles of oxygen.
- » Experiment B produced 9 bubbles of oxygen.

11 Mitochondria are known as the powerhouses of the cell. What structures in mitochondria lend themselves to this claim and how?

12 Find out which cells in the human body might have a net production of 30 ATP and which might have a net production of 32 ATP. Discuss why this might occur.

13 How is energy stored in ATP?

14 What is considered to be a waste product of cellular respiration? Is it harmful? How does the body remove it?

15 Which pair of molecules are products of aerobic and anaerobic cell respiration in some organisms?

	Aerobic cellular respiration	Anaerobic cellular respiration
a	ATP	CO_2
b	CO_2	$C_6H_{12}O_6$
c	O_2	Pyruvate
d	Lactate	ATP

16 During exercise under low oxygen conditions, muscles can switch to only anaerobic forms of respiration. What aspects of this process are useful and what aspects are dangerous?

Unit 3, Area of Study 2 review

Multiple choice

Question 1 ©VCAA 2019 Q12 ADAPTED MEDIUM

During which process would the production of ethanol be observed?

- A Aerobic cellular respiration
- B Photosynthesis
- C Fermentation in yeasts
- D Fermentation in animals

Question 2 ©VCAA 2014 Q7 ADAPTED MEDIUM

During photosynthesis in chloroplasts, energy is used to convert carbon dioxide into carbohydrates. This occurs

- A during the light-dependent reaction in the grana.
- B during the light-independent reaction in the stroma.
- C on the membrane of the thylakoids during the light-independent reaction.
- D on the surface of the outer chloroplast membrane during the light-dependent reaction.

Question 3 ©VCAA 2014 Q8 ADAPTED MEDIUM

When the atmospheric CO_2 level increases, the resulting increase in the rate of photosynthesis is due to the rate of the

- A light-independent reactions on the thylakoid membranes of the chloroplasts increasing.
- B light-dependent reactions on the thylakoid membranes of the chloroplasts increasing.
- C light-independent reactions in the stroma of the chloroplasts increasing.
- D light-dependent reactions in the stroma of the chloroplasts increasing.

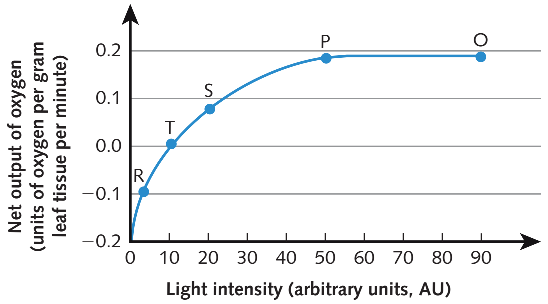
Question 4 ©VCAA 2018 Q9 ADAPTED MEDIUM

The respective inputs and outputs of the Krebs cycle in an animal cell are

	Inputs	Outputs
A	NAD^+, pyruvate, ADP, P_i, FAD^+	CO_2, NADH, ATP, $FADH_2$
B	NADH, ADP, water, P_i, $FADH_2$	ATP, NAD^+, oxygen, FAD^+
C	NADH, ADP, oxygen, P_i, $FADH_2$	ATP, NAD^+, water FAD^+
D	NADPH, ADP, pyruvate, P_i, $FADH_2$	$NADP^+$, ATP, oxygen, FAD^+

Use the following information to answer Questions 5 and 6.

The graph below shows the net amount of oxygen that geranium leaves release as light intensity is increased in an experiment. The temperature is always kept constant.



Question 5 ©VCAA 2017 Q13 ADAPTED MEDIUM

Which one of the following conclusions can be made based on the graph?

- **A** At point O, all photosynthesis has ceased.
- **B** The least amount of photosynthesis occurs at point T.
- **C** At point P, the amount of oxygen output is twice that at point R.
- **D** Above 10 AU of light, the plant is absorbing more CO_2 from the atmosphere than it is producing.

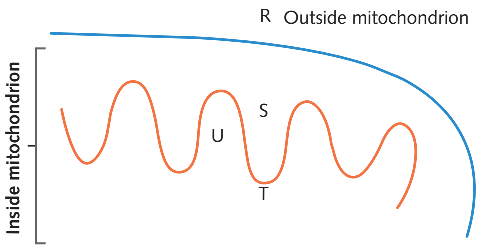
Question 6 ©VCAA 2017 Q14 ADAPTED MEDIUM

The rate of oxygen output remains constant between points P and O because

- **A** lactic acid build-up has denatured the enzymes involved in the photosynthesis reactions.
- **B** the rate of photosynthesis is limited by the concentration of available carbon dioxide.
- **C** the light has stimulated more respiration in the mitochondria.
- **D** oxygen has stopped being produced around the geranium leaves because of an accumulation of oxygen there.

Question 7 ©VCAA 2018 Q8 ADAPTED MEDIUM

The diagram below shows a section through a part of a mitochondrion.



The sites of the pathways in aerobic respiration are

- **A** R – glycolysis, S – Krebs cycle, T – electron transport chain.
- **B** R – glycolysis, U – Krebs cycle, T – electron transport chain.
- **C** U – glycolysis, T – Krebs cycle, R – electron transport chain.
- **D** T – glycolysis, R – Krebs cycle, S – electron transport chain.

Question 8 ©VCAA 2019 Q13 ADAPTED EASY

In human cells, the rate of aerobic cellular respiration may increase if the

- **A** temperature of the cell is increased from 37°C to 40°C.
- **B** rate of facilitated diffusion of glucose into the cytosol of the cell decreases.
- **C** carbon dioxide concentration in the cytosol of the cell increases.
- **D** amount of glucose available to the mitochondria increases.

Question 9 ©VCAA 2017 Q11 ADAPTED HARD

Radioactively labelled glucose was added to a culture of animal cells. The cells were then monitored for three minutes. After this time, radioactively labelled atoms would be present in which cellular chemical?

- **A** Inorganic phosphate
- **B** Adenosine triphosphate
- **C** Oxygen
- **D** Carbon dioxide

Question 10 ©VCAA 2010 EXAM1 Q15 ADAPTED HARD

The starting compounds and end products of a chemical reaction that results in a net reduction of ATP are

	Starting compounds	End products
A	DNA	Nucleotides
B	Glucose	Starch
C	Peptides	Amino acids
D	Triglycerides	Fatty acids and glycerol

Question 11 ©VCAA 2011 EXAM 1 Q20 ADAPTED HARD

The reaction ADP + $P_i \rightarrow$ ATP

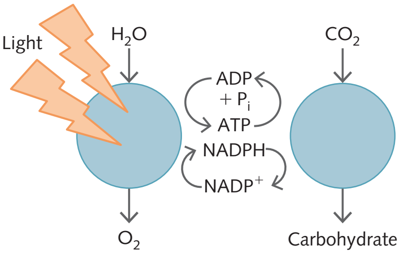
- **A** is not reversible.
- **B** only occurs in eukaryotic cells.
- **C** occurs in the absence of enzymes.
- **D** occurs in yeast cells during anaerobic respiration.

Question 12

In the three stages of aerobic cellular respiration, the yield of ATP per glucose molecule is:

	Glycolysis	Krebs cycle	Electron transport chain
A	26	2	2
B	2	2	28
C	4	26	2
D	30	1	1

Question 13 ©VCAA 2018 Q15 ADAPTED HARD



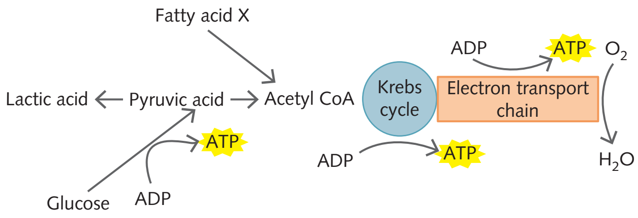
From the diagram above you can tell that

- **A** the light-dependent reaction builds ATP and the light-independent reaction releases ADP and P_i.
- **B** $NADP^+$ is the loaded form of NADPH.
- **C** the oxygen released by photosynthesis comes from the carbon dioxide.
- **D** the light-independent reaction has no output other than carbohydrate.

Question 14 ©VCAA 2014 Q12 ADAPTED HARD

If a cell has insufficient glucose for cellular respiration, fatty acids can be changed to acetyl CoA. Each fatty acid X molecule produces eight molecules of acetyl CoA.
The pathways for the breakdown of fatty acid X and glucose are summarised in the diagram below.

The number of molecules produced in each step is *not* shown.



Referring to the information above and your knowledge of cellular respiration, which one of the following conclusions can be made?

A More ATP is made in the Krebs cycle than in glycolysis.

B One glucose molecule produces less ATP in aerobic conditions than one fatty acid molecule.

C No ATP is formed from the breakdown of glucose under anaerobic conditions.

D Pyruvic acid is converted to lactic acid under aerobic conditions.

Question 15 ©VCAA 2018 Q7 ADAPTED EASY

A series of experiments were performed to investigate the effect of four different variables on the rate of an enzyme-catalysed reaction that normally occurs in the human body. The students each changed one of the following variables: substrate concentration, pH, temperature and enzyme concentration. After recording their data, the results were displayed in a series of graphs, as shown below. Each graph is a line of best fit for their data.

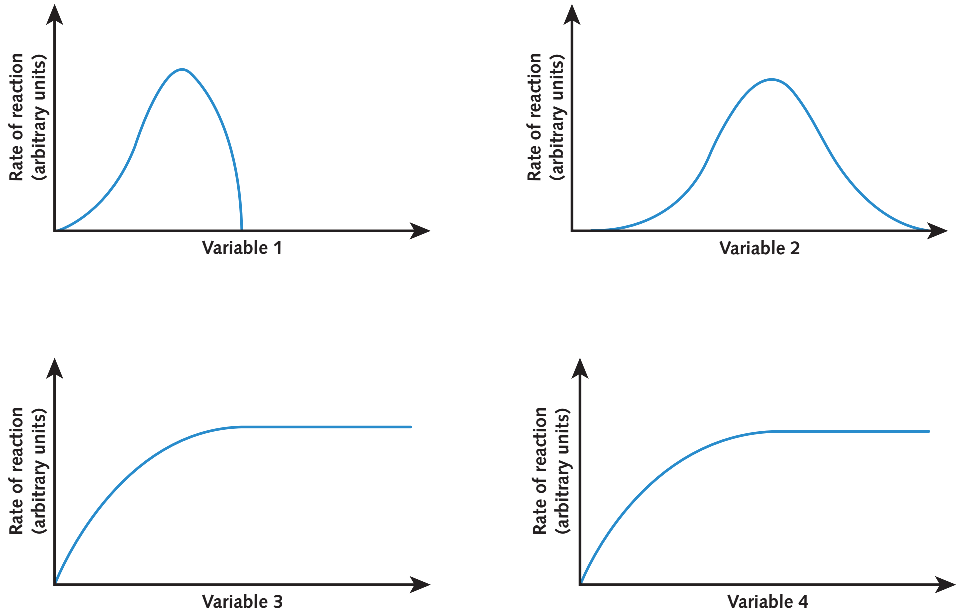
The students did not label the horizontal axis on any of their graphs. From your knowledge of enzyme functioning you can conclude that if

A more enzyme were added to the enzyme–substrate mixture, the graph of Variable 1 would show an increase in rate of reaction.

B more substrate were added to the enzyme–substrate mixture, the graph of Variable 2 would show an increase in rate of reaction.

C the enzyme–substrate mixture were cooled to 25°C, the graph of Variable 3 would drop to zero.

D the enzyme–substrate mixture were heated to 45°C, the graph of Variable 4 would drop to zero.



Short answers

Question 1 ©VCAA 2013 SEC B Q1 ADAPTED

Yeast is a single-celled, microscopic fungus that uses sucrose as a food source. The cellular respiration by a particular species of yeast was investigated in an experiment. Yeast cells were placed in a container and a sucrose solution was added. The experiment was carried out at room temperature. An airtight lid was placed on the container. The percentages of ethanol and oxygen in the container were recorded over a one-hour period. The results are shown in the following table.

	Oxygen (%)	Ethanol (%)
Start of experiment	21	0
End of experiment	18	4

a Explain why a change in the oxygen level was observed during the experiment.

1 mark

b Name the process that would account for the change in the ethanol level observed during the experiment.

1 mark

c The experiment was allowed to continue for the next 24 hours. Predict what you think would happen to the levels of oxygen and ethanol over this extended period and provide an explanation of your prediction.

2 marks

Question 2 ©VCAA 2013 SEC B Q1 ADAPTED

Increasing the efficiency of photosynthesis in food crops has always been of interest to scientists, particularly the effect of the concentration of carbon dioxide on photosynthesis.

a During which stage of photosynthesis is carbon dioxide used?

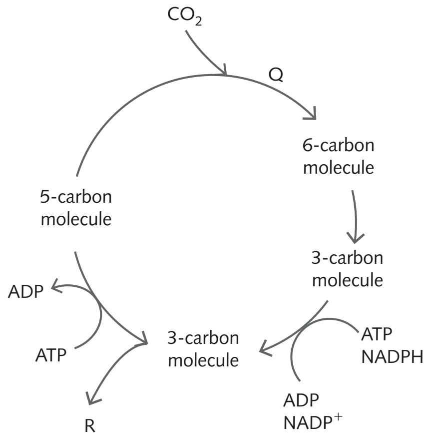
1 mark

b Name the stage of photosynthesis that does not require carbon dioxide as an input. List two inputs of this stage and describe the role played by each in this stage of photosynthesis.

2 marks

Question 3 ©VCAA 2007 E1 SEC B Q4 ADAPTED

The light-independent reactions (Calvin–Benson cycle) of photosynthesis are summarised in the diagram below.



a State the name of Q.

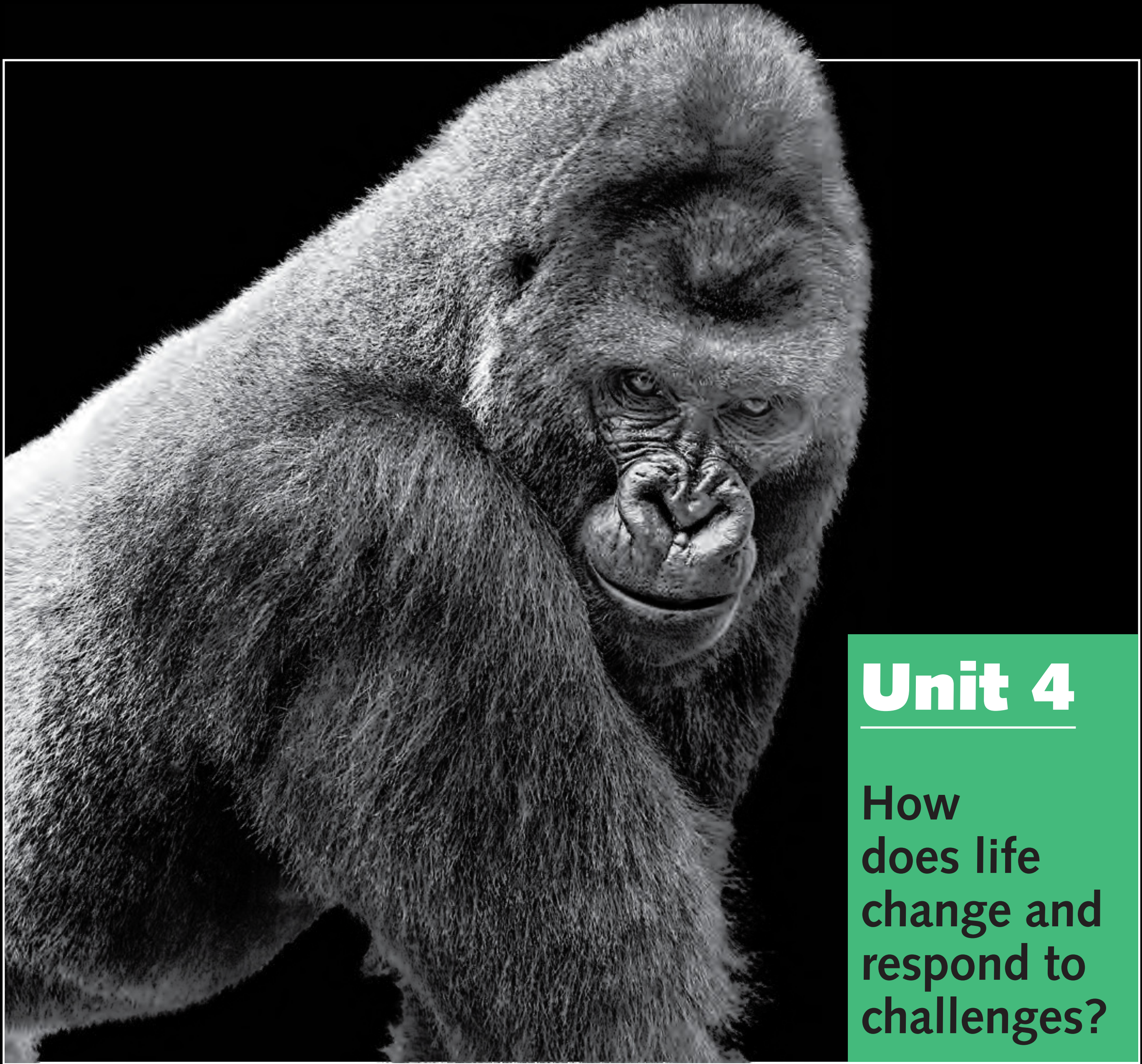
1 mark

b NADPH and ATP are used during the light-independent reactions. What are the roles of NADPH and ATP?

1 mark

c What is compound R?

1 mark



Getty Images/Dean Fikar

Unit 4

How does life change and respond to challenges?

Area of Study 1: How do organisms respond to pathogens?

Area of Study 2: How are species related over time?

Area of Study 3: How is scientific inquiry used to investigate cellular processes and/or biological change?

Responding to antigens

6

By the end of this chapter you will have covered the following material.

Key knowledge

Responding to antigens

- physical, chemical and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants pp. 204–214
- the innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins and interferons pp. 214–223
- initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens pp. 222–233

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- formulate hypotheses to focus investigation pp. 208–210

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 208–210

Generate, collate and record data

- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 208–210

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 208–210
- repeat experiments to ensure findings are robust pp. 208–210
- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors pp. 208–210

Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 208–210

Online Chapter Map
Chapter 6 map

6 Responding to antigens

How does the flu virus enter your body? What does your body do about it?

6.1 Physical, chemical and microbiota barriers in animals – first line defence

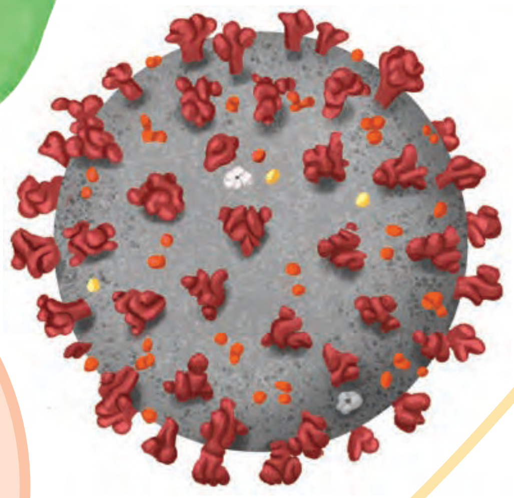
p. 204

Understanding and mitigating pathogens is a primary concern of the medical profession. Humans, animals and plants have developed sophisticated lines of defence against attack by pathogens. The first line of defence includes external barriers and strategies to keep the pathogen out.

6.2 Physical and chemical defences in plants

p. 211

Plants also need to protect themselves against pathogens. Plants have developed physical and chemical defences to fight pathogens; for example, the thick bark of trees.



6.3 Innate immune response in animals – second line of defence

p. 214

If our external barriers cannot prevent infection, then we have a second line of defence – our innate immune system. White blood cells destroy foreign pathogens in our bloodstream. Some use enzymes to rupture pathogens, while others release histamines to aid healing.

p. 224

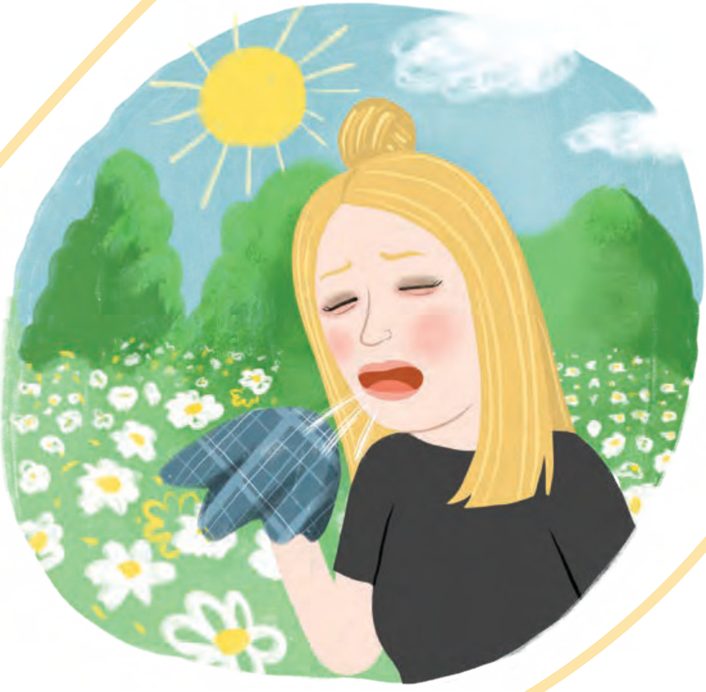
6.4
Antigens and pathogens

It is important that our immune system can distinguish our own cells from foreign cells. Antigens are parts of substances that induce an immune response in our body. Pathogens vary widely. Some of them can reproduce quickly; others do not reproduce, but exist within host cells, which reproduce them; for example, HIV, SARS or SARS-CoV-2.

p. 231

6.5
Allergens

In some people, the most seemingly innocuous pollen can set off extreme anaphylactic shock. Allergies begin with sensitisation whereby IgE antibodies are first produced. Treatment can include desensitisation, which aims to build immune tolerance.



p. 232

6.6
Phagocytosis

Phagocytes such as macrophages and neutrophils engulf pathogens, isolate them inside a membrane-bound vesicle and then digest them.

Your body has three lines of defence to protect you from infection and disease. The first line of defence consists of chemical and physical barriers to prevent invaders entering your body. If this fails, the second line of defence destroys invaders in a generalised way. If that does not work, your body activates the third line of defence, which targets specific invaders.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 6 map (p. 200)

Online Key Terms:
- Chapter 6 flashcards (p. 202)

Weblinks:
- The body's first line of defence (p. 208
- Immune response in plants (p. 211)

Online Worksheets:
- First line of defence (p. 208)
- Plant immunity (p. 211)

Online Key Concepts:
- Chapter 6: Summary of key concepts (p. 236)

Online Key Terms
Chapter 6 Flashcards

Know your key terms

adaptive immune response
allergen
allergy
anaphylactic shock
antibiotic
antigen
apoptosis
bacteria
bacterial capsule
cellular pathogen
chemokine
chemotaxis
cilia
companion plant
complement
cytokine
defensins
degranulation
dendritic cell
desensitisation
disease
eosinophil
first line of defence
flagellum
fungi
granulocyte
histamine
host
immune system
immune tolerance
infectious disease
inflammation
innate immune response
interferon
keratin
leukocyte
lymphocyte
lysis
lysozyme
macrophage
mast cell
microbiome
microbiota
microflora
monocyte
mucous membrane
natural killer cell
necrosis
neutrophil
non-cellular pathogen
Non-infectious diseases
non-self antigen
non-specific response
obligate parasite
opsonisation
pathogen
phagocyte
phagocytosis
phagolysosome
phagosome
platelet
prion
protist
second line of defence
secondary metabolite
self-antigen
sensitisation
sterile inflammation
transmitted
vasodilation
virus

Remember

WB
REMEMBER
PAGE 129

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1. A disease is any condition that affects the structure or normal functioning of an organism.
2. A bacterium is a prokaryotic organism that can cause diseases such as gastroenteritis and cholera.
3. Plants have structures, such as thick waxy cuticles and hairs, that assist in preventing disease-causing pathogens from entering.
4. Plants have stomata on their leaf surface, which open and close to allow gas exchange. Disease-causing pathogens can enter the plant through the stomata.

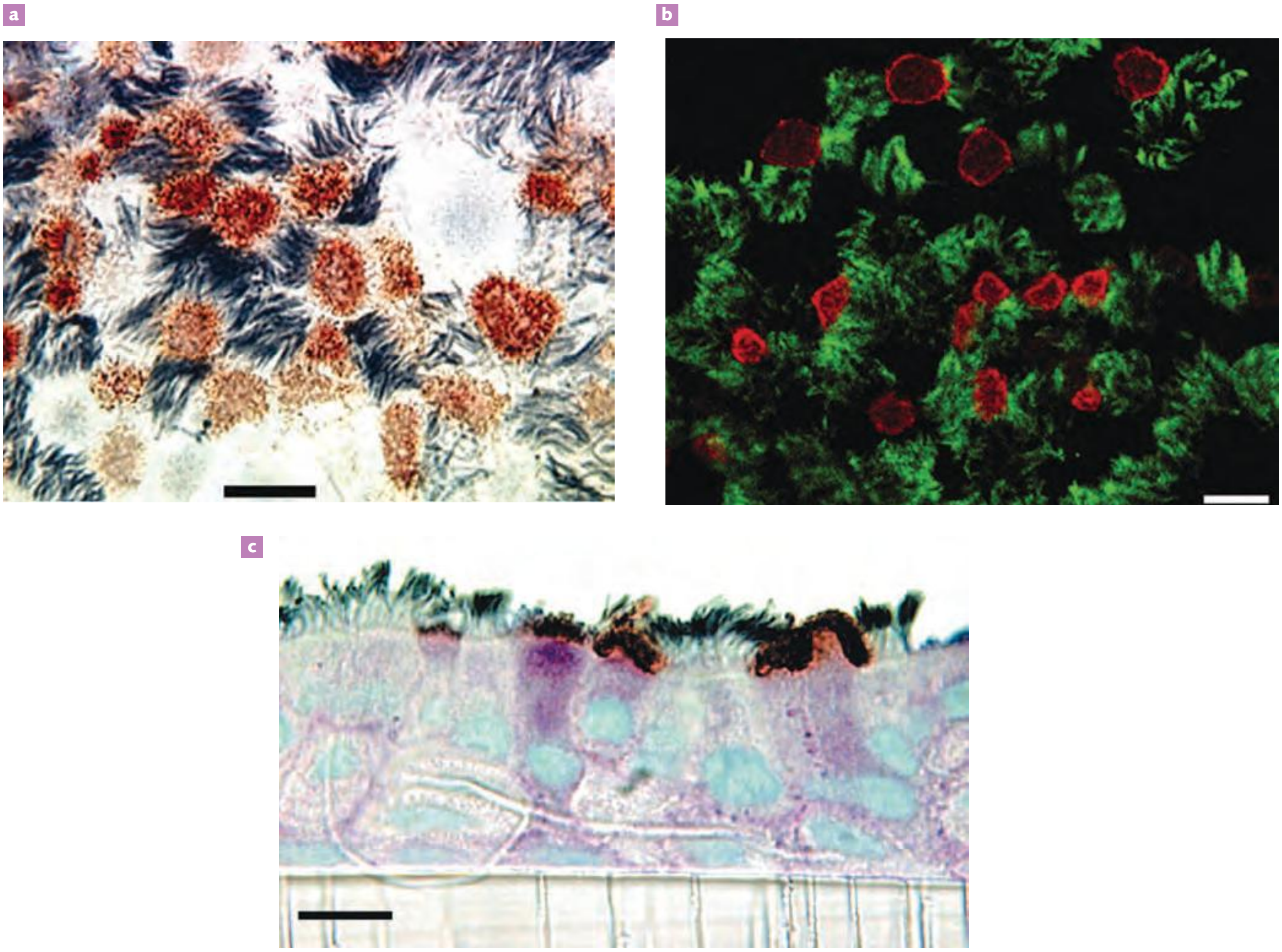


Figure 6.1 Influenza virus particles binding to cilia cells in the respiratory tract as seen by **a** light microscopy (virus is red and cilia are grey), and **b** fluorescent confocal microscopy (virus is red and cilia are green). **c** Influenza virus shown targeting secretory cells (virus is red and cilia are grey). Bars = 10 μm.

A **disease** is any condition that interferes with how an organism, or any part of it, functions. Diseases can be grouped according to their cause. **Non-infectious diseases** are those caused by environmental, genetic, or lifestyle factors and not by pathogens. Examples include obesity, diabetes, haemophilia, melanoma cancers from uv exposure, lung cancer fro smoking or asbestos exposure. **Infectious diseases** are those that are caused by an infective agent that can be passed from one organism to another. The infected organism is the **host**. An infectious agent that causes disease is called a **pathogen**.

Disease is often described in terms of battles and wars – attacks on the body and invasions by pathogens. The immune system is commonly referred to as the defence system, fighting pathogens using lines of defence, like a walled city under siege. If the first line of defence is breached and pathogens enter the body, they are attacked by second and third lines of defence. Hence, despite significant exposure to invading micro-organisms and parasites, in most cases we are able to resist infection. All organisms have evolved various types of defence mechanisms to inhibit the entry of pathogens and to deal with them should they gain a foothold. This is often referred to as an 'evolutionary arms race' between pathogens and their hosts. Even simple, single-celled organisms such as bacteria can defend themselves against viruses by using the CRISPR-Cas9 system.

Influenza is an acute infectious condition. It makes an individual ill quite quickly, but hopefully clearing within a relatively short time period. The clearance of influenza from the body is not a passive process. It involves a highly coordinated series of responses from molecules, cells and the body as a whole.

CONNECT

You will learn about the third line of defence in Chapter 7.

CONNECT

See page 87 for more about CRISPR-Cas9 in bacteria.

Coughing and sneezing are common ways in which your body tries to rid itself of the influenza virus before it reaches your cells. This is called the **first line of defence** of your body's immune system. As part of the first line of defence, cells in mucous membranes produce mucus, which traps viral particles, and ciliated epithelial cells in the trachea beat their cilia, moving the contaminated mucus away from the lungs and towards the throat for swallowing. Some influenza virus particles may escape these mechanisms and infect the epithelial cells in the respiratory tract to initiate an infection. The symptoms that usually follow include muscle aches, fatigue and fever. These are also signs that the body's **second line of defence** has been initiated. In the second line of defence, chemicals are released that ultimately hinder the virus's ability to reproduce inside your cells. The second line of defence is a **non-specific response** from the immune system in that it detects and responds to the pathogen the same way each time it encounters the pathogen. A suite of signalling molecules are released that protect surrounding cells from further invasion and activates other cells to destroy the cells that are infected, safely containing the infection.

WB
6.1.1
PHYSICAL AND CHEMICAL BARRIERS
PAGE 130

6.1 Physical, chemical and microbiota barriers in animals – first line of defence

The most effective way of preventing the colonisation of an organism by pathogens is to keep them out of the body in the first place. This first line of defence comprises various physical, chemical and microbiota barriers that stop or impede the entry of pathogens and other foreign substances. The scales of reptiles (Figure 6.2), the exoskeleton of arthropods such as insects and crustaceans, the shells of eggs and human skin are examples of physical barriers that protect the animal from invasion.

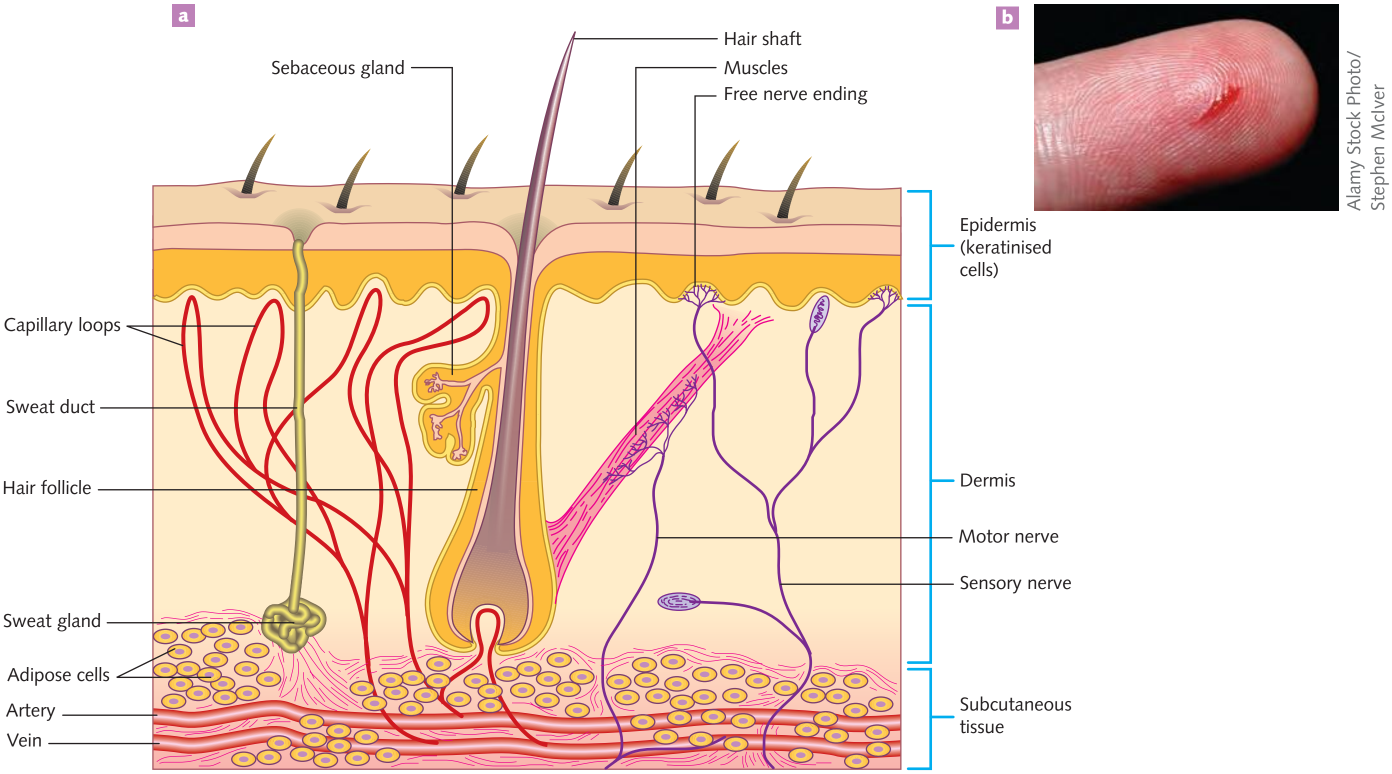
Alamy Stock Photo/Jody Neice

Figure 6.2 **Reptiles such as this iguana have tough, scaly skin that helps defend against the entry of some pathogens.**

Skin: a tough physical barrier

The skin is the largest organ in the human body, and acts as a tough physical barrier between the body and the outside world. Like all the inner and outer linings of the body, the skin is made from epithelial cells. The epithelial cells become keratinised, a process in which the structural protein **keratin** is deposited, and form a hard outer layer that is impervious to water and micro-organisms (Figure 6.3a). The importance of the skin as a barrier can be seen in burns victims who lose a large proportion of their skin. If they survive the effects of heat and dehydration, they may still die as a result of multiple infections caused by invading micro-organisms that overwhelm the immune system.

Damaged skin is an ideal site for infection. In addition to burns, other injuries such as cuts and abrasions provide a potential site for the entry of pathogens. When the skin is cut and blood vessels are damaged, cell fragments in the blood, called **platelets**, are quickly attracted to the site of the wound. As they stick to the damaged tissue, they send out chemical messages. These messages trigger the formation of a web-like mesh of fibrin protein that stabilises the aggregation of platelets and traps red blood cells to form a clot. This plugs the break in the vessel wall, forming a scab that seals the wound and keeps out micro-organisms while the skin is healing. As long as it remains unbroken, our tough waterproof skin is an effective barrier against invading micro-organisms and pathogens.



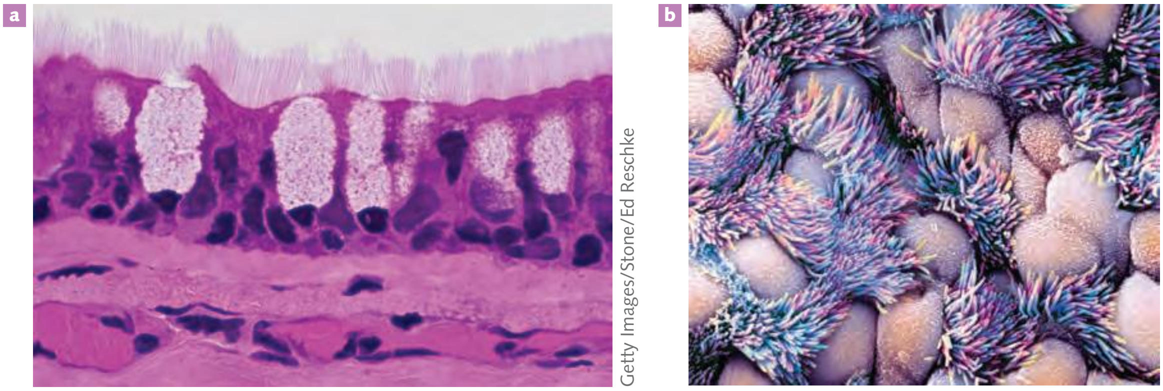
Alamy Stock Photo/Stephen McIver

Figure 6.3 a A cross-section of human skin. **b** A cut or scratch breaks the barrier of the skin and can be a site of pathogen entry into the body.

Tissue secretions

The external openings of the respiratory, digestive, excretory and reproductive systems are ideal entry points for micro-organisms. Tissues secrete chemical substances that physically trap and expel invading micro-organisms and other foreign particles. These tissue secretions include mucus, sebum (an oily secretion) and tears.

The human respiratory, gastrointestinal and reproductive tracts are lined with epithelial cells that secrete mucus. For this reason, they are called **mucous membranes**. Slender hair-like structures called **cilia** line the respiratory tract (Figure 6.4). Their beating pushes mucus up to the throat,



Getty Images/Stone/Ed Reschke

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Figure 6.4 a A light micrograph of a mammalian trachea, showing, in vertical section, the cilia lining the wall. Cilia help to trap pathogens and move them up and out of the body **b** A scanning electron micrograph showing the cilia of cells lining the respiratory system; the structures between the cilia are mucus-secreting cells.

where it can be coughed or sneezed out or swallowed. People with defective mucus secretion or inhibited ciliary movement frequently develop lung infections caused by **bacteria** colonising the epithelial surfaces.

The eyes have ducts that secrete tears, which help to flush pathogens from the eye surface. **Lysozyme**, which is an enzyme in tears, saliva and mucus, acts as an antimicrobial agent, breaking down the cell wall of certain types of bacteria and causing them to undergo **lysis**, or bursting, as shown in Figure 6.5.

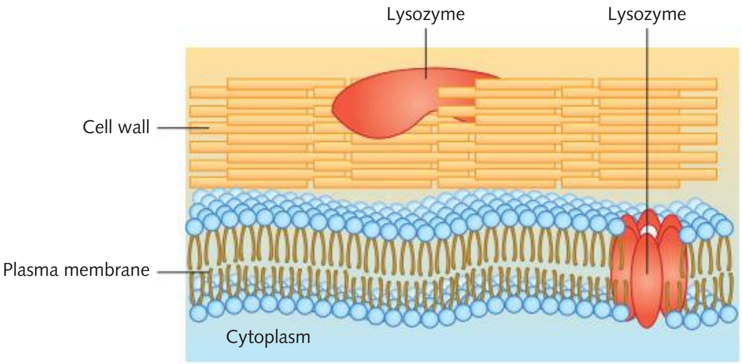


Figure 6.5 The action of lysozyme on the bacterial cell wall as it binds to the cell wall, breaks apart bonds and exposes the plasma membrane to further action of the enzyme.

The skin secretes sebum, which has antimicrobial properties to protect from surface invaders.

Urine passing through the urethra has a flushing effect on micro-organisms that are trying to enter the body via the urethra.

Skin secretions such as sweat and oil mean that skin has a pH of 3–5, which is acidic enough to prevent colonisation by many pathogenic species. The low pH of the vagina also prevents the overgrowth of infectious agents. The highly acidic environment of the stomach kills many micro-organisms in food and drinks, as do the digestive enzymes secreted by the stomach and small intestine.

WB
6.1.2
MICROBIOTA AS A BARRIER
PAGE 130

Microbiota as a barrier

During birth, a baby acquires micro-organisms from its mother that become permanently associated with it. The symbiotic micro-organisms that live in close association with each other on and in our bodies are our normal **microbiota** (also called **microflora**) and include bacteria, fungi, protists and viruses. By taking up space and using nutrients, our normal microbiota prevent growth of, and colonisation by other micro-organisms that may be pathogenic. Through the substances they produce, normal microbiota also set up a chemical micro-environment that supports the growth of other beneficial, non-pathogenic, micro-organisms. These bacteria are known as commensals because they help the human body in a variety of ways, including digestion and assisting the immune system; for example, by consuming invading pathogens. This results in a microbial community of species that contributes to our health, and effectively forms a barrier against pathogenic bacteria. When non-pathogenic bacteria are killed by **antibiotic** treatments, any pathogenic micro-organisms with antibiotic resistance may replace them and cause disease.

Other first lines of defence

In the gut, peristalsis is an important mechanism for keeping both food and infectious agents moving through. Failure of peristalsis is typically accompanied by overgrowth of bacteria within the intestinal lumen.

Figure 6.6 and Table 6.1 summarise the first lines of defence of humans.

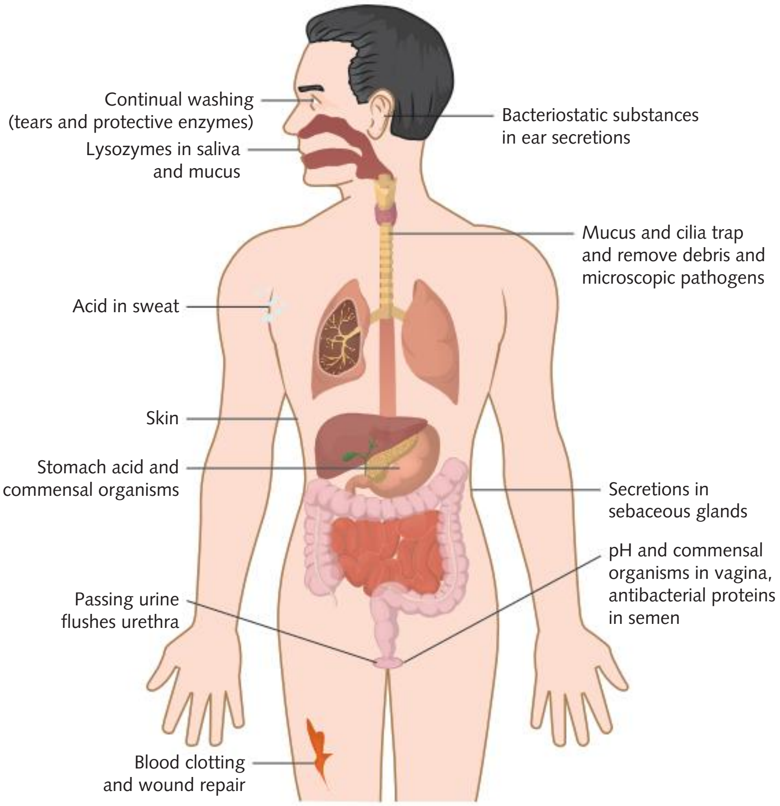


Figure 6.6 A summary of the physical and chemical barriers to pathogenic infections in a human. Peristalsis also helps by moving food and infectious agents through the digestive system.

Weblink
The body's first line of defence

Online worksheet
First line of defence

EXAM TIP
Make sure you know the first lines of defence in humans and can provide an example of each.

Table 6.1 A summary of first lines of defence barriers in humans

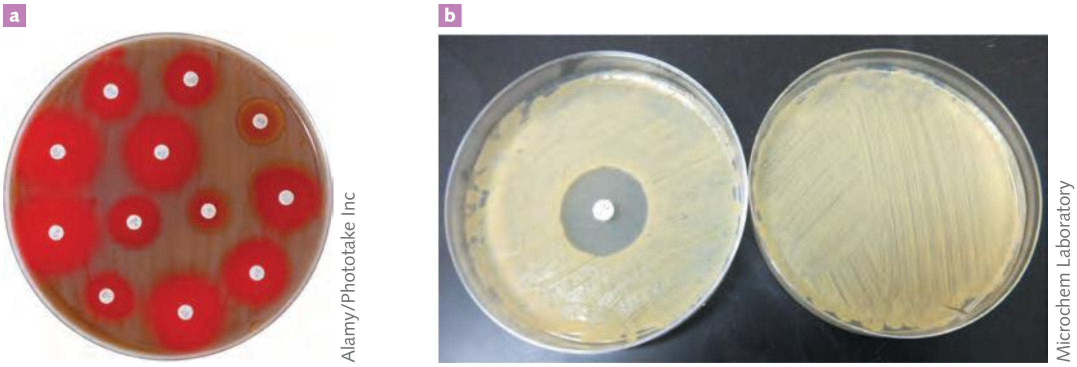
Type of barrier	Location	Barriers or mechanisms to prevent entry of pathogen
Physical	Skin	Keratinised skin cells, rapid blood clotting, rapid wound healing, antiseptic action of acidic secretions
Chemical	Skin	Secretion of sebum, which contains antimicrobial substances such as lysozymes
Chemical	Digestive system	Lysozymes in saliva and mucus, enzymes and strong acids in stomach
Physical and chemical	Respiratory system	Mucus traps dirt and small pathogens; cilia lining trachea move this upwards
Physical and chemical	Reproductive tract	Mucus with acidic pH moving fluids flush out pathogens
Chemical	Urinary tract	Urine flushes out pathogens and its acidity inhibits bacterial growth
Physical and chemical	Sense organs	Ear wax and hairs, eyelashes and nostril hairs trap pathogens; tears wash away pathogens and contain lysozyme
Microbiota	Skin and digestive tract	Their presence prevents the growth of pathogenic micro-organisms

INVESTIGATION 6.1

Second-hand data analysis: is lysozyme an effective barrier against bacteria?

Eyes are warm and moist, which makes them an ideal entry point for bacteria into the human body. Tears contain the powerful antibacterial enzyme lysozyme, which destroys pathogens rapidly by lysing their cells. This investigation uses agar plates, spread with a culture of bacteria, to compare the bactericidal effectiveness of lysozyme, an antiseptic and a disinfectant. Bacteria grown on agar plates produce a bacterial 'lawn', a cloudy film of millions of bacteria on the surface of the agar plate. If paper discs containing antibacterial substances are placed on the agar, they produce clear areas, known as zones of inhibition, where bacteria cannot grow (Figure 6.7).

You will analyse the results obtained from the investigation. The materials and method are provided so that you can reflect on them as part of your analysis.



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Microchem Laboratory

Figure 6.7 Examples of bacterial plates with zones of inhibition shown as cleared areas around antibiotic-soaked paper discs. The size of the zones of inhibition indicates the sensitivity of the bacteria to the antibacterial substance on the discs. **a** Red areas are blood agar where no bacteria is growing. Blood agar contains 5% sheep blood and provides a range of nutrients for bacteria that wouldn't otherwise grow *in vitro*. **b** In this example, the bacterial lawn is off-white and the agar is clear.

Aim

To compare the antibacterial effectiveness of lysozyme from tears with that of an antiseptic and a disinfectant

Materials

Per class:

- Broth culture of *Escherichia coli*
- Incubator set to 25°C
- Lab coats
- Safety glasses
- Gloves

Per group:

- 3 nutrient agar plates
- Filter paper
- Onion
- 10 mL each of disinfectant, antiseptic and distilled water
- Dilute disinfectant solution; for example, bleach
- Sterile 5 mL pipette
- Forceps
- Glass spreader
- Bunsen burner
- Sticky tape
- Ruler

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Although lab strains are usually harmless, bacteria may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at the end of the investigation. Decontaminate benches before and after the investigation. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Onions contain substances that irritate the eyes and nose.	Ensure the onion is held close to eyes, but does not actually come in contact with face or eyes.
Ethanol may be used to sterilise the bench top and is highly flammable.	Be careful to avoid ignition of ethanol liquid or fumes when using the Bunsen burner.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach, ensure the residue is wiped off; ensure lab coat sleeves are rolled down and gloves are worn.

Method

Record your hypothesis before beginning this investigation.

Note: To minimise contamination, wipe the bench with bleach or ethanol before you start.

1. Fold a piece of filter paper into quarters and use a hole punch to make four filter-paper discs.
2. Label the base of one agar plate with the date and the name of your group, and then divide the agar into four quarters. Near the edge of the plate, label each of the four quarters: ‘Water’, ‘Lysozyme’, ‘Antiseptic’ and ‘Disinfectant’.
3. Remove 1 mL of *E. coli* culture with the pipette, lift the lid off the labelled plate and transfer the bacteria to the surface of the agar.
4. Either replace the lid quickly and spread the liquid evenly by swirling, or spread the liquid evenly with the sterilised glass spreader, then replace the lid. Leave the plate on the bench for 2 minutes to allow the bacteria to penetrate the agar.
5. Make your eyes water by holding a cut onion near them, and blink to release tears.
6. Sterilise the forceps in the Bunsen burner flame, allow them to cool, then pick up a filter-paper disc and carefully dip it into one of the tears. Quickly touch the edge of the disc to the remains of the folded filter paper to blot, then gently place the disc on the quarter of the agar plate labelled ‘Lysozyme’.
7. Prepare small quantities (10 mL) of disinfectant and antiseptic solutions by diluting according to directions on the bottles.
8. Resterilise the forceps and moisten a disc by dipping it into the antiseptic and blotting, then gently place the disc on the correctly labelled quarter of the agar plate.
9. Repeat step 8 for the disinfectant and the distilled water.
10. Repeat steps 1–9 twice to make a total of three replicates.
11. Seal the plates with sticky tape and incubate at 25°C for 24 hours.
12. Ensure the bench is wiped down with bleach and wash your hands thoroughly.
13. The next day, observe the plates for the presence or absence of growth near the discs.
14. Measure the diameter of the zone of inhibition, which is the clear area around each disc. This shows the degree of sensitivity of the bacteria to each substance.

Results

This table shows the data that one group of students obtained when following this method. Copy the table into your logbook. Calculate the mean values and draw a suitable graph to represent the data.

Data for a group of students

	Diameter of zone of inhibition (mm) for each substance			
Trial	**Lysozyme**	**Antiseptic**	**Disinfectant**	**Water**
1	11	13	15	1
2	16	17	13	2
3	12	12	16	1
Mean				

Analysis of method

1. What steps in the method were taken to ensure there was no cross-contamination?
2. Explain the role of the disc dipped in water.
3. Explain the purpose of the three agar plates.
4. Identify one other risk and how you would manage it.

Analysis of results

Describe the results by stating the order of effectiveness of each of the solutions as bactericides.

Discussion

Compose a discussion of the findings (minimum of 300 words) as per the scientific method.

Conclusion

Draw a conclusion for this investigation.

KEY CONCEPTS

- Structural, chemical and biological features can act as barriers to pathogens as a first line of defence.
- The skin is a tough physical barrier made from keratinised epithelial cells that can prevent the entry of pathogens.
- When the barrier of the skin is broken, platelets quickly form a plug, or scab, that upholds the barrier until the skin is repaired.
- Mucus traps pathogens that invade mucous membranes, and cilia beat the mucus to a place where it can be expelled. Urine and tears flush out micro-organisms and peristalsis keeps them moving through the gut.
- Chemical barriers that prevent pathogen colonisation include low (acidic) pH on skin and in the stomach, digestive enzymes produced in the gut, and lysozyme in tears, saliva and mucus.
- Symbiotic organisms form our microbiota, which take up space and use nutrients. This prevents colonisation by pathogens.

Concept questions 6.1

1. Name the three types of barriers that form the first line of defence against disease.
2. List three openings in the skin that can allow the entry of pathogens.
3. Outline the role of mucous membranes.
4. Describe three ways in which the body can flush out micro-organisms.
5. Recount the role of platelets in blood clotting.

HOT Challenge

6. There are several main places in the human body where low pH kills pathogens as part of the first line of defence. Draw up a table that lists these places and the normal pH of these sites. Research what chemicals determine the pH at each site.

6.2 Physical and chemical defences in plants

Plants are prone to attack by parasites, pests and disease just as animals are. They are subject to attack by a huge array of mites, insects, nematodes (roundworms), fungi, bacteria and viruses (Figure 6.8), yet plants often survive these attacks. Plants also have mechanisms of defence. An understanding of plant defences may help scientists to reduce crop losses caused by plant disease. This research is critical to the wellbeing of humans, because plants are a vital component of our ecosystems. We depend on plants for food, as well as for valuable materials such as wood, plastics, textiles, medicines, dyes, inks and industrial chemicals.

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Figure 6.8 **a** Leaves of a healthy tobacco plant, *Nicotiana sylvestris*, and **b** a plant infected with tobacco mosaic virus

First line of defence

Plants have physical and chemical barriers to infection that form their first line of defence against invaders. As these are present before contact with the pathogen, they are termed 'passive defences'.

WB

6.2.1
PLANTS –
FIRST LINE OF
DEFENCE
PAGE 133

Physical barriers

Physical barriers in plants include the thick bark of stems and a thick and waxy cuticle (leaf surface) (Figure 6.9). Waxy cuticles and vertically hanging leaves may also prevent the formation of moisture films on leaves. This inhibits bacteria and roundworms that require water for motility, and fungal spores that germinate only in water. Hairs and thorns may also deter vectors of particular pathogens. Stomatal openings are weak spots, because they offer an entry point. Some plants have hairs that guard these openings and others may have sunken stomata that make access difficult.

Weblink
Immune responses in plants

Online Worksheet
Plant immunity

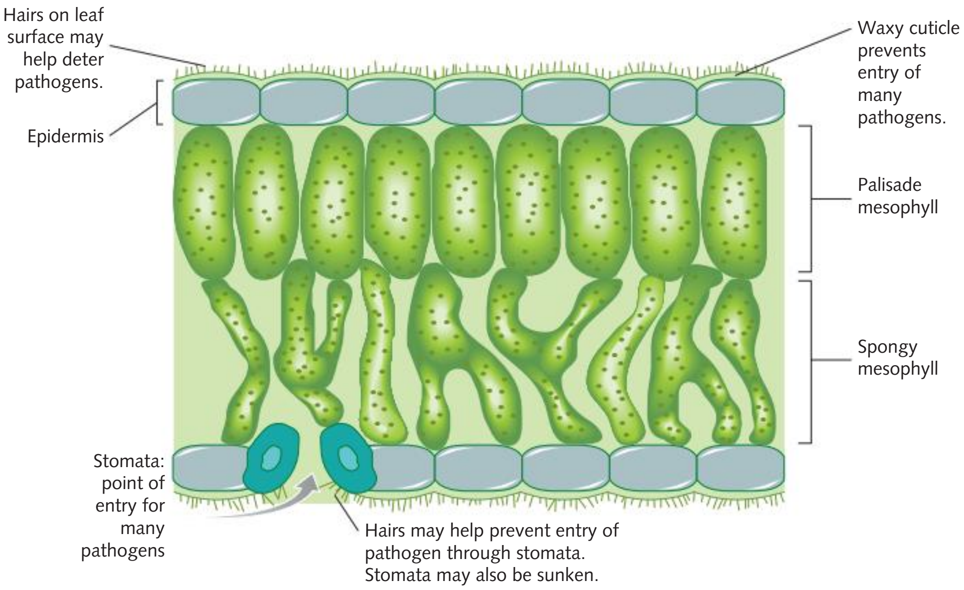

Figure 6.9 A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

Chemical barriers

The first line of defence in plants also includes chemicals that inhibit the growth and development of pathogens. These chemicals include enzymes that help to destroy pathogens, peptides that inhibit their growth, and organic compounds called **secondary metabolites** that are not involved in normal growth, development and reproduction, but which are used as defence mechanisms by the plant.

Some secondary metabolites are released into the environment. For example, asparagus plants produce asparagusic acid, which inhibits the eggs of nematode parasites from hatching. The chemicals that plants such as asparagus secrete into the soil are toxic to nematodes, making the plants good **companion plants** for tomatoes, which are commonly attacked by these parasitic roundworms (Figure 6.10). Other substances remain in the plant, ready to stop invaders. These substances include wetting agents that destroy fungal plasma membranes, and phenols and other compounds on leaf surfaces that discourage herbivore feeding and inhibit many potential pathogens.

Alamy Stock Photo/Science Photo Library

Science Photo Library/Biophoto Associates

Figure 6.10 a The root knot nematode is a common plant pathogen, infecting more than 2000 plant species and causing about 5% of global crop losses. **b** Nematophagous fungi trap and eat nematodes such as the root knot nematode and are considered to be biological control agents of nematodes.

Defensins

Defensins are small, stable peptides that can inhibit the development of fungi, as well as bacteria, viruses and insects. More than 300 defensin-like genes have been identified in plants. Defensins may constitute up to 10% of the total proteins in some types of seeds, and they are also present in the cells of flowers, leaves, fruit, bark and tubers. Their antimicrobial action comes from their ability to reduce membrane permeability and inhibit the action of enzymes and ribosomes. Because of their anti-feeding activity against insects, defensins can also provide a defence against insect-transmitted viruses.

As well as protecting plants from pathogens, defensins appear to be involved in cellular signalling, growth regulation and heavy metal tolerance. Many defensins accumulate during normal plant development; others are produced in response to attack by pathogens or environmental stress such as drought, salt and cold. Defensins have been shown to inhibit the human cancer cell cycle, so they may potentially be used to treat human diseases.

WB
6.2.2
PLANTS – SECOND LINE OF DEFENCE
PAGE 211

Second line of defence

Despite the many barriers, pathogens still enter plants. When they do, plants mount a strong defence. These innate immune responses may be very rapid, with host gene expression beginning minutes or even seconds after exposure to pathogens. Unlike animals, plants do not have a circulatory system that can efficiently transport their defence mechanism. Instead, their responses tend to be more localised, with most cell types retaining the capacity to express a broad range of antimicrobial defences.

Note:

Information about PAMPs and DAMPs has been included to complete the story about the defence against pathogens but you do not need to know it for the VCE Biology exam. You do need to know about receptors and antigens.

Detection of plant pathogens

Plants recognise invaders in much the same way as the cells of animals do. The broad molecular patterns commonly shared by pathogens (such as flagellin, glycoproteins, lipopolysaccharides and chitin) are recognised as being foreign to the plant. These pathogen-associated molecular patterns (PAMPs) are recognised by pattern recognition receptors (PRRs) that are found on the surface or in the cytoplasm of a variety of cells. PRRs also detect components of plant cells and tissues that should not normally be released, but which can be present after damage to the plant. These damage- or danger-associated molecular patterns (DAMPs), such as breakdown products of plant cell walls, can also stimulate the defence responses of plants, preparing the plant for attack by invading pathogens and initiating the clearance of compromised cells and repair of damaged tissues.

Note:

Chemicals in the first line of defence in plants are secreted almost continuously. Chemicals in the second line of defence are secreted in response to detection of a pathogen.

Responses to plant pathogens

Once invaders have been detected, plants can synthesise a toxic cocktail of antimicrobial compounds that includes defensins and phytoalexins. Phytoalexins are low molecular weight antimicrobial compounds that can puncture cell walls, delay maturation, disrupt metabolism, or prevent reproduction of the pathogen. The effectiveness of these defences is shown by plants in which phytoalexin biosynthesis is inhibited. Such plants are more susceptible to infection and are extensively colonised by pathogens. More than 350 phytoalexins have been found in more than 100 plant species from 30 families of plants.

Another chemical response to invasion is the production of a burst of highly reactive oxygen molecules. These substances have a direct antimicrobial action, and are also highly toxic to plant cells. They cause rapid and localised programmed cell death (**apoptosis**) at the site of pathogen invasion. This has the effect of producing a physical barrier around the area of infection, which acts to isolate the pathogen from the rest of the plant.

Several other mechanisms help stop the spread of infection through the plant. Some plants shed infected parts such as leaves and branches (Figure 6.11), an option not available to animals. Wounds caused by a pathogen can be quickly plugged by resin, and cells can thicken and fortify their walls, thereby preventing the spread of pathogens into nearby cells.

Shutterstock.com/Henk Vrieselaar

Figure 6.11 Plants shed infected leaves to rid themselves of infection.

After the initial reaction to invasion, plant tissues may become resistant to a broad range of pathogens for an extended period of time. This is called systemic acquired resistance. It occurs because a signal travels through the vascular system to activate synthesis of antimicrobial proteins in distant tissues when a pathogen attacks a plant. This brings about a heightened state of readiness in which the whole plant, not just the part initially attacked, is prepared for further invasion. Systemic acquired resistance is effective against a broad range of plant pathogens, making it fundamentally different from the adaptive immune response of mammals.

Despite these many plant defences, pathogens frequently reduce plant growth, reducing productivity and yields in crops. Crop breeding programs often select plants with strong innate defences that will provide the individual plant with resistance to one or more important diseases.

KEY CONCEPTS

- Plants have physical and chemical barriers to prevent invasion by pathogens. These include thick bark, waxy cuticles, hairs and production of molecules such as defensins that are toxic to pathogens.
- Plants use PRRs (pattern recognition receptors) to detect PAMPs (pathogen-associated molecular patterns) on invading pathogens. They mount a rapid innate immune response to invasion by pathogens.
- After invasion by a pathogen, plants attain a state of systemic acquired resistance that makes them more prepared to fight invasion by a broad range of pathogens.

Concept questions 6.2

1 Provide three reasons that explain why it is important for us to understand disease and defence in plants.
2 Describe five physical adaptations that prevent the entry of pathogens into plants.
3 Summarise the chemical defences of plants in a suitable table, including their names and the ways in which they act.
4 Describe the interactions of a companion plant with a crop plant. Use an example to support your response.

HOT Challenge

5 Describe the mechanism by which defensins kill invading micro-organisms and why the response may be localised.

6.3 Innate immune response in animals – second line of defence

If a pathogen breaches an animal's first line of defence, it will be detected and dealt with by the host's immune system. The **immune system** is often described as having two components. The initial response to a pathogen is rapid and general and occurs in the same way every time any pathogen invades the body. This response is called the **innate immune response** and is sometimes described as being the second line of defence. The third line of defence is the **adaptive immune response**, which develops into a potent, coordinated battle against a pathogen and involves the activation of immune cells called **lymphocytes**. These cells have the capacity to 'remember' the pathogen and make a faster, stronger response to it the next time it appears.

Some specific cells of the innate immune response

WB
6.3.1
SOME SPECIFIC CELLS OF THE INNATE IMMUNE RESPONSE
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Several different cell types are involved in mediating innate immune responses, and collectively these cells are called **leukocytes**, or white blood cells. Figure 6.12 and Table 6.2 summarise these different cell types. Leukocytes that engulf and digest foreign pathogens are called **phagocytes** (meaning 'eating cells'). **Granulocytes** are leukocytes that have granules in their cytoplasm. The granules contain enzymes, which help digest pathogens.

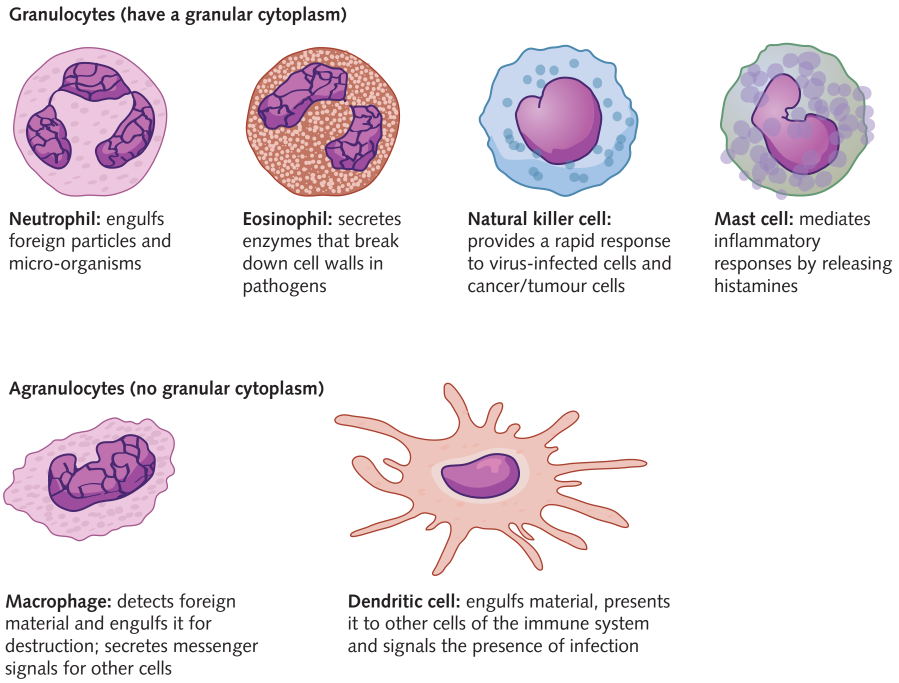
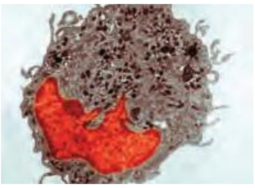
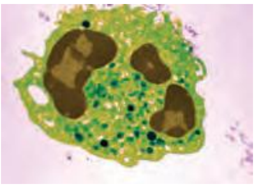


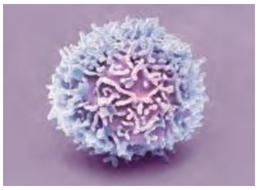
Figure 6.12 Different types of white blood cells (leukocytes)

Table 6.2 The cellular components of the immune system

Cell	Function
Leukocyte	A general term describing white blood cells. Includes all of the cell types below.
Phagocyte	A general term describing a white blood cell that engulfs and digests foreign pathogens in a process called phagocytosis. Macrophages, neutrophils and dendritic cells are phagocytes.
Granulocyte	A general term describing white blood cells that are granulated (neutrophils, basophils and eosinophils).They have granules in their cytoplasm, which contain enzymes that digest pathogens.
Macrophage  Alamy Stock Photo/ Cultura Creative	A large phagocyte found in body tissues that becomes a powerful stimulator of an immune response when it engulfs a pathogen.
Neutrophil  Science Photo Library/ David M. Phillips	Classed as a granulocyte and a phagocyte and found in the blood and tissues. Rapidly enters sites of inflammation, engulfing the pathogen and then dying in large numbers. Pus contains the debris of dead neutrophils.

EXAM TIP

Even though spelling is not assessed in the VCE Biology exam, make sure you know how to spell key terms because incorrectly spelt terms lack clarity and may lead to a failure to gain marks.

Dendritic cell  Alamy Stock Photo/ agefotostock	A phagocyte with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system.
Eosinophil  Alamy Stock Photo/ Science Photo Library	A granulocyte with secretory vesicles that contain powerful enzymes that rupture (lyse) cell walls of pathogens. Important in combating parasites such as worms and flukes. Their chemicals are toxic to the tissues of both parasites and host.
Natural killer cell  Alamy Stock Photo/ Science Photo Library	A granulated lymphocyte that secretes chemicals that lyse cancer cells and cells that are infected with viruses. They attach to the glycoproteins on the surface of infected cells, and kill them.
Mast cell  Alamy Stock Photo/ Science Photo Library	A granulocyte that releases histamines; also involved in healing wounds. Concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin.

Macrophages

Macrophages are a type of white blood cell. They tend to survive for a long time, sometimes months. They develop from **monocytes** that have left the blood vessels and entered the tissues in response to signs of infection.

As potent activators of other immune cells, macrophages are specialised to switch on inflammation. Macrophages have receptors on their surface that recognise **antigens** (substances that trigger an immune response) on invaders, such as bacteria, that are not normally found in the body. Macrophages activated in this way produce a number of cell signalling molecules called **cytokines**, which include **interferons** and various interleukins. Cytokines are important because they act as signalling molecules, stimulating cells to move towards the sites of inflammation. Macrophages also destroy pathogens and clear apoptotic cells and damaged tissue by **phagocytosis**.

Neutrophils

A **neutrophil** is a type of granulocyte that is abundant in blood. Neutrophils have irregular, multilobed nuclei and granular cytoplasms. Neutrophils rarely survive longer than a few days, so reinforcements from the blood are constantly required. At least 80 million neutrophils are produced by the bone marrow every minute. Like macrophages, neutrophils carry out phagocytosis (so these cells are sometimes collectively called ‘phagocytes’). They produce a wide range of cytokines that can induce **chemotaxis**, which is the movement of cells towards the source of a chemical gradient. Cytokines can also trigger mast cells to release **histamine**, a chemical that stimulates inflammation. Neutrophils also produce defensins, which are peptides that act as powerful natural antibiotics with wide antimicrobial activity.

An important feature of neutrophils is that, as well as having a very short lifespan, they die rapidly after they have phagocytosed a pathogen. This adaptation ensures that pathogens cannot propagate in neutrophils and spread through the body. Pus contains the cellular debris of large numbers of neutrophils that have died in this way. Some pathogens are able to evade death during phagocytosis. In as little as 30 seconds after ingestion, *Rickettsia*, an intracellular parasite of phagocytes, uses an enzyme to free itself into the cytoplasm. *Legionella* bacteria survive by preventing lysosomes from fusing with the phagosome, and pathogenic *Streptococci* cause lysosomal granules to explode and release their lethal contents into the cell, thus killing the phagocyte and releasing the pathogen.

Dendritic cells

Dendritic cells are derived from monocytes. They are large cells with a non-granular cytoplasm and are named for their dendrites, or finger-like structures, which reach in between cells in tissues and sample whatever is in the microenvironment. If this contains antigens, the dendritic cell becomes activated and can engulf and destroy invaders, and send out signals to other cells of the immune system to bring about the beginning of a coordinated immune response. Dendrites on the surface of the cell help them to take up, process and present antigens to lymphocytes efficiently.

Dendritic cells have similar functions to those of macrophages, including phagocytosis of antigens and secretion of cytokines that bring about an immune response. However, dendritic cells differ in that they are highly specialised to take up the pathogen at the site of the infection and transfer it to organs of the lymphatic system. This is where the dendritic cells present the antigen to lymphocytes, cells of the adaptive immune system. Dendritic cells are therefore important in linking innate immune responses with the adaptive immune system.

Eosinophils

Eosinophils are leukocytes with a role in the innate immune response. Eosinophils secrete powerful enzymes that can form destructive holes in the cells of multicellular pathogens such as blood flukes and parasitic worms.

Natural killer cells

Natural killer cells circulate around the body, acting as security guards. They check the cells they encounter for suitable surface markers that identify the cell as self. Any suspicious cells whose markers have changed, such as those infected with virus or transformed by cancer, are destroyed by an attack on their plasma membranes. This leads to apoptosis, ensuring the destruction of both the cell and the virus inside. The importance of natural killer cells in the initial response to infection by a virus is shown by patients deficient in natural killer cells being highly susceptible to the early phases of *Herpes* infection.

Mast cells

Physical damage such as a cut in the finger can rupture body cells and release danger signals that stimulate **mast cells**. Mast cells are located in the tissues. When activated by the danger signals, or the detection of an antigen through binding to surface receptors called IgE (p. 231), they release their granules, which are loaded with histamine, a major stimulus for the initiation of inflammation. Mast cells also secrete heparin, which prevents blood clotting in the injury site, although a clot forms around the outside of the injury site to prevent the spread of the pathogen.

Histamine, together with cytokines (both are signalling molecules) released by macrophages, promotes **vasodilation** (widening of blood vessels, especially arterioles) in the damaged region. With increased blood flow comes a battalion of cells and chemicals to fight off an infection. This increased blood flow is the cause of the redness and swelling seen at the site of an injury. As blood also transfers heat, swollen areas often become very warm. Histamine changes the permeability of capillaries in the inflamed area, making it easier for leukocytes, blood plasma and blood proteins to squeeze out through the walls and into affected tissue. This will be discussed further on page 220 in the section on inflammation.

Complement proteins

The **complement** system gets its name from its role in helping, or complementing, the immune system. The complement system consists of many small proteins that circulate in the blood and tissue fluids.

These proteins are secreted mainly by the liver, but also by macrophages, monocytes and other body cells. Complement proteins are normally inactive and are designated by a C and a number (e.g. C3). The inactive precursor proteins become activated immediately when they encounter antigens from a foreign body, such as an invading bacterium. Activation of a complement protein (e.g. inactive C3 becomes activated C3a and C3b) has a cascade effect, stimulating the activation of other complement proteins (e.g. inactive C5 becomes activated C5a and C5b), which then activate other proteins. This is known as a complement cascade.

These proteins have three main ways of defending the body.

- An important product of the complement cascade is the membrane attack complex (MAC). MAC forms pores in the membranes of target cells, disrupting the phospholipid bilayer. With membrane integrity destroyed, osmotic cytolysis occurs because of a sudden influx of fluid, which causes the cell contents to spill out into the extracellular fluid. Cell death follows (Figure 6.13).

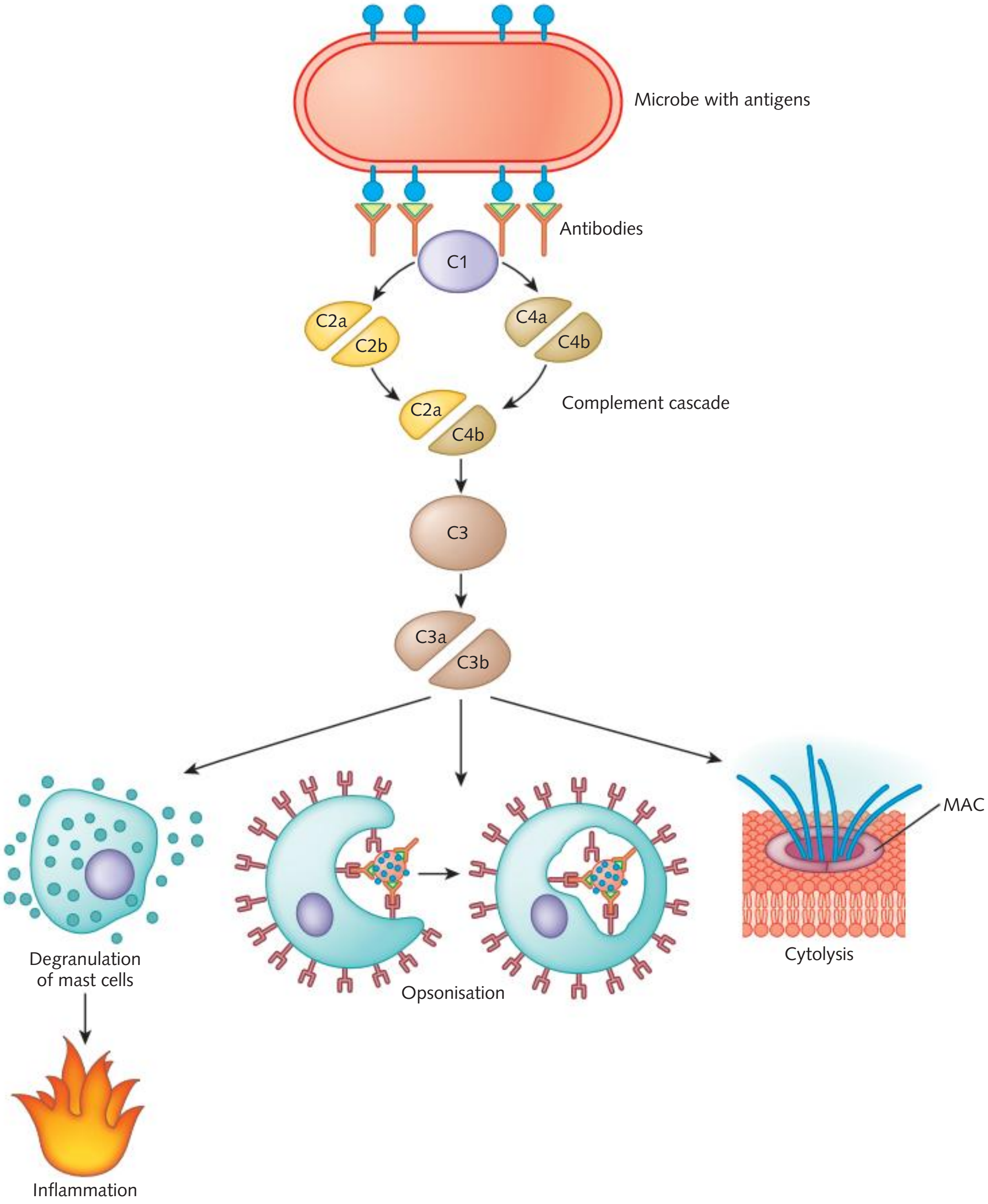


Figure 6.13 Activation of the complement system leads to a complement cascade, which causes opsonisation, cytolysis and inflammation.

- C3b molecules coat the surfaces of microbial cells such as yeasts and bacteria and function as opsonins, acting as a tag to help their detection and uptake by phagocytes, which have complement receptors on their surface. C3b molecules induce chemotaxis by creating concentration gradients that attract phagocytes and other white blood cells to the damaged or infected site. Complement activates these phagocytes by increasing their ability to ingest and destroy pathogens. This process is called **opsonisation** (Figure 6.13).
- Degranulation of mast cells (release of their granules) leads to inflammation (p. 220).

With its powerful and potentially dangerous effects, the complement system is tightly controlled. One important safeguard is that activated complement proteins are rapidly inactivated unless they bind to the surface of a pathogen. In addition, MAC-inhibitory proteins are expressed on all body cells. This protects the body's own cells by inhibiting MAC formation in their membranes. People lacking the gene for the MAC-inhibitory protein suffer episodes of intravascular red blood cell lysis caused by activated complement.

Interferons

Complement proteins and lysozymes are extremely effective against bacteria but cannot destroy viruses. Instead, some virus-infected cells secrete cytokines called interferons, which induce resistance to viral infection in the surrounding cells. Interferons act as warning signals from the doomed cell and cause changes in the surfaces of the surrounding cells, making it more difficult for a virus to infect them. By targeting multiple points in the viral life cycle, interferons also prevent replication of virus particles inside the host cell.

Interferons also enhance many of the functions of other cells of the immune system. For example, natural killer cells are activated by the release of interferons and cytokines targeting macrophage responses. Natural killer cells contain cytotoxins that are released to eliminate both virus-infected cells and cancer/tumour cells. Within their cytoplasm are granules containing toxins such as perforin and other proteases that, on release, induce apoptosis. Apoptosis produces small blebs (bulges in the plasma membrane) of cell contents, which are efficiently engulfed and processed by phagocytes. Apoptosis is the preferred method of cell eradication because it ensures that both the infected cell and its contents are eliminated without further contaminating surrounding tissue. In necrosis, the plasma membrane has no control over the process, so when the membrane is breached both the cell contents and virions (virus particles outside a cell) are released and can then move into surrounding cells (Figure 6.14). This would do little to stem the further progression of the virus into healthy cells and tissue.

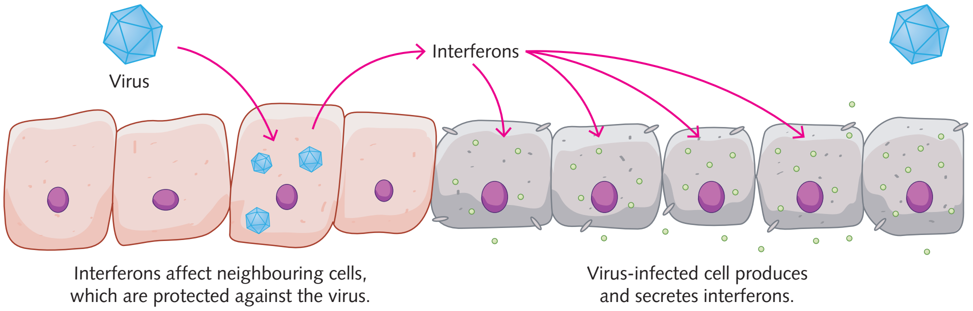


Figure 6.14 **Interferons are secreted by virally infected cells and act on neighbouring cells to prevent viral entry and viral replication. Cells shown in grey are in a protected state.**

Note:

Cytokines such as interferons also play an important role in the adaptive immune response.

Because of their particular effects on cells, individual interferons have proved useful in the treatment of a number of diseases such as multiple sclerosis, rheumatoid arthritis, hepatitis C and some cancers. Like complement proteins, interferons are non-specific in their effects, being secreted in response to the detection of any viral invader.

KEY CONCEPTS

- Innate immune responses are non-specific and are inborn features of the body.
- Leukocytes (white blood cells) mediate the innate immune response by engulfing and digesting foreign pathogens.
- Immune cells such as macrophages begin the immune response after their receptor sites bind with foreign antigens.
- Neutrophils are a type of phagocyte that are abundant in blood. They readily migrate from capillaries to sites of infection, where they phagocytose pathogens and then self-destruct to stop pathogen spread.
- The complement system consists of a number of small molecules in the blood that have important roles in inflammation, including opsonisation and deposition of the membrane attack complex (MAC).
- Interferons are produced by virus-infected cells and alert neighbouring cells to the danger, causing changes in gene expression that make them more resistant to viral infection and replication.
- All plants and animals mount innate immune responses to pathogens.

Concept questions 6.3a

1 Define 'innate responses to infection' and state the type of organism that can generate these responses.

2 Describe the role of phagocytic leukocytes in the immune system.

3 List one similarity and one difference between:
  a macrophages and eosinophils
  b neutrophils and basophils.

4 State three outcomes when the complement system is activated.

HOT Challenge

5 Suggest why phagocytes, such as macrophages, typically contain large numbers of ribosomes and lysosomes.

WB 6.3.2 INNATE IMMUNE RESPONSE – INFLAMMATION PAGE 139

Innate immune response – inflammation

Inflammation is a major feature of the innate immune response. It consists of a complex series of reactions and processes involving the activities of many different cell types and signalling molecules, which act together to initiate and maintain inflammation until the danger is cleared.

Inflammation has two roles:

- to destroy the cause of the infection and remove it and any products it has produced from the body. If this fails, then inflammation works to limit the spread of the infectious agent by confining the infection to a small area
- to replace or repair tissue damaged by the infection by improving blood flow.

The first signs of infection in a cut finger are usually pain, heat, redness and swelling. These are the four physical signs of inflammation. Inflammation occurs in tissues where cells are killed or damaged by physical injury or invading pathogens, and is the key weapon of the innate immune response. The pain, heat, redness and swelling occur because blood vessels in the inflamed area become permeable (leaky), allowing cells of the immune system to move out of the blood vessels into the area to mount a strong response against invading pathogens. Once the pathogen is cleared, the cells die or migrate elsewhere, the four signs of inflammation disappear and the site returns to normal.

Initiation of inflammation

Inflammation is triggered by the recognition of a non-self organism or part of a non-self organism. If pathogens enter the body, they are likely to encounter white blood cells (such as macrophages and dendritic cells). It is important to note that even though these cells are generally classed as white blood cells, many occur in all body tissues, especially in the skin, liver, lungs, kidneys, spleen and lymph nodes, where they act as resident sentinels.

Infection by a pathogen is not essential for inflammation. Intracellular molecules, which are usually hidden from the immune system, can be released through injury or tissue damage. These can be detected as DAMPs by PRRs and may trigger **sterile inflammation** (inflammation arising in the absence of infection). This type of inflammation may be more in anticipation of infection, and with a greater emphasis on promoting effective wound healing.

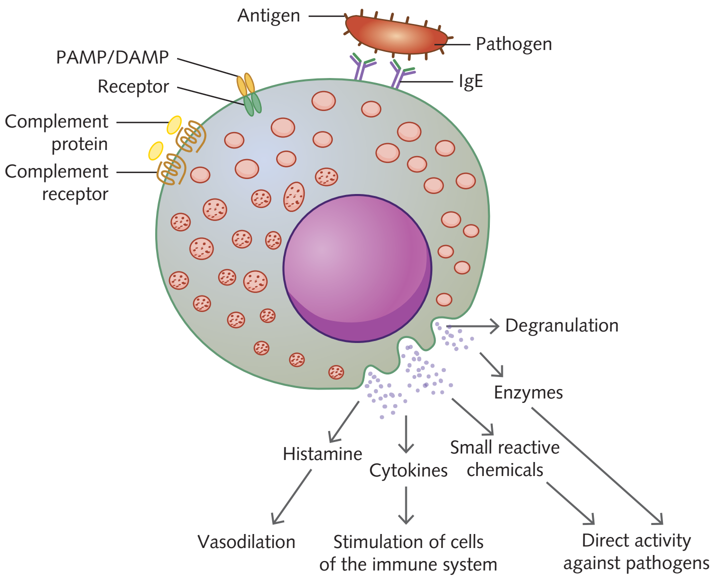


Figure 6.15 **Mast cells degranulate in response to inflammatory signals, releasing histamine and other molecules. IgE stands for immunoglobulin E, an antibody.**

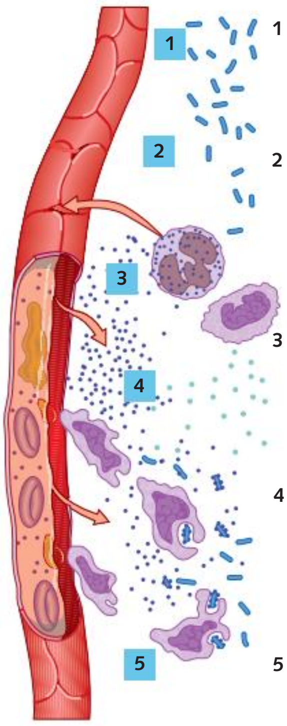
Inflammation takes place in the following steps.

1. Blood flow increases to the site of damage. This causes redness and swelling and brings more immune cells to the site.
2. Mast cells release histamine. Histamine makes blood vessel walls more permeable and causes them to dilate (vasodilation). This allows immune cells such as phagocytes to move out of the blood vessel more readily and enter the tissue along with tissue fluid that makes the area swell.
3. Blood clotting occurs. Platelets and fibrin (protein) form a clot, which prevents other pathogens entering the damaged site.
4. Phagocytes engulf pathogens or foreign material.
5. Other immune cells detect antigens and produce immune signalling molecules, including interferon and complement. This brings more immune cells to the site to help with the immune response.

Inflammation involves cellular migration

During the resting state, and especially once an immune response is initiated, cells of the immune system undergo chemotaxis. This is an important way in which cells involved in the inflammatory response are recruited from the blood to sites of infection or tissue damage. During chemotaxis, white blood cells move towards increasing concentrations of cytokines called **chemokines**, which are any molecules that induce chemotaxis. Chemokines include molecules released by micro-organisms, activated macrophages and other cells. There are many types of chemokines, each with specific receptors expressed by particular target cells. Only cells expressing a particular chemokine receptor will undergo chemotaxis in response to that chemokine.

In response to chemokines, two types of leukocytes, monocytes and neutrophils, squeeze out through the capillary walls into the tissues. After monocytes enter the tissues, they mature into macrophages. The



1 Bacteria can invade cells or tissues where they can either kill the cells or release harmful by-products of metabolism.

2 Upon detection of bacterial PAMPs, granulocytes in infected tissues secrete histamines, and phagocytes and other body cells secrete cytokines to recruit other immune cells.

3 Histamines can affect the permeability of small blood vessels so that plasma fluid and proteins can seep into surrounding tissue.

4 Cytokines cause phagocytes to move through the leaky blood vessel walls and into tissue where they are activated by detection of PAMPs.

5 Bacteria are engulfed by phagocytic white blood cells.

Figure 6.16 The steps that occur in acute inflammation after invasion by a bacterial pathogen

swelling caused by the action of histamine, which increases leakage of blood plasma and leukocytes into the inflamed area, also causes some localised pain. Feeling pain is an important process, as it reduces voluntary movement in that area, thus limiting further tissue damage and speeding up the repair process.

Resolving inflammation

After an infection, inflammation does not resolve passively but in a highly coordinated, active process that is controlled by several factors, including cytokines and other signalling molecules. These switch off movement of leukocytes to the site of inflammation, reverse vasodilation, and reduce the permeability of fine blood vessel walls to the level before the inflammation. They also stimulate macrophages to safely dispose of material that has accumulated at the site of infection. This includes dead neutrophils, fibrin and exudate, the fluid that leaks out of blood vessels at the site of inflammation. During phagocytosis, macrophages detect and recognise a molecule on the surface of neutrophils that have died by apoptosis. This triggers the release of cytokines, including those that promote the resolution of inflammation. Sometimes the signal for apoptosis fails and the neutrophils die by **necrosis**, unprogrammed cell death that occurs as a result of injury or infection (Figure 6.17). Whereas apoptosis is an immunologically ‘silent’ form of cell death that does not normally activate the immune system, necrosis stimulates inflammation, and macrophages release cytokines that further enhance inflammation rather than suppressing it.

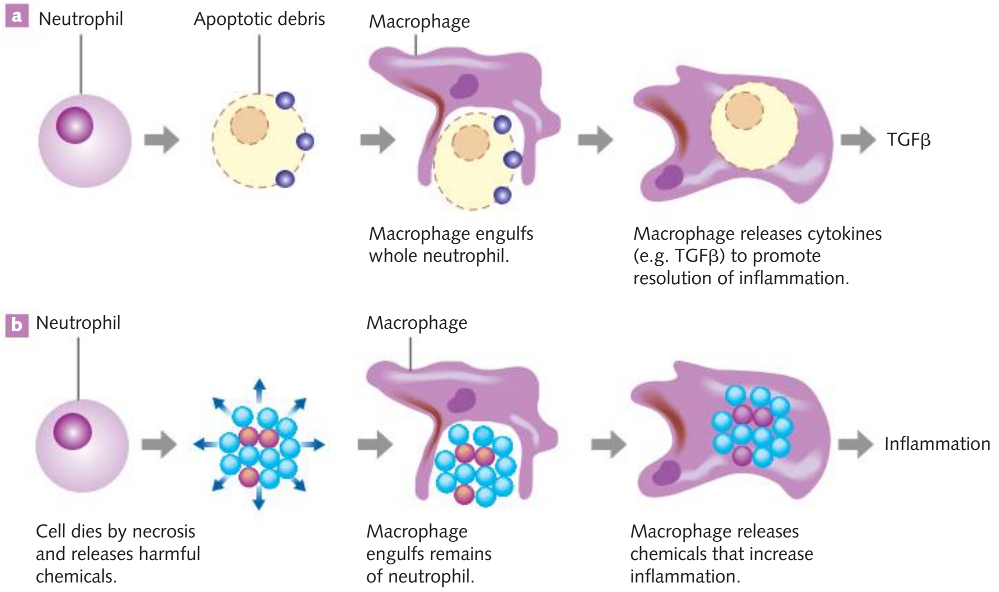


Figure 6.17 Different signals, from a apoptosis and b necrosis, can resolve or prolong inflammation.

Successful resolution of inflammation limits excessive tissue injury and reduces the opportunity for chronic, or long-term, inflammation. Defects in these clearance mechanisms appear to be associated with persistent tissue inflammation and autoimmune responses directed against cellular contents. Repeated bouts of inflammation, as happens with autoimmune conditions such as Crohn’s disease and rheumatoid arthritis, result in ongoing tissue damage.

Distinguishing self from non-self

In order for an organism to detect when a pathogen has gained access, the organism must be able to distinguish self from non-self. Both plants and animals are alerted to invasion of pathogens by physical and chemical changes that occur in their cells or tissues, which enable them to distinguish self from non-self. The presence of foreign molecules, either on the outer surface of the invaders or in the toxins and enzymes they secrete, stimulates host immune responses that usually lead to the destruction and removal of the pathogen.

Cells of the immune system recognise non-self molecular patterns (PAMPs) that are characteristic of microbes but are not found on host cells. Immune cells have evolved receptors (PRRs) to recognise these molecules because they are unique to pathogens, their structure is probably essential for the pathogen to function properly.

KEY CONCEPTS

» Inflammation is characterised by pain, heat, redness and swelling. Inflammation is the key weapon of the innate immune response, destroying invading pathogens before they can establish an infection.

» Chemotaxis is the process of cellular migration towards regions of higher concentrations of chemokines. Each cytokine has a specific receptor that is expressed by particular leukocyte types.

» Inflammation is initiated when macrophages, dendritic cells or other cells detect antigens or signalling molecules and begin to produce cytokines that bring in and activate other immune cells.

» Pain reduces voluntary movement to assist the repair process.

» Immune cell recruitment to sites of inflammation is assisted by histamine, which is secreted by mast cells and increases the permeability of capillaries.

» The resolution of inflammation is a highly active process that is necessary to limit and repair tissue damage caused by the inflammatory response.

» The innate and adaptive immune responses rely on being able to tell self from non-self.

Concept questions 6.3b

1 List the four physical signs of inflammation.

2 Some white blood cells are called granulocytes. Describe:
  a one feature that distinguishes granulocytes from other white blood cells
  b their function.

3 Explain the steps that cause your ankle to swell if you sprain it.

4 Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of the immune system. Cytokines are a category of signalling molecules that mediate and regulate immunity, inflammation and other processes. IL-10 (interleukin-10) is a cytokine with potent anti-inflammatory properties, repressing the expression of other inflammatory cytokines by activated macrophages. Some chemokines initiate inflammation; others shut it down. Why is this important?

5 Discuss what is involved in the resolution of inflammation and why this is important for limiting tissue damage at sites of infection.

HOT Challenge

6 a Describe the mechanism by which macrophages, having detected a danger signal (i.e. a PAMP or a DAMP by a PRR), alert other cells of the immune system of the danger.
  b Describe the importance of an organism being able to distinguish between self and non-self.

WB

6.4
ANTIGENS AND PATHOGENS
PAGE 140

6.4 Antigens and pathogens

The body experiences threats from a range of different sources, many of which are extracellular and pathogenic in nature. However intracellular threats such as invasion from viruses and accumulative mutations can also occur. Other threats to the immune system also include allergens and malfunctions in the immune system. Many threats to the human body are extracellular and pathogenic in nature or other forms that are allergens or malfunctions in the immune system. Other threats are intracellular, within the body cells, such as invading viruses or cumulative mutations causing cells to become cancerous. The trigger that alerts the body and initiates an immune response is the presence of antigens. Depending on where they come from, antigens can be cells, molecules attached to the outer surface of a cell, or just free-floating molecules. These antigens detected as foreign and not belonging to the individual are called **non-self antigens**. Appropriate and effective immune responses depend on the ability of the immune system to distinguish self-components from non-self antigens, derived from a foreign agent such as a pathogen.

Antigens can be made of many different substances, including proteins, peptides, lipids or polysaccharides, and are often embedded in the membranes of cells or the outer coat of viruses. Antigens can also be intracellular components of pathogens, including nucleic acids, proteins, carbohydrates and lipids. The genetic information of the organism to which they belong determines their exact shape and structure. Just as DNA is unique to an individual, it follows that the antigens produced by an organism are unique to that organism.

Cells normally in the body are marked by molecules located on the surface of the cells called **self-antigen** which indicate they are not foreign and so the immune system does no attack them. In humans, the most important self-antigens are called major histocompatability complex proteins (MHC). They are a group of proteins found on all nucleated cells that allow the immune system to distinguish self from non-self. There are two main types of MHC proteins that will discussed in Chapter 7. Some responses are specific to a particular antigen and others are more generalised, and responses can be localised or systemic (throughout the systems in the body, not just at the site of infection). Some antigens are **allergens**, substances that trigger an allergic response. An **allergy** is a specific type of immune response to normally harmless stimuli such as food, pollen or house dust mites. More harmful unwanted immune responses occur when the antigen is a self-antigen. This can cause an autoimmune disease, in which the body's own cells and tissues are mistakenly targeted for destruction by the immune system. To a normally functioning immune system, self-antigens are invisible, and the immune system does not mount a response when it encounters them.

Pathogens are sources of non-self antigens. An immune response will be launched against the non-self antigens with the aim of destroying or neutralising the pathogen. Pathogens can be cellular or non-cellular. **Cellular pathogens** are able to reproduce and function by themselves. Cellular pathogens include bacteria, fungi, protists and parasites. Fungi cause diseases such as thrush and tinea; bacteria cause sore throats and gastroenteritis. Protists are the disease-causing agents in malaria, amoebic dysentery and giardiasis. A parasite is an organism that lives on or in its host for all or part of its life, causing harm to and gaining nutrition from the host. Examples of parasites are arthropods (mites and ticks) and helminths (worms and flukes).

Non-cellular pathogens are not made of cells and cannot reproduce and function without being inside a host, in most cases inside a host cell. Non-cellular pathogens include prions and viruses. Viruses are responsible for diseases such as smallpox, anthrax, HIV/AIDS, and coronavirus diseases such as SARS-CoV, which causes severe acute respiratory syndrome, and SARS-CoV-2, which causes COVID-19. Prions cause kuru and mad cow disease.

Bacteria

Bacteria may have been the first cellular life form on Earth, and today they are still the most abundant and most diverse group of organisms. Only a relatively small number of bacteria cause disease. Billions of bacteria live on your skin and in your body, many of which are commensal. All of the genomes of the microbes in this microbiota are collectively referred to as the **microbiome**, and this can be used to identify the organisms of the microbiota.

Structure of bacteria

Typically bacteria are 1–10 μm (micrometres) in length and 0.20–2 μm in diameter. Like all cells, bacteria have a plasma membrane that encloses the cytoplasm. The plasma membrane is surrounded by a cell wall (Figure 6.18).

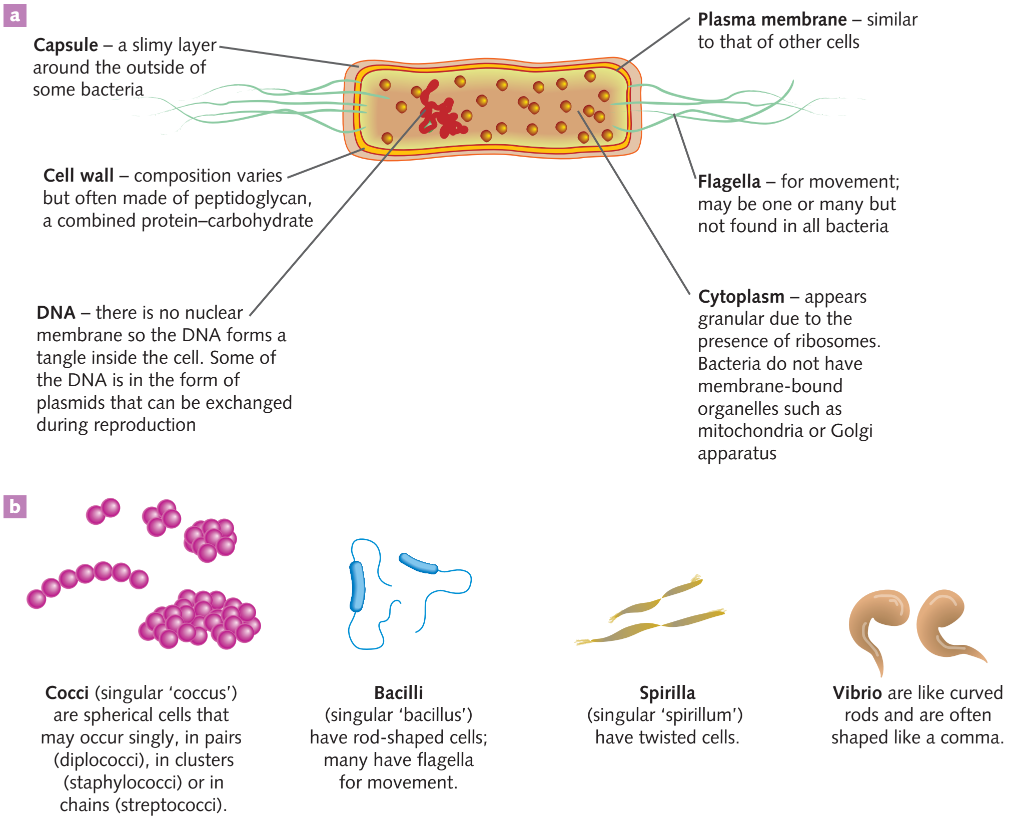


Figure 6.18 a The structure of a typical bacterial cell. b Bacteria are classified according to their cell shape.

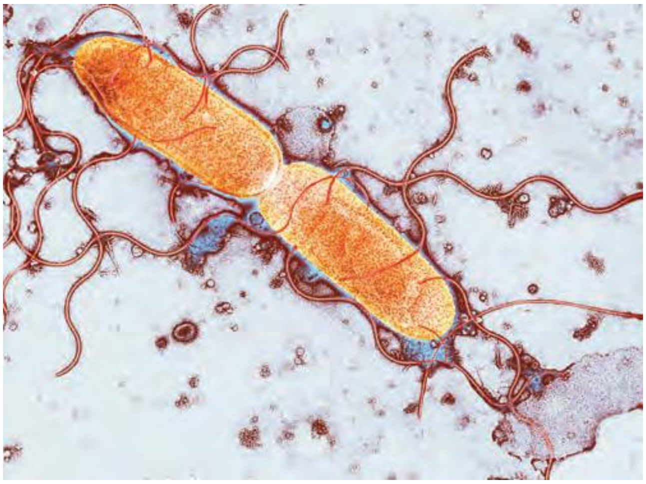

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Figure 6.19 Transmission electron micrograph of *E. coli* dividing into two by binary fission. Note the flagella, which appear like hairs extending from the cells.

As they are prokaryotes they have no membrane-bound organelles or nucleus; however, bacteria do have ribosomes and a single circular chromosome. The cell wall is made of peptidoglycan (a protein–carbohydrate compound). These are known as gram-positive bacteria. Some bacteria also have an additional outer membrane made of a lipopolysaccharide component. These are known as gram negative bacteria.

Some bacteria have a **flagellum** (a hairlike appendage) or multiple flagella, which help them to move about (Figure 6.19). Some species have a slimy **bacterial capsule**, which they use to help the bacteria stick to surfaces such as teeth or mucous membranes. The capsule is a large, well-organised layer outside the cell wall. It usually increases the virulence of a species (the degree to which it causes disease) because it makes it harder for the body's immune system or antibiotics to recognise and attack the inner bacterium.

Bacteria are important pathogens of humans, plants and animals. Bacteria can be transmitted from one host to another in several ways: by direct contact, in food and water, and in droplets of moisture in the air. Biting insects, such as ticks and fleas, can also transfer bacteria on their biting parts.

Once inside a host, bacteria can divide rapidly. Different features of bacteria cause disease in different ways. Some bacteria damage host tissues directly, while others produce toxins (often their own metabolic wastes) that disrupt the functioning of cells nearby or even further away. For example, toxins produced by diphtheria bacteria in the throat affect tissues throughout the body. Many parts of the bacterial cell are highly pathogenic to the host. External molecules such as lipopolysaccharides (lipid–carbohydrate compounds) or peptidoglycans are such examples. These antigens can stimulate immune responses that are sometimes so strong that they damage host cells and tissues. Some bacterial strains interfere with the host's immune system, making the host susceptible to other pathogens.

Some bacteria produce exotoxins and endotoxins (Figure 6.20). Exotoxins are toxic substances produced by the bacterial cell and then released to act outside of the bacteria. The toxins damage the cells of the host organism and thus spread disease through the ongoing necrosis of affected cells. Endotoxins are lipid-based bacterial toxins located within bacterial cells and are released into the body's internal environment (plasma and interstitial fluid) upon lysis of gram-negative bacteria.

EXAM TIP
In biology, *exo* means outside or external and *endo* means inside or internal. Even if you don't know what a word means, you can sometimes work it out.

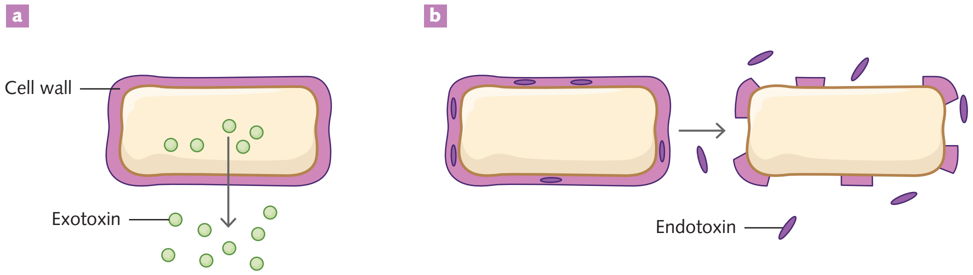

Figure 6.20 a Exotoxins are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are secreted or released, following lysis, into the surrounding medium. **b** Endotoxins are part of the outer portion of the cell wall of gram-negative bacteria. They are released when the bacteria die and the cell wall breaks apart.

The success of the treatment of any of these bacterial infections is determined by the effect of both the immune system and medications on bacterial cells. The most common treatment for bacterial infections are antibiotic medications. If the antibiotic works by rupturing the plasma membrane, there is then the added complication of whether that bacterial species will release toxins. So even though the discovery of antibiotics has been ground-breaking, treatments are often complicated because bacteria are very good at evolving and surviving assaults from human technologies and treatments.

Image courtesy of Robert Johnson, Zoologica

Figure 6.21 A yellow fungus infection in a bearded dragon lizard. The fungus causes the lizard's scales to break off and leave swollen and painful ulcerated lesions.

Fungi

The fungal world includes large organisms such as mushrooms and toadstools, as well as tiny forms that can only be observed under a microscope. These microscopic fungi include unicellular yeasts and moulds. **Fungi** (singular: fungus) are eukaryotes that reproduce by spores and have cell walls made of chitin rather than cellulose. Microscopic fungi are generally larger than bacteria. Not all fungi cause disease, but some of them are pathogenic, causing disease in a wide range of organisms including plants and animals.

Most fungal diseases in animals are external, where they irritate and inflame the skin (Figure 6.21). A common example is ringworm, a fungal skin infection of rabbits, dogs, cats, horses and humans. Tinea is another fungal skin disease of humans. Symptoms include a rash and itchy skin. Both diseases are easily transmitted from one individual to another. As they grow on the skin, fungi produce spores, and as the infected skin flakes off it carries these spores with them. If the spores come into contact with damaged or broken skin, they may cause new fungal infections. Spores are very long lived, an adaptation that improves transmission rates. They can remain alive for years in bedding, furniture and grooming tools, germinating when conditions are suitable.

Some fungal infections can be internal, particularly in people with suppressed immune systems, in which the fungal invasion represents a significant clinical problem.

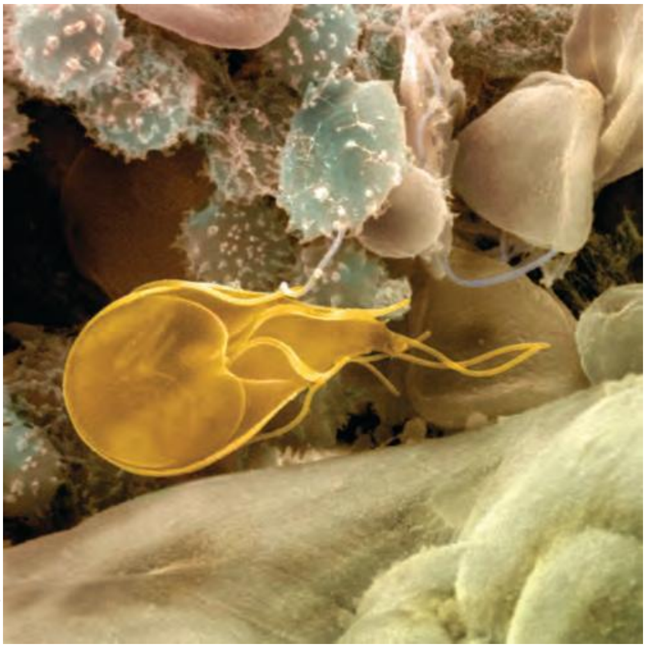

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Figure 6.22 A scanning electron micrograph of *Giardia lamblia* (yellow) in the human small intestine. This flagellated protist contaminates drinking water, causing intestinal upsets.

Protists

Protists are unicellular, eukaryotic organisms. They reproduce both sexually and asexually. Of the 65 000 known species of protists, fewer than 24 species cause diseases in humans, but these few infect hundreds of millions of people each year. We still do not have effective preventatives against many of them and the drugs we have to treat them are limited in their effectiveness.

Examples of pathogenic protists include *Giardia lamblia* (Figure 6.22), *Trypanosoma* (which causes African sleeping sickness) and the protists that cause chlamydia, cryptosporidiosis, amoebic dysentery and malaria. Malaria has been plaguing humans for many thousands of years. It is caused by protists from the *Plasmodium* genus that are transmitted to the host by the bite of a female *Anopheles* mosquito. The *Plasmodium* protists infect host red blood cells, and reproduce inside them. Infected red blood cells eventually rupture, releasing more parasites and their metabolic wastes into the bloodstream. This toxic release induces the classic malarial headaches, chills and a burning fever. These symptoms eventually subside but can recur when more cells are lysed, releasing more parasites. If left untreated, the host may develop an enlarged liver and spleen or, in the case of cerebral malaria, brain injury that leads to death in severe cases.

KEY CONCEPTS

- Pathogens are cellular or non-cellular infectious agents that cause disease.
- Antigens are intracellular or extracellular components of proteins, peptides, lipids or polysaccharides present in pathogens.
- Bacteria are prokaryotes; some are beneficial to their hosts and some are pathogenic.
- Bacteria produce endotoxins or exotoxins that can also cause disease.
- Fungi are eukaryotic organisms that are usually external pathogens that reproduce and spread through their spores.
- Protists are unicellular eukaryotes, a small number of which are significant pathogens of humans. Examples of protist pathogens are *Giardia*, amoebas, *Trypanosoma* and *Plasmodium* species.

Concept questions 6.4a

1 Define ‘antigen’.
2 How is an antigen different from an allergen?
3 State three ways that a bacterial pathogen can harm its host.
4 Antibiotics can help fight bacterial disease but not fungal disease or protist pathogenic disease. Explain why.
5 Describe the advantages to a bacterium of:
  a having a capsule
  b forming endospores.

HOT Challenge

6 Malaria kills more people than any other infectious disease. Malaria is a vector-borne disease. The continent of Africa has the highest burden of disease in the world, according to the World Health Organization.
  a Distinguish between malaria and *Plasmodium*.
  b What is a vector-borne disease?
  c Why is malaria so difficult to control?
  d What measures are used to control the pathogen and the vector?
  e Propose a hypothesis about why mainland Australia is currently malaria free.

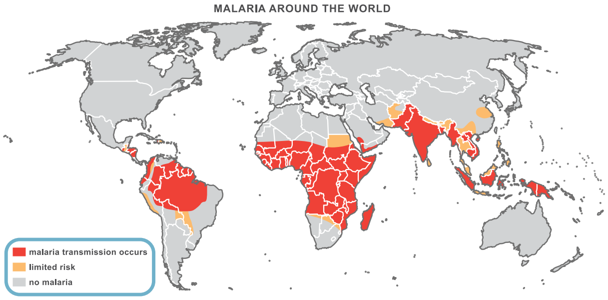

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Figure 6.23 Malarial distribution in the world

Viruses

A **virus** is a non-cellular agent composed of a protein coat and nucleic acid (Figure 6.24), either DNA or RNA (either single stranded or double stranded forms of both types of nucleic acid), but never both. A virus is often referred to as an **obligate parasite** because it cannot function and reproduce outside the host cell.

Viruses essentially vary in the:

- nucleic acid sequence within their core
- array of viral proteins incorporated into the phospholipid envelope.

CONNECT

The importance of these surface proteins in the development of various vaccines is discussed in detail in Chapter 7.

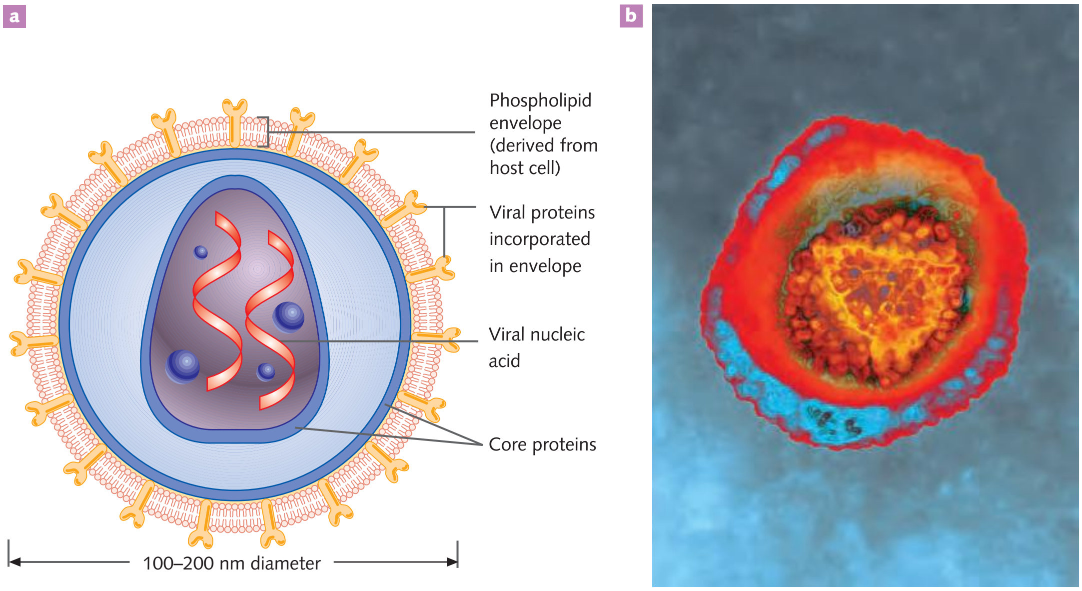

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Figure 6.24 a Viruses consist of a nucleic acid core surrounded by a protein coat. **b** The virus that causes herpes in humans.

The nucleic acid sequence determines the 'shape' of the viral proteins, which the body's immune system detects as being non-self. Some viruses, such as the variola virus that causes smallpox, remain relatively stable and therefore so do their surface proteins. Others mutate rapidly and thus change their surface proteins; for example, influenza and HIV. The immune response centres on identifying specific surface proteins as non-self and then mounting a response.

When a virus infects an organism, it injects its nucleic acid into a host cell. Once inside the host cell, viral proteins hijack normal functioning of the cell, and the viral nucleic acid directs the host cell to make multiple copies of the viral protein coat and nucleic acid. These then assemble into new viral particles and can bud from the host cell, becoming encapsulated by the host plasma membrane as they leave. Viruses can also replicate in vast numbers within the cells, becoming released when the host cell undergoes lysis due to the huge viral load. This releases many new viral particles, which can infect other cells within the host and can be **transmitted**, or transferred, to other host organisms. This life cycle is called a lytic cycle, and the viral genome stays separate from that of the host. Viral nucleic acids and proteins differ enough from those of the host that they can be identified as non-self antigens, stimulating an immune response to viral infection. Some viruses can incorporate their DNA into a host's chromosome and remain dormant, hiding from the immune system and being replicated along with the host's chromosome every time the cell divides.

Each virus is usually highly specific to the host cell or organism it can infect. For example, the SARS-CoV-2 virus specifically infects cells of the lung, causing the disease COVID-19. This is because the virus is able to recognise and bind to receptors that are expressed in respiratory tract epithelia. Even a small change in DNA sequence (such as might exist between closely related species) can result in an amino acid change in the target receptor protein, so that the host protein structure is different enough that the viral protein can no longer bind.

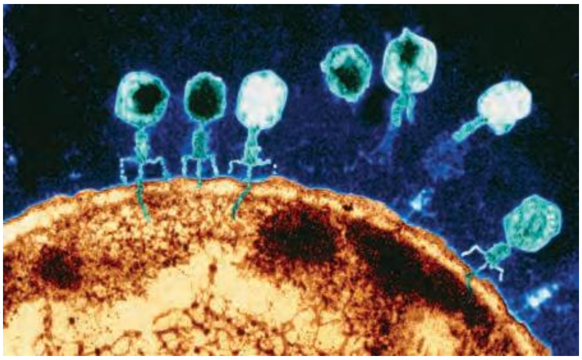

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Figure 6.25 A coloured transmission electron micrograph of T-bacteriophage viruses attacking a bacterial cell of *Escherichia coli*. Seven virus particles are seen (blue), each with a head and a tail. Small blue strands of genetic material (DNA) are being injected into the bacterium.

Bacteriophages: viruses of bacteria

Bacteria have their own group of viral pathogens, known as bacteriophages. Bacteriophage means 'bacteria eater' and these viruses lyse and destroy their bacterial host cells. Bacteriophages have a DNA genome surrounded by a protein shell. They inject their DNA into the host bacterium, which is then transcribed and translated to produce many copies of the bacteriophage genome and protein coat (Figure 6.25). These accumulate within the bacterium until the bacterium bursts and releases its contents. Some research efforts are focused on understanding whether bacteriophages (or phages for short) can be used as the basis of a therapy to kill bacterial pathogens that are resistant to multiple antibiotics.

Prions

A **prion** is a protein that has a different conformation (shape) from the normal conformation. The normal prion protein cellular form is denoted as PrP^C, and the disease-causing prion protein form such as scrapie is shown as PrP^{Sc}. When a PrP^{Sc} protein molecule encounters a normal PrP^C form, it causes a structural change that converts it to the harmful form. This now harmful prion can in turn convert other normal forms to harmful forms (Figure 6.26). When there are sufficient numbers of the harmful PrP^{Sc} form, they aggregate (clump together) to form filaments. These fibres kill brain cells (Figure 6.27), leaving holes in the brain tissue and affecting muscle coordination and brain function as a consequence.

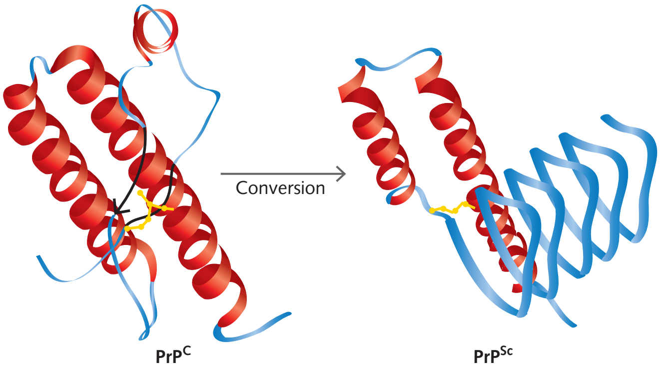

Figure 6.26 The process of converting a normal prion to an abnormal prion.

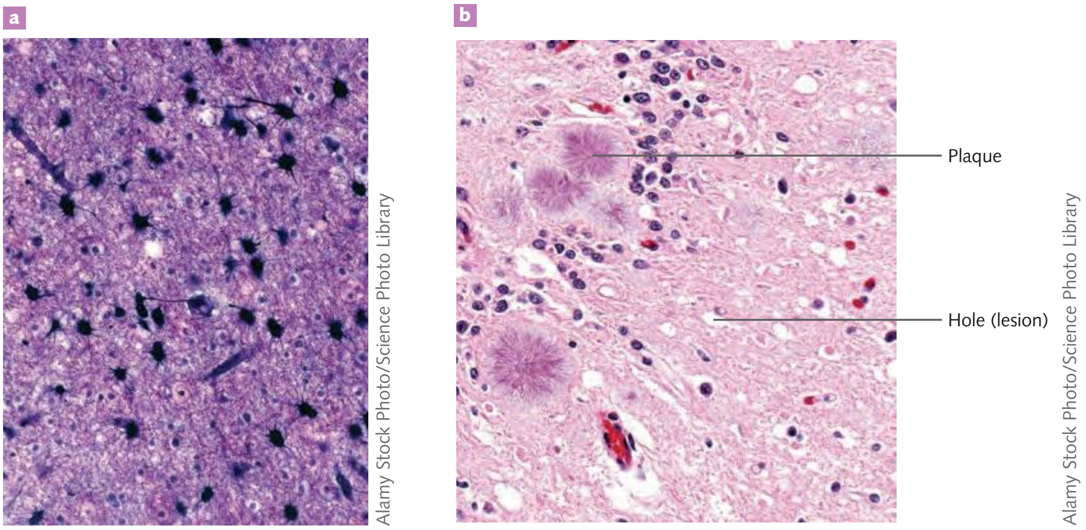

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Figure 6.27 a Healthy brain tissue. **b** Brain tissue from a victim of Creutzfeldt–Jakob disease. Note the plaques, and the holes that give the brain tissue a spongy appearance.

Prions occur in different species. In cattle, prions cause mad cow disease, which is called bovine spongiform encephalopathy (Figure 6.28). In humans, a prion disease called Creutzfeldt–Jakob disease has been identified. Scrapie is a prion disease in sheep and goats. Prion diseases can be transmitted when infected flesh is consumed. The disease may remain dormant for many years before the symptoms appear.

Science Photo Library/Eurelios/C.V.L.

Figure 6.28 This cow is infected with bovine spongiform encephalitis (BSE), commonly known as mad cow disease, which is caused by a prion.

KEY CONCEPTS

- Viruses are non-cellular pathogens, which are obligate parasites, because they must infect a host cell to reproduce.
- Viruses consist of a nucleic acid (DNA or RNA, single or double stranded) surrounded by a protein capsule. They use host machinery to replicate.
- Bacteriophages are viral pathogens because they lyse and destroy their bacterial host cells.
- Prions are infectious non-cellular protein pathogens that cause transmissible spongiform encephalopathies.
- The pathogenic prion protein form PrP^{Sc} can convert the normal cellular form (PrP^{C}, found in healthy brain tissue) to the PrP^{Sc} form that causes neurodegenerative disease.

Concept questions 6.4b

1 Define 'obligate parasite'.
2 Justify this statement: 'All viruses are pathogens'.
3 Viruses infect only specific host cells. Explain how this specificity comes about.
4 Outline the steps involved for a virus to reproduce.
5 Research on the Internet to find four diseases that are classified as transmissible spongiform encephalopathies (TSEs).

HOT Challenge

6 Research into Alzheimer's disease has demonstrated that it may be a double prion disorder. Two proteins central to the pathology of Alzheimer's disease act as prions. It is not believed that Alzheimer's is infectious by any common definition of the term. Prion diseases seem to be transmitted to humans through contaminated animal samples such as blood, meat or nervous tissue. Infectious prion diseases are more common in non-human species.
   a Outline how altered prion proteins in infectious prion diseases such as Creutzfeldt–Jakob disease affect the brain.
   b Alzheimer's disease can be genetic or idiopathic (of unknown cause) in origin but has not been shown to be infectious. Given the current issues with cross-species contamination of pathogens not normally infectious to humans, list five measures you think should be followed when eating animal tissues.

6.5 Allergens

WB 6.5 ALLERGENS PAGE 142

Some people's immune systems are excessively sensitive to certain substances, allergens, that cause allergic responses such as asthma, hay fever and anaphylaxis. Allergic reactions are rapid and most occur when IgE antibodies are produced. These antibodies bind to receptors on mast cells. The antibodies can bind specifically to antigens that are normally harmless. These antigens may be pollens, air pollutants, house dust, animal fur, fungal spores, cosmetics or other substances, which are known collectively as allergens. When the allergen–antibody complex is formed, the antibody attached to a receptor on a mast cell causes the mast cell to release its granules full of histamine, cytokines and other inflammatory molecules. This process is called **degranulation** (Figure 6.29). The abnormal amounts of histamine cause such effects as swollen bronchioles in lungs. The incidence of asthma and hay fever is around 15% in Australians and is increasing. These disorders are forms of allergies – exaggerated innate or adaptive immune system responses to usually harmless antigens.

These molecules bring immune cells to the affected area, causing localised inflammation. Histamine acts on smooth muscle to cause tightening of the airways, and symptoms also include excessive mucus production in the affected area. The inflammatory response is traditionally viewed as an innate immune system response.

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Figure 6.29 Mast cell response to allergen

Allergies begin with a **sensitisation** stage, in which IgE antibodies start being produced. These antibodies arise from an adaptive immune response, involving allergen phagocytosis and presentation to B lymphocytes and T_H cells in a secondary lymphoid organ – in a lymph node. Most allergens are not blood-borne and would therefore not cause a response in the spleen. It is not known why allergens cause an adaptive response in some people and not others, but it seems partly genetically determined and partly environmentally determined. For example, repeated exposure to the allergen can increase the risk of sensitisation.

Allergies can sometimes be treated by **desensitisation**, in which the affected individual is repeatedly exposed (usually by injection) to small doses of the allergen. This brings about a state of **immune tolerance**, stopping or reducing the production of antibodies to the allergen.

In most cases, allergies are simply annoying. However, they can be life-threatening if they result in **anaphylactic shock**. This is when inflammatory responses race through the body, leading to constriction of the airways and loss of fluid into body tissues from leaky capillaries. The loss of fluid into body tissues is due to high levels of histamines and results in a sudden drop in blood pressure, which may lead to a heart attack. Victims of anaphylactic shock need medical treatment urgently to counteract this exaggerated immune response.

6.6 Phagocytosis

One of the key actions of inflammation is to destroy invading pathogens before they can establish an infection.

Macrophages and neutrophils carry out phagocytosis in the same way that an amoeba engulfs food particles (Figure 6.30).

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Figure 6.30 **A scanning electron micrograph of macrophages with cytoplasmic extensions. These extensions engulf foreign particles.**

Macrophages and neutrophils are stimulated into action when receptors on their cell surfaces detect either bacterial products or signalling molecules such as complement proteins or cytokines. On their cell surfaces there are also receptors that bind to receptors found on pathogens. The macrophages and neutrophils are attracted to the site of infection and move out of the capillaries and migrate towards the damaged cells. They are attracted by the increased concentration of the chemicals that triggered their activation.

The attracted cells then attach to the pathogen-infected cells in one of two ways.

- Unenhanced attachment is associated with the innate response. The macrophage's receptor binds to the receptor on the surface of the infected cells or pathogens.
- Enhanced attachment occurs between the receptors and specific immunoglobulins (IgG) that are on the surface of the pathogen-infected cells, opsonising them, and takes place as part of the adaptive immune response (p. 260).

The cellular response for both macrophages and neutrophils that follows is now similar. During phagocytosis, the pathogen is engulfed and destroyed within a membrane-bound vesicle called a **phagosome**. A lysosome fuses with the phagosome to form a **phagolysosome**, which becomes increasingly acidic, as seen in Figure 6.31. An array of digestive enzymes and antimicrobial compounds, often including a burst of highly reactive oxygen molecules, helps to destroy the invader.

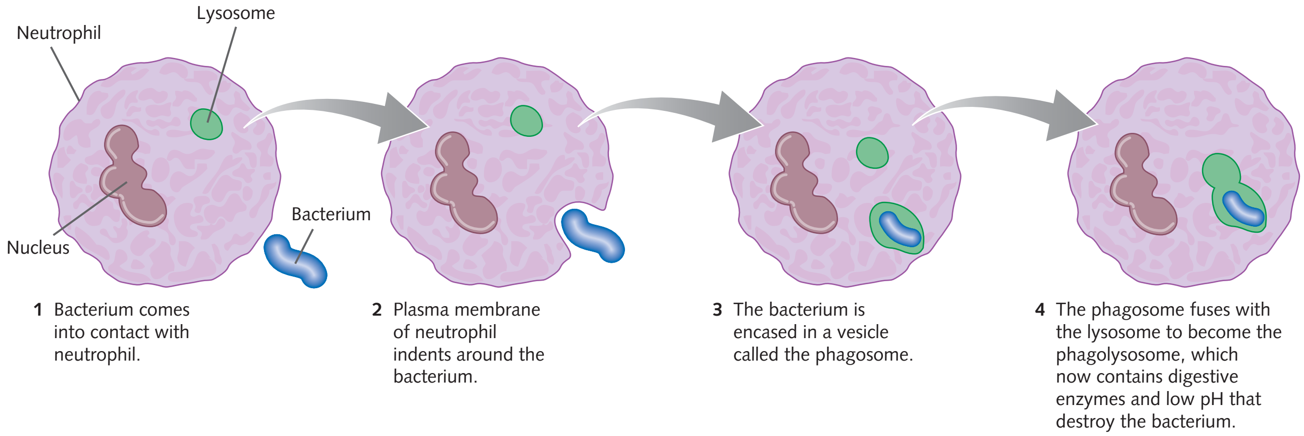


Figure 6.31 Phagocytosis and lysosomal degradation of a bacterium within a neutrophil

KEY CONCEPTS

- An allergic reaction occurs in response to an allergen.
- An allergen is a normally harmless antigen that produces IgE in large quantities, causing an adaptive immune response.
- Phagocytosis (internalisation) of a pathogen is followed by fusion of the phagosome to the lysosome. The resulting phagolysosome contains digestive enzymes and an acidic environment to break down the pathogen.

Concept questions 6.5/6.6

1 Describe the role of phagocytic leukocytes in the immune system.

2 Arrange the following points in order, to illustrate the sequence of events that would occur when a macrophage encounters a bacterium.
   - Lysosome fuses with vacuole.
   - Macrophage recognises bacterial surface molecules as non-self.
   - Powerful enzymes digest bacterium.
   - Vacuole forms around bacterium.
   - Macrophage envelops bacterium with its plasma membrane.

3 What cells release histamine during an allergic response?

4 What happens in an allergic response in the immune system?

5 a Are allergies the same as anaphylaxis?
   b Many people with allergies can be desensitised.
      i What does this mean?
      ii Can people with anaphylaxis be desensitised?

HOT Challenge

6 Are allergies a sign of a weak immune system? Discuss.

BRANCHING OUT

The war against bacterial infections

Penicillin is a widely used antibiotic. It is a chemical that blocks the action of an enzyme called glycopeptide transpeptidase. Many bacteria need this enzyme to build cell walls. Penicillin irreversibly binds to the active site of this enzyme so it cannot function and cell walls can no longer be built. Bacteria require a cell wall to stop them from bursting. Without the ability to build a cell wall the bacteria stop dividing and the infection is eradicated.

Any bacteria that use the enzyme glycopeptide transpeptidase to build cell walls will be sensitive to the action of penicillin. Bacteria that don't use glycopeptide transpeptidase are not susceptible to penicillin. Scientists have discovered antibiotics to treat infections caused by these non-susceptible strains. There are now hundreds of antibiotics available to treat infections by different bacteria.

Bacteria reproduce asexually by binary fission. If conditions are right, one bacterium can give rise to a billion cloned bacteria in just 10 hours. All essential genes coding for the proteins required for survival are found on a large circular chromosome in the nucleoid. Any mutations arising during chromosome replication are passed on to all clones. Other non-essential genes are carried on small circular pieces of DNA called plasmids. These non-essential genes often confer extra, very useful, functions, including antibiotic resistance.

Sometimes bacteria from different species swap plasmids through the process of conjugation. One bacterium builds a tube-like structure called a pilus that transfers plasmids to a second bacterium. Through mutations and plasmid transfer, bacteria are constantly evolving, acquiring new traits so they can survive in new environments.

The use of antibiotics applies an environmental pressure to bacteria populations. Those that survive because they contain a gene for antibiotic resistance pass on the gene to their clones when they divide, and their clones are then also resistant. Several mechanisms have evolved in bacteria to provide antibiotic resistance. Some make the antibiotic inactive by changing its shape or properties, others remove it from the cell, while others change the target site so the antibiotic can no longer bind. This evolutionary process has resulted in many bacteria developing resistance to the action of antibiotics.

Staphylococcus aureus, commonly known as golden staph, is a species of bacterium that lives on our skin and in our noses. It is generally harmless. However, if it enters our body through punctures in the skin, it can cause a range of infections. Some are mild infections such as boils and school sores. Others are severe, such as toxic shock syndrome, meningitis (brain), osteomyelitis (bone), pneumonia (lungs), septic phlebitis (veins) and endocarditis (heart valves). If the infection gets into the bloodstream, it can spread to other organs, causing severe infections known as sepsis. Sepsis can lead to multiple organ failure and death.

At the peak of antibiotic discovery and development, 15–20 new antibiotics were released onto the market every 10 years. As of June 2019, approximately 42 new antibiotics with the potential to treat serious bacterial infections were in clinical development. The success rate for clinical drug development is low; only one in five infectious disease drugs that enter the human testing phase are approved for patients.

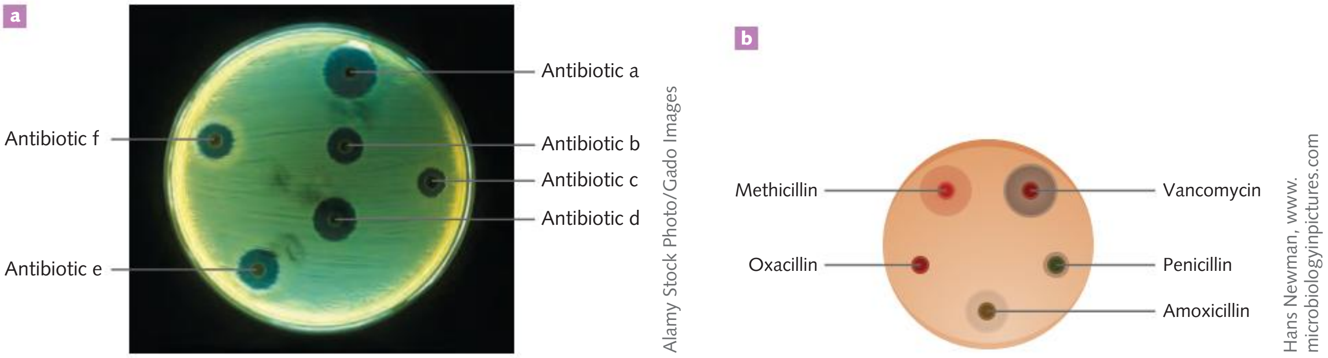
Some companies are turning away from the development of new antibiotics for various reasons, including:

- the high cost of developing and testing a new drug
- the limited lifetime of the drug if resistance develops
- doctors are starting to prescribe antibiotics more sparingly
- sales may be limited because antibiotics are only prescribed for a short duration to overcome the bacterial infection.

Questions

1 Explain how the antibiotic penicillin works at a cellular level.

2 **a** Figure 6.32a shows the *Staphylococcus aureus* sensitivity test. The grey-green background of the plate is a 'lawn' of densely growing bacterial cells. Discs soaked in antibiotics are placed on the lawn and rings of bacterial inhibition or death around the discs can give a measure of the susceptibility or resistance of the bacterial strain to the antibiotics. Use Figure 6.32a to decide which antibiotic(s) would be effective against *Staphylococcus aureus*.



Alamy Stock Photo/Gado Images

Hans Newman, www.microbiologyinpictures.com

Figure 6.32 a ***Staphylococcus aureus*** **sensitivity and b test for patient X**

b A patient in a hospital, patient X, had a catheter inserted into his arm to deliver intravenous fluids. A golden staph infection emerged at the puncture site and the patient was given methicillin to treat the infection. Over time, the infection became worse and patient X's doctor realised she was dealing with a methicillin-resistant strain of *Staphylococcus aureus* (MRSA). She ordered an antibiotic sensitivity test to determine the best antibiotic to treat this MRSA infection. The results of this test are shown in Figure 6.32b. Use Figure 6.32b to decide which antibiotic would be most effective against MRSA for treatment of patient X.

3 Many companies are no longer developing new antibiotics. Antibiotics are complex to develop and new products cannot be sold freely on the open market. They instead must be stockpiled and kept in reserve as a 'drug of last resort'. This is not appealing to potential investors who are looking for a good financial return on their invested money.

a Identify the bioethical issue in this situation.

b Identify the perspectives of the drug company, an investor and a member of the public.

c What is your position on this situation? Develop a course of action that you would like to see undertaken and write a fully reasoned argument for its adoption.

Online key concepts
Chapter 6: Summary of key concepts

6 Summary of key concepts

6.1 Physical, chemical and microbiota barriers in animals – first line of defence

KEY CONCEPTS

p. 204

- Structural, chemical and biological features can act as barriers to pathogens as a first line of defence.
- The skin is a tough physical barrier made from keratinised epithelial cells that can prevent the entry of pathogens.
- When the barrier of the skin is broken, platelets quickly form a plug, or scab, that upholds the barrier until the skin is repaired.
- Mucus traps pathogens that invade mucous membranes, and cilia beat the mucus to a place where is can be expelled. Urine and tears flush out micro-organisms and peristalsis keeps them moving through the gut.
- Chemical barriers that prevent pathogen colonisation include low (acidic) pH on skin and in the stomach, digestive enzymes produced in the gut, and lysozyme in tears, saliva and mucus.
- Symbiotic organisms form our microbiota, which take up space and use nutrients. This prevents colonisation by pathogens.

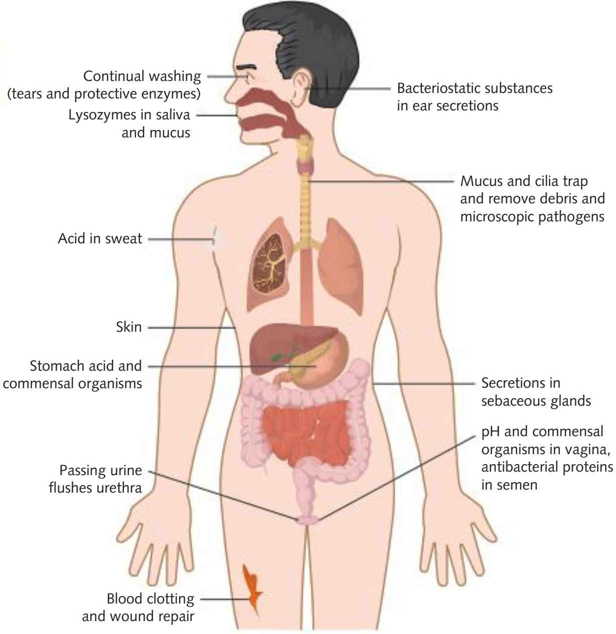


Figure 6.6 A summary of the physical and chemical barriers to pathogenic infections in a human. Peristalsis also helps by moving food and infectious agents through the digestive system.

6.2 Physical and chemical defences in plants

KEY CONCEPTS

p. 211

- Plants have physical and chemical barriers to prevent invasion by pathogens. These include thick bark, waxy cuticles, hairs and production of molecules such as defensins that are toxic to pathogens.
- Plants use PRRs (pattern recognition receptors) to detect PAMPs (pathogen-associated molecular patterns) on invading pathogens. They mount a rapid innate immune response to invasion by pathogens.
- After invasion by a pathogen, plants attain a state of systemic acquired resistance that makes them more prepared to fight invasion by a broad range of pathogens.

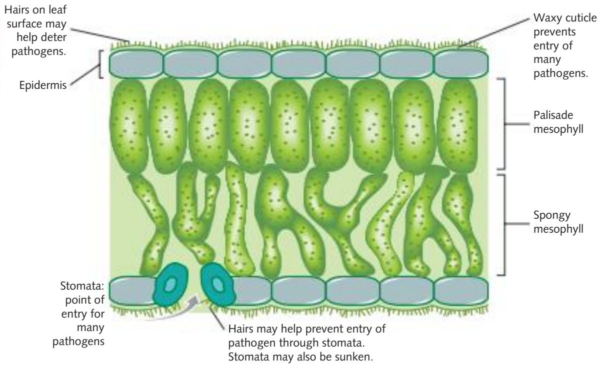


Figure 6.9 A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

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6.3 Innate immune response in animals–second line of defence

KEY CONCEPTS

p. 214

- Innate immune responses are non-specific and are inborn features of the body.
- Leukocytes (white blood cells) mediate the innate immune response by engulfing and digesting foreign pathogens.
- Immune cells such as macrophages begin the immune response after their receptor sites bind with foreign antigens.
- Neutrophils are the type of phagocyte that are abundant in blood. They readily migrate from capillaries to sites of infection, where they phagocytose pathogens and then self-destruct to stop pathogen spread.
- The complement system consists of a number of small molecules in the blood that have important roles in inflammation, including opsonisation and deposition of the membrane attack complex (MAC).
- Interferons are produced by virus-infected cells and alert neighbouring cells to the danger, causing changes in gene expression that make them more resistant to viral infection and replication.
- All plants and animals mount innate immune responses to pathogens.
- Inflammation is characterised by pain, heat, redness and swelling. Inflammation is the key weapon of the innate immune response, destroying invading pathogens before they can establish an infection.
- Chemotaxis is the process of cellular migration towards regions of higher concentrations of chemokines. Each cytokine has a specific receptor that is expressed by particular leukocyte types.
- Inflammation is initiated when macrophages, dendritic cells or other cells detect antigens or signaling molecules and begin to produce cytokines that bring in and activate other immune cells.
- Pain reduces voluntary movement to assist the repair process.
- Immune cell recruitment to sites of inflammation is assisted by histamine, which is secreted by mast cells and increases the permeability of capillaries.
- The resolution of inflammation is a highly active process that is necessary to limit and repair tissue damage caused by the inflammatory response.
- The innate and adaptive immune responses rely on being able to tell self from non-self.

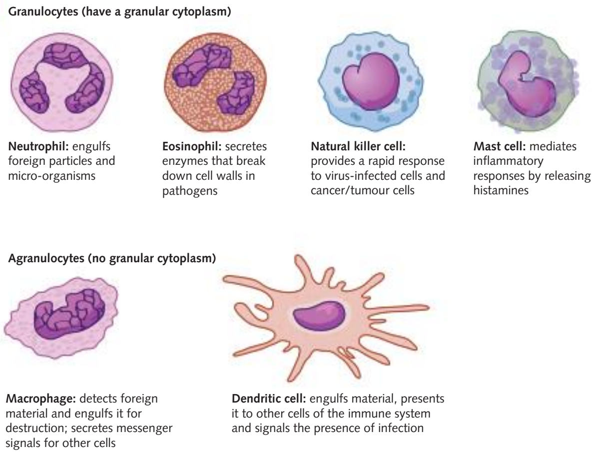
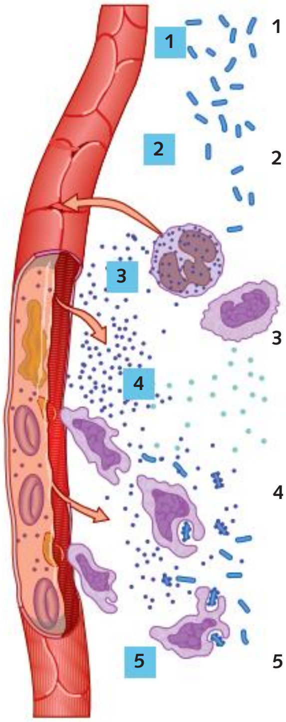


Figure 6.12 Different types of white blood cells (leukocytes)



1. Bacteria can invade cells or tissues where they can either kill the cells or release harmful by-products of metabolism.
2. Upon detection of bacterial PAMPs, granulocytes in infected tissues secrete histamines, and phagocytes and other body cells secrete cytokines to recruit other immune cells.
3. Histamines can affect the permeability of small blood vessels so that plasma fluid and proteins can seep into surrounding tissue.
4. Cytokines cause phagocytes to move through the leaky blood vessel walls and into tissue where they are activated by detection of PAMPs.
5. Bacteria are engulfed by phagocytic white blood cells.

Figure 6.16 The steps that occur in acute inflammation after invasion by a bacterial pathogen

6.4 Antigens and pathogens

KEY CONCEPTS

p. 231

- Pathogens are cellular or non-cellular infectious agents that cause disease.
- Antigens are intracellular or extracellular components of proteins, peptides, lipids or polysaccharides present in pathogens.
- Bacteria are prokaryotes; some are beneficial to their hosts and some are pathogenic.
- Bacteria produce endotoxins or exotoxins that can also cause disease.
- Fungi are eukaryotic organisms that are usually external pathogens that reproduce and spread through their spores.
- Protists are unicellular eukaryotes, a small number of which are significant pathogens of humans. Examples of protist pathogens are *Giardia*, amoebas, *Trypanosoma* and *Plasmodium* species.
- Viruses are non-cellular pathogens, which are obligate parasites, because they must infect a host cell to reproduce.
- Viruses consist of a nucleic acid (DNA or RNA, single or double stranded) surrounded by a protein capsule. They use host machinery to replicate.
- Bacteriophages are viral pathogens because they lyse and destroy their bacterial host cells.
- Prions are infectious non-cellular protein pathogens that cause transmissible spongiform encephalopathies.
- The pathogenic prion protein form PrP^{Sc} can convert the normal cellular form (PrP^{C}, found in healthy brain tissue) to the PrP^{Sc} form that causes neurodegenerative disease.

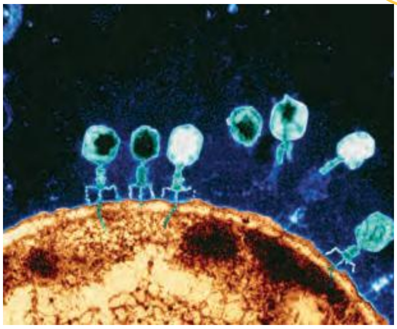


Figure 6.29 A coloured transmission electron micrograph of T-bacteriophage viruses attacking a bacterial cell of *Escherichia coli*. Seven virus particles are seen (blue), each with a head and a tail. Small blue strands of genetic material (DNA) are being injected into the bacterium.

6.5 Allergens & 6.6 Phagocytosis

KEY CONCEPTS

p. 232

- An allergic reaction occurs in response to an allergen.
- An allergen is a normally harmless antigen that produces IgE in large quantities, causing an adaptive immune response.
- Phagocytosis is an example of a signal transduction pathway involving a cell receptor, signal transduction and cellular response, in this case to engulf the targeted foreign material.
- Phagocytosis (internalisation) of a pathogen is followed by fusion of the phagosome to the lysosome. The resulting phagolysosome contains digestive enzymes and an acidic environment to break down the pathogen.

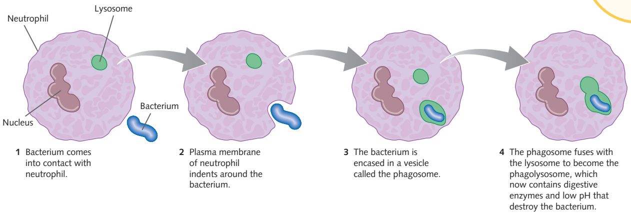


Figure 6.31 Phagocytosis and lysosomal degradation of a bacterium within a neutrophil

WB
6.6.1
KEY TERMS –
SPOT THE ERRORS
PAGE 144

6 Chapter glossary

adaptive immune response an immune response directed against a specific antigen; it retains memory of that antigen so that, on subsequent exposure to the same antigen, it responds with a secondary response

allergen an antigen that is normally innocuous but can sometimes cause an over-reaction from the immune system known as an allergy

allergy an immune response characterised by IgE production to an innocuous substance

anaphylactic shock a severe allergic reaction that causes widespread swelling, including of the face and neck, which can lead to difficulty breathing and a life-threatening reaction

antibiotic a naturally or synthetically produced compound that is toxic to bacteria

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cells by various enzymes, including caspases

bacteria unicellular prokaryotes that can be pathogenic and therefore carry disease

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

cellular pathogen a disease-causing pathogen that is made up of one or more living cells such as bacteria or fungi

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or a cell along a chemical concentration gradient either towards or away from a chemokine

cilia slender hair-like structures projecting from a cell surface that beat against fluid

companion plant a plant that is grown with another plant because one species improves the growth of the other

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

cytokine a signalling molecule that coordinates inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

defensin a type of small antimicrobial peptide secreted by nearly all plants and animals

degranulation a cellular process in which the granules of neutrophils, mast cells, basophils or eosinophils are emptied into extracellular surroundings

dendritic cell a phagocyte with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

disease any condition that interferes with how an organism, or any part of it, functions

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular organisms

first line of defence physical and chemical barriers that keep pathogens from entering the body of a living thing

flagellum a helical filament that rotates to give bacteria locomotion

fungus a heterotropic organism made up of one or many cells; has cell walls but is not a plant

granulocyte a white blood cell that has granules in the cytoplasm

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

host the organism in which a parasite lives

immune system a complex network of cells, tissues and organs in the body that detect differences between self-molecules and foreign (non-self) organisms, and mounts an immune response that results in the formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

infectious disease a disorder caused by bacteria, viruses, fungi and other organisms, that can often be transmitted to other members of a population

inflammation an innate response to infection or damage that causes pain, swelling, heat and redness

innate immune response a response to a pathogen that is not specific to the antigen, only that it has been identified as being non-self; the response does not generate antibodies or memory lymphocytes

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

keratin the tough, fibrous protein of the outer epidermis layer

leukocyte the general term for a white blood cell

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

macrophage a large white blood cell that phagocytoses pathogens; originates as monocytes in circulation

mast cell a cell that is located in the tissues and releases granules containing histamines when activated

microbiome the bacteria, viruses and fungi that live in the gut plus their released metabolites and nucleic acids that exist in a specific environment

microbiota a community of micro-organisms, including fungi and bacteria, that live in or on another organism

microflora *see* microbiota

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

natural killer cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

necrosis cell death that results from tissue damage or infection when the plasma membrane is breached; results in inflammation

neutrophil a phagocytic leukocyte found in the blood and tissues

non-cellular pathogen a disease-causing pathogen that is not made of living cells; for example, viruses and prions

non-infectious diseases are caused by environmental, genetic or lifestyle factors and not by pathogens

non-self antigen a molecule that is not recognised by the immune system as being part of the organism itself

non-specific response a response that is the same regardless of the type of antigen

obligate parasite a parasite that cannot complete its life cycle without a suitable host; without a host, the parasite cannot reproduce

opsonisation the process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

pathogen an infective agent foreign to the body and capable of causing disease

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis a process by which phagocytes engulf a particle or cell

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

platelet a cell fragment found in the blood involved in blood clotting

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

protist a unicellular eukaryotic organism

second line of defence non-specific immune responses including fever and inflammation

secondary metabolite an organic compound produced by bacteria, fungi or plants; its role is to sustain functional and homeostatic health of cells within organs by assisting cells to excrete wastes and toxic substances

self-antigen an antigen or a molecule that is a normal body component

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

sterile inflammation inflammation resulting from the detection of damage- or danger-associated molecular patterns released during tissue injury in the absence of infection

transmitted when an infection is passed from one person or organism to another

vasodilation widening of blood vessels, particularly arterioles

virus an obligate intracellular pathogen that can use the host cell's machinery to replicate itself; usually consists of a nucleic acid surrounded by a protein coat

WB
6.6.2
EXAM MARKING
PAGE 146

6 Chapter review

Remembering

1 State two important differences between a bacterium and a virus. Give two examples of diseases that are caused by each of these pathogens.

2 State two diseases caused by fungi, and two diseases caused by protists.

3 Are viruses living organisms? If not, why not?

4 Identify the type of change (physical or chemical) that PRRs detect in host tissues after a pathogen has entered.

5 Outline one role of the bone marrow in the defence system.

6 Outline the advantage of keratinisation of skin cells.

Understanding

7 Describe the unique feature of a prion that distinguishes it from other non-cellular infectious agents.

8 Describe two changes to the structure of prion proteins that lead to Creutzfeldt–Jakob disease.

9 *Staphylococcus aureus* causes food poisoning by releasing a heat-stable toxin. Describe the effect of reheating food on the potential of this pathogen to cause food poisoning.

10 Defensins are peptides, so their synthesis requires significant amounts of nitrogen compounds, which are generally a scarce resource for plants. Yet, up to one-tenth of some seeds are defensins. Suggest an important advantage to a plant of putting very large quantities of such an 'expensive' chemical into their seeds.

11 Describe the events that follow the activation of complement.

12 Explain what is meant when we say the body can discriminate between 'self' and 'non-self'.

Applying

13 Eating diseased tissue that contains abnormal prion proteins can cause the brain to become infected. Predict a property you would expect prions to have, given that they manage to enter the bloodstream without being digested. Provide evidence to support your answer.

14 Cigarette smoke decreases ciliary beat frequency and reduces the number of ciliated cells in the airway epithelium. Predict the effect of smoking on the body's defences.

15 Both an infection in your foot and a sprained ankle cause the local area to swell, become red and painful, throb and feel hot. Explain why these two different events lead to the same response by the body.

Analysing

16 Figure 6.33 shows infection by a fungus responsible for rust in wheat and rye.

- **a** Identify the part of the plant the fungus probably gains access through.
- **b** Describe the damage the fungus causes to its host.
- **c** Predict, with reasons, whether antibiotics would be useful in controlling its spread. Design a controlled experiment to test your hypothesis.
- **d** Describe two methods that could be used to control the fungus.

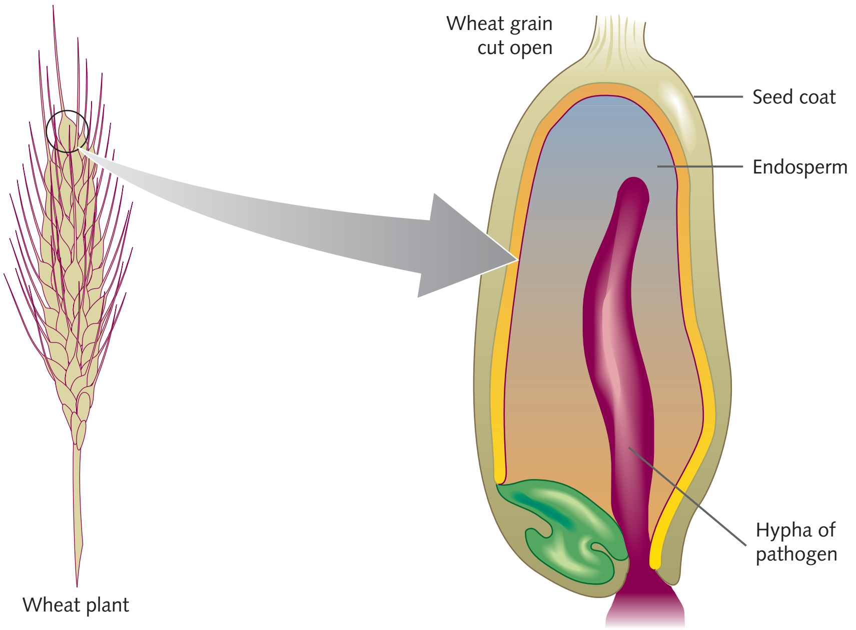


Figure 6.33 A wheat plant and magnified cut wheat grain, showing infection by a pathogen

17 Figure 6.34 shows the number of cattle infected with the prion causing BSE (also known as mad cow disease) in the UK for the years 1985–2000. Since 1992, feedstuff containing animal neurological tissue, such as brain and spinal cord, has been banned.

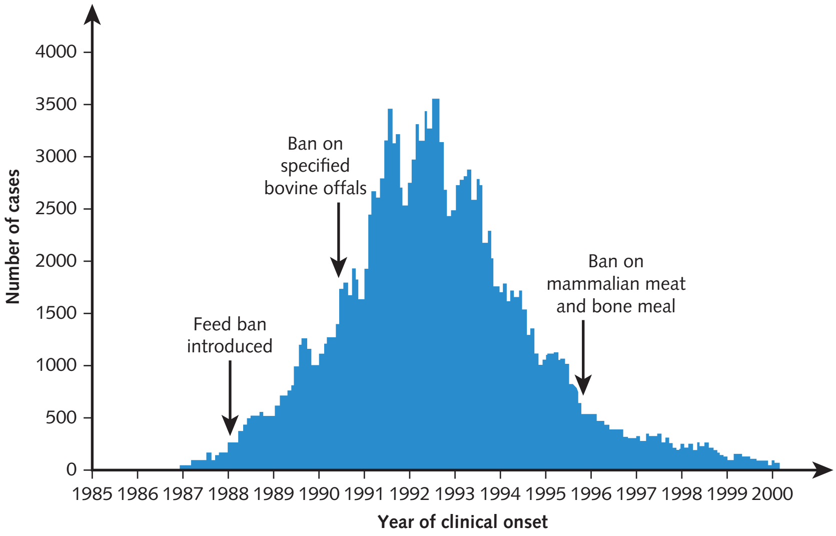


Figure 6.34 The number of cattle infected with BSE from 1985 to 2000

a Describe the trend in numbers of BSE-infected cattle in the UK from 1985 to 1995.
b Describe the action of a prion when it causes disease.
c Suggest a reason for the decline in the incidence of BSE since 1994.
d There are fears that the infectious agent causing BSE is now infecting humans, causing Creutzfeldt–Jakob disease. Describe measures that could reduce the transmission of this disease.

18 Consider the stages in the replication of a virus. Imagine you are a chemist trying to find antiviral medicines. Describe two points at which a virus would be susceptible to antiviral chemical therapies.

Evaluating

19 Given the increase in antibiotic resistance in recent years, discuss whether we should restrict the use of antibiotics to only those people with a life-threatening illness.

Creating

20 It has been said that we underestimate the effectiveness of our innate immune system because we do not usually become aware of the potential infections that it prevents. Design an investigation using mice and an immunosuppressant to test this idea.

21 When macrophages engulf bacteria, the phagosomes fuse with lysosomes to form phagolysosomes, where the bacteria are killed and broken down. One sample, the control, contains normal bacteria. The two other samples contain bacteria that can evade immune destruction, one by surviving inside the phagosome and the other by escaping into the cytoplasm from the phagosome.

Design an investigation to distinguish between these three samples, ensuring that you describe the results that you would expect from each sample of bacteria. If you use macrophages with red fluorescent labelled phagolysosomes and bacteria with green fluorescent proteins in their cytoplasm, you can see:

- disappearance of green bacteria as they are digested in the phagolysosome
- green bacteria in the cytoplasm when they escape the phagolysosome
- green bacteria remaining in the red fluorescently labelled phagolysosomes.

22 Prepare a flow chart to summarise the steps involved in inflammation.

Reflecting

23 Consider the observation that plant defensins have been shown to inhibit the growth of human cancer cells. Reflect upon why this might be.

Acquiring immunity

7

By the end of this chapter you will have covered the following material.

Key knowledge

Acquiring immunity

- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes pp. 249–253
- the characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T and cytotoxic T cells pp. 253–268
- the difference between natural and artificial immunity and active and passive strategies for acquiring immunity pp. 270–272

Key science skills

Analyse, evaluate and communicate scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 268–269

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To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 7 map (p. 246)

Online Key Terms:
- Chapter 7 flashcards (p. 248)

Weblinks:
- The immune system explained (p. 249)
- The lymphatic system as part of the body's defence (p. 250)
- Types of immune responses (p. 254)
- Different levels of defence (p. 260)

Online Worksheets:
- The lymphatic system's role in immunity (p. 250)
- Types of immune responses (p. 254)

Video:
- The T and B cells of the immune system (p. 254)

Online Key Concepts:
- Chapter 7: Summary of key concepts (p. 274)

Online Chapter Map
Chapter 7 map

7 Acquiring immunity

If you had chicken pox when you were young, you probably won't get it again because your third line of defence produces memory cells that remember how to fight a specific disease.

p. 249

7.1 Adaptive immune response – third line of defence

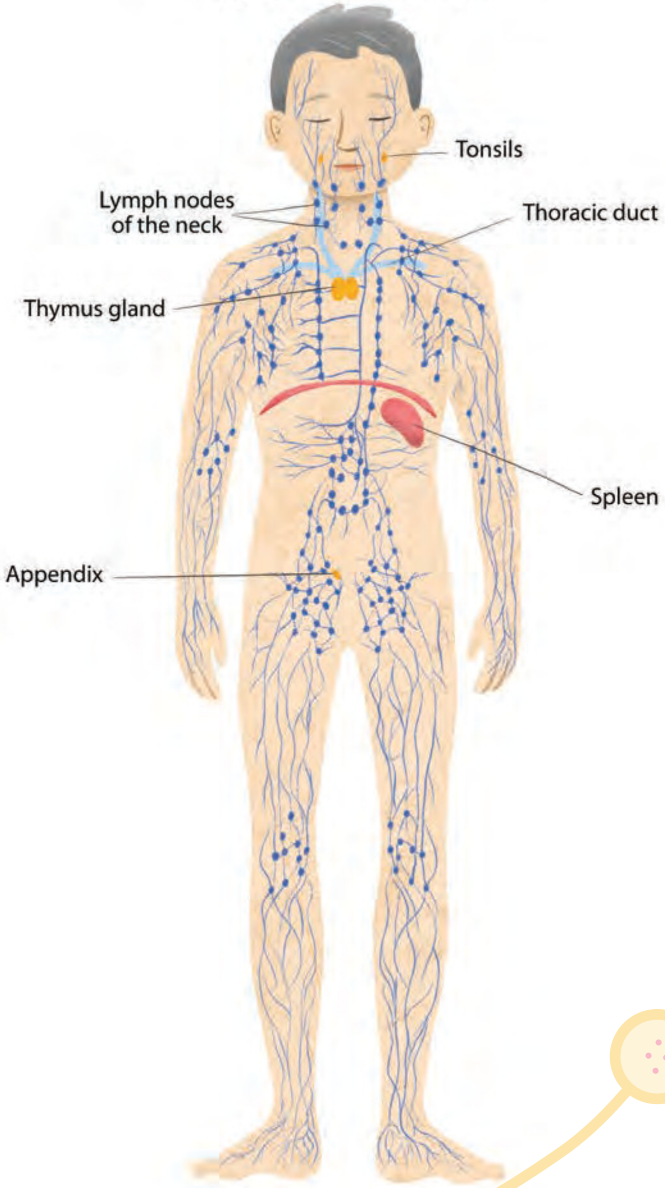
Your body's immune response is sophisticated. B and T cells are the key players in the third line of defence. B cells produce antibodies and T cells bring about potent antiviral and antitumour responses. B and T cells can also become memory cells that can rapidly mobilise to protect against reinfection.

p. 260

7.2 Humoral immunity

Antibodies are Y-shaped molecules bound to the surface of B cells and secreted into circulation. They spread through the body in the bloodstream and attack pathogens. T helper cells can respond to the same antigen, and activate B cells.

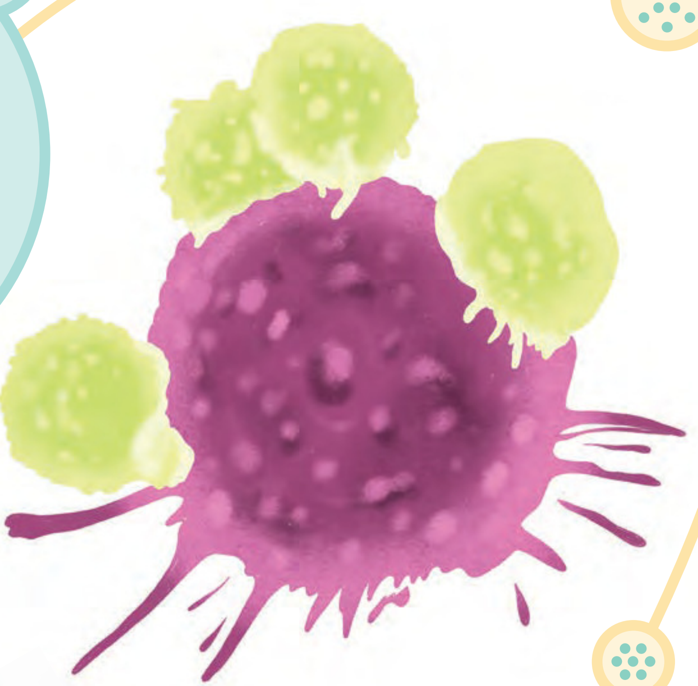
The lymph and immune systems



7.3 Cell-mediated immunity

p. 264

There's always a back-up plan. Some viruses have evolved to prevent T receptor cells from recognising the virus. T_c cells have evolved to kill infected cells by releasing chemicals that bring about a process called apoptosis, or programmed cell death.



7.4 Active and passive immunity

p. 270

Active immunity is when the immune system, responds and produces memory cells that remember the invader over many years. Passive immunity is when antibodies are provided from an external source such as vaccination.

Your body is adapted to combat pathogens that it may encounter. Science has also produced ways to assist your body to defend itself.

Online Key Terms
Chapter 7 Flashcards

Know your key terms

- active immunity
- agglutination
- antibody
- antigen-presenting cell (APC)
- autoimmune disease
- B cell
- B cell receptor
- B plasma cell
- bone marrow
- cell-mediated immunity
- clonal selection
- cytotoxic T cell (T_C cell)
- epitope
- helper T cell (T_H cell)
- humoral immune response
- immune
- immunoglobulin (Ig)
- interleukin
- interstitial fluid
- lymph
- lymph node
- lymphatic system
- major histocompatability complex (MHC)
- memory cell
- MHC restriction
- mucosal-associated lymphoid tissue (MALT)
- neutralisation
- passive immunity
- primary lymphoid organ
- primary response
- regulatory T cell (T_{reg} cell)
- secondary lymphoid organ
- secondary response
- self-tolerance
- specific response
- spleen
- T cell receptor
- thymus

Remember

REMEMBER
PAGE 148

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 The innate immune response is non-specific.
2 PAMPs are pathogen-associated molecular patterns on the surface of pathogens.
3 DAMPs are damage- or danger-associated molecular patterns that are released from damaged or dying cells.
4 PRRs are pattern recognition receptors on cells of the innate immune system (e.g. macrophages) that recognise PAMPs and DAMPs.

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Figure 7.1 Measles is a viral infection that triggers an adaptive immune response and results in life-long protective immunity against the disease.

In Chapter 6 you learnt about the innate immune response. In this chapter you will learn about other components of the immune system, which work together to bring about the adaptive immune response. These are **specific responses** that target non-self antigens and pathogens when they have been detected by particular components of the immune system. Once the infection is over, some of the cells of the adaptive immune response (third line of defence) remain as specific **memory cells**. If you are exposed to the same non-self antigen or pathogen again, these cells rapidly respond, often overcoming the infection without you even knowing your body had been exposed to that specific pathogen again. Immunisations work to trigger your body to produce specific immune memory cells so next time you encounter the substance you were immunised against (e.g. the measles virus in Figure 7.1), your memory cells will be activated to prevent infection.

Weblink
The immune system explained

7.1 Adaptive immune response – third line of defence

Spacesuits protect astronauts from the extremes of outer space, allowing them to survive in an environment too hostile for human existence. Filled with potential pathogens, Earth's atmosphere is also hostile, but the constant efforts of our immune system allow us to survive.

David Vetter was born in the United States in 1971 without an adaptive immune system, affected by a condition known as severe combined immunodeficiency (SCID). Without an immune system, his risk of catching a fatal infection was so high that he was raised from birth in a sterile isolator unit, or bubble, designed by NASA to keep all pathogens out. Not even his family was allowed into the bubble. At 5 years of age, David was able to walk outside for the first time using a special suit, also designed by NASA and based on their spacesuits. Although he lived more and more at home as he grew older, he died at the age of 12.

AAP

Figure 7.2 David Vetter was born without an immune system. He was raised from birth in a sterile isolator unit designed by NASA to protect him from pathogens.

Today, medical knowledge about SCID has improved and children with the disorder no longer have to be raised in such isolation. This rare disease demonstrates the critical role that the cells of the adaptive immune system, B and T lymphocytes, play in fighting pathogens.

Adaptive responses, which exist only in vertebrates, target pathogens only after they have been specifically identified by particular components of the immune system. They are termed 'adaptive' because they are capable of change in response to the experience of an antigen. These responses are highly specific because they attack only the pathogen that stimulated the response. Because of this specificity, the body requires some time to tailor its customised response, meaning that adaptive responses are not as rapid as innate responses. Adaptive responses occur in specialised structures in specific tissues and organs of the **lymphatic system**.

People surviving diseases such as smallpox and the bubonic plague seldom contract the disease again. This feature is called immunological memory, and we say the person has become **immune** to the effects of that pathogen.

WB
7.1.1
LYMPHATIC SYSTEM – AN ANALOGY
PAGE 149

Weblink
The lymphatic system as part of the body's defence

Online worksheet
The lymphatic system's role in immunity

Lymphatic system

Under normal circumstances, when inflammation is not occurring, blood capillaries allow a small amount of plasma to leak out through their walls. This fluid that surrounds the body cells is called tissue fluid, or **interstitial fluid**. Although most tissue fluid returns to the capillaries, some, now called **lymph**, is drained away by lymph vessels of the lymphatic system. The lymphatic system transports fluid, wastes, immune cells and, unfortunately, pathogens around the body and back into the bloodstream. The lymphatic system (Figure 7.3) consists of lymphoid organs (Table 7.1) and transport vessels that carry lymph between these organs and back to the blood. Blood plasma, tissue fluid and lymph are essentially the same fluid with differing amounts of wastes and nutrients depending on where the fluid is in the body.

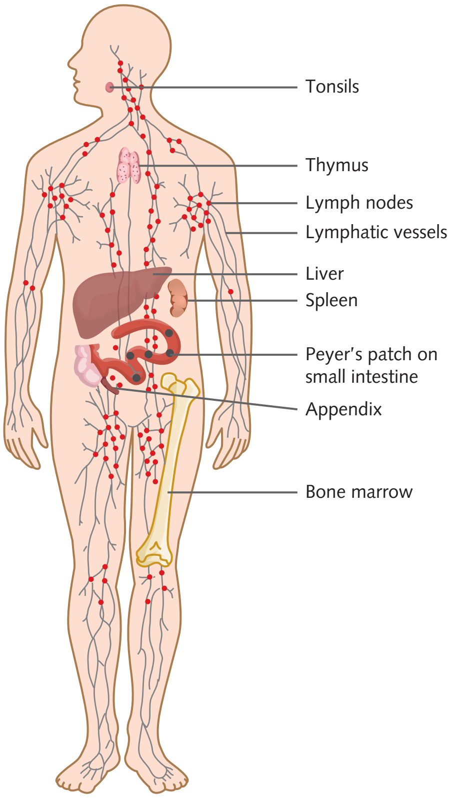


Figure 7.3 The location of organs and tissues involved in the lymphatic system in the human body

Primary lymphoid organs

The **primary lymphoid organs**, the **bone marrow** and **thymus**, are responsible for the production and development of the cells of the immune system. The bone marrow is the soft tissue in the centre of bones. It is where leukocytes are differentiated from stem cells. These stem cells produce approximately 200 billion blood cells every day. Some cancers of the bone marrow and leukocytes result in a person not having enough white blood cells for a sufficiently functional immune system. These patients are treated with bone marrow transplants. In this procedure, the recipient's stem cells are ablated (killed) and replaced with healthy donor bone marrow stem cells, which should repopulate the immune system with fully functional cells. In the time it takes for the donor bone marrow stem cells to fill the immune system with fully developed healthy cells, the individual remains highly susceptible to infections.

The thymus gland is located in the chest above the heart, and is where some immune cells mature. The thymus gland is relatively large in children, then shrinks (involutes) in adults. This accompanies a general reduction in the amount of new T lymphocytes that develop in the thymus as people grow older. Some immune cells are called T cells because they mature in the thymus gland. These cells are produced in the bone marrow, but at an early stage in their development they migrate to the thymus, where they complete their developmental steps before maturing and entering circulation.

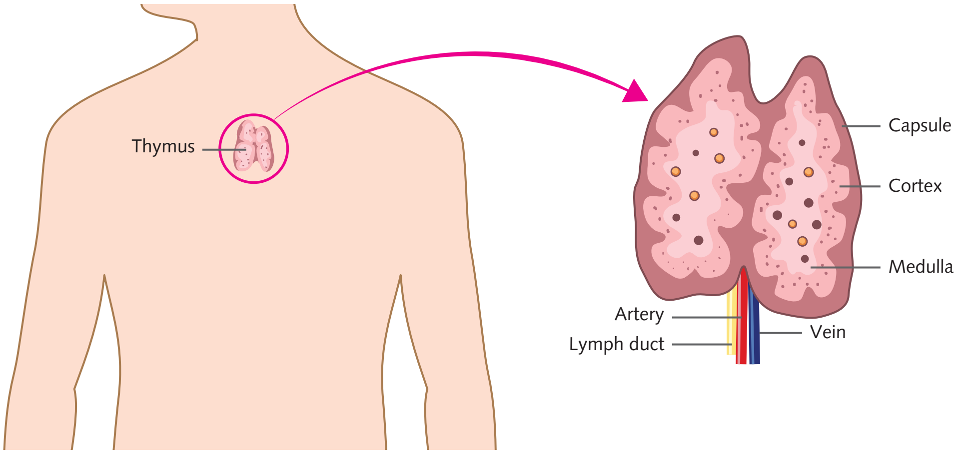


Figure 7.4 **The thymus gland is the site where some immune cells mature.**

Secondary lymphoid organs

The **secondary lymphoid organs** hold mature immune cells and provide the environment for the initiation of the immune response. In the **spleen**, B lymphocytes may be stimulated by the presence of an antigen, or by other immune cells, to differentiate and proliferate into **B plasma cells**. Phagocytosis also occurs in the spleen. As well as supporting the maturation of B lymphocytes, and acting as a source and storage site for several types of leukocyte, the spleen filters the blood to remove aged red blood cells. Because of the blood filtration that occurs there, and the cells that are present in well-organised structures within it, the spleen is an organ that is specialised for detecting and responding to systemic infections, in which the pathogen is present in the bloodstream.

Mature resting naïve B and T lymphocytes, as well as other white blood cells in the circulation, move around the body through blood vessels and the lymphatic system. Until they intercept their specific antigen, they are called ‘naïve’ cells. Along the way, they move through lymph nodes, scanning for antigens that they recognise through receptors that are particular to each individual lymphocyte. Within **lymph nodes**, antigens are usually displayed to passing lymphocytes by macrophages that have collected them out of the circulation (mainly by complement receptors on the macrophages binding to opsonised antigens), or by dendritic cells that have picked them up in body tissues and migrate with them to lymph nodes for this purpose.

The secondary lymphoid organs also include **mucosal-associated lymphoid tissue (MALT)** – an extensive system of lymphoid tissue that initiates immune responses along mucosal areas such as the gastrointestinal tract, eyes and lungs.

Table 7.1 provides a summary of the lymphatic system.

Table 7.1 Components of the human lymphatic system

Organ or tissue		Location	Role
Primary lymphoid organs Responsible for the production and maturation of the cells of the immune system	Bone marrow	Central shaft of most bones, with a substantial amount in the thigh and pelvic bones	All blood cells develop from bone marrow stem cells. These stem cells can develop into all types of blood cells (are multipotent).
	Thymus	Inside the rib cage, made up of two pinkish grey lobes	Involved in the development of T cells (a type of lymphocyte) and shrinks (involutes) with age, beginning soon after birth
Secondary lymphoid organs Provide the environment for the initiation and progression of the immune response	Lymph nodes	Small, bean-shaped structures in specific locations throughout the body, including throat, armpits, groin, abdomen and chest	Filter the extracellular fluid (lymph) that drains from limbs and mucosal tissues, trapping foreign material, and are where lymphocytes can come across antigens and begin to respond to them.
	Spleen	Large, dark red organ located just above the stomach	Filters the blood, recognises and destroys old and faulty red blood cells, detects foreign invaders and produces antibodies. Generally the site for immune responses directed against blood-borne pathogens.
	Mucosal-associated lymphoid tissue (MALT)	Clusters of immune cells, including lymphocytes found in association with the wet mucosal surfaces of the body, such as those of the respiratory, digestive and female reproductive systems	Cells in these structures survey the mucosa for pathogens and protect the body from an enormous variety of invaders. Tonsils and adenoids are more complex examples of MALT. In the gastrointestinal tract, this is called gut-associated lymphoid tissue (GALT) and includes Peyer's patches, small clumps of white blood cells sitting in the wall of the intestine. Other examples include bronchus-associated lymphoid tissue (BALT) in the lungs and nasal-associated lymphoid tissue (NALT) in the nose.

Lymph nodes

Unlike the blood circulatory system, the lymphatic system has no pump. It relies on muscle contraction and one-way valves to move the lymph away from the tissues towards the heart. Lymph vessels coming from the tissues eventually join with the circulatory system by draining into the bloodstream near the heart. Lying along the course of lymphatic vessels, sometimes in chains, are lymph nodes. Approximately 500–600 lymph nodes are distributed throughout the body, with clusters in the armpits, groin, neck and chest, and abdomen. These collect and monitor material drained from the arms, legs, oral and nasal passages, and gut, respectively. They range in size from a few millimetres to about 1–2 cm in diameter and are tightly packed with white blood cells (Figure 7.5). As lymph moves along the lymph vessels, the lymph nodes act as filters or traps for foreign particles and invading pathogens. When an antigen is present in a lymph node, white blood cells become activated, causing an influx of more white blood cells and enlargement of the node as an immune response begins to occur within.

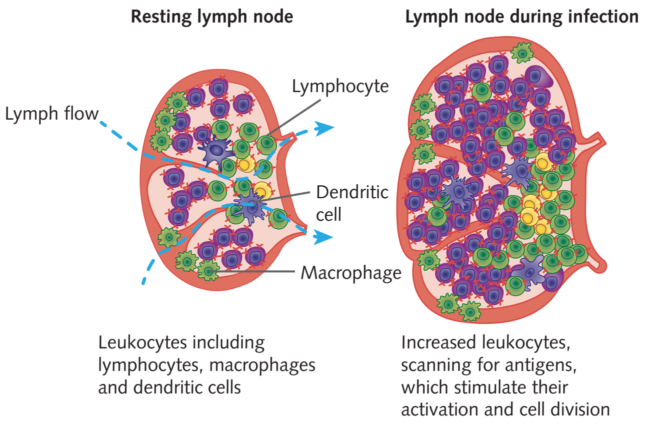


Figure 7.5 Lymph nodes are sites where lymphocytes scan for antigens and can initiate responses when they come across a particular antigen.

The lymphoid system, including the secondary lymphoid tissues, is a transport system linking innate and adaptive immune responses. When an infection occurs (for example, if a cut on the arm becomes infected), localised inflammation will occur at the cut site as part of an innate response. This inflammation causes chemotaxis of white blood cells into the area, including neutrophils, macrophages and dendritic cells. There, the phagocytes engulf foreign material, damaged cells and apoptotic debris, and secrete cytokines that stimulate further influx and activation of immune cells into the site (p. 221).

Dendritic cells, upon taking up the antigen, become activated and leave the inflamed site. They enter the lymphoid system and travel through lymphoid vessels to lymph nodes. Along the way, they change their surface receptor expression, down-regulating receptors that help detect and engulf antigens and up-regulating receptors that help present these to B and T lymphocytes of the adaptive immune system. When dendritic cells carrying antigens arrive in lymph nodes, where lymphocytes are concentrated, they also spread out their membrane to ensure a large surface area on which lymphocytes can scan for antigens. Dendritic cells are named for their small finger-like projections, or dendrites, that are on the surface of the cell that help them to take up, process and present antigens to lymphocytes efficiently. At this stage, they become **antigen-presenting cells (APCs)**.

KEY CONCEPTS

- All blood cells are produced from stem cells in the bone marrow. Some leukocytes reside in lymphoid organs and others circulate in the blood and lymphatic system. Others are resident in the tissues.
- Primary lymphoid organs include the bone marrow and thymus, where cells of the immune system are produced and mature.
- Secondary lymphoid organs include lymph nodes, spleen and mucosal-associated lymphoid tissue (MALT), where immune responses are initiated and carried out.
- The lymphoid system, including the secondary lymphoid tissues, is a transport system linking innate and adaptive immune responses.

Concept questions 7.1a

1 Describe the role of lymph nodes in the immune system.
2 Where is an immune response likely to be initiated when the pathogen:
  a enters through a cut in the hand?
  b is a gastrointestinal pathogen that causes ‘food poisoning’?
  c enters and circulates in the bloodstream?
3 How does the innate immune response differ from the adaptive immune response, considering the aim of both is to confer defence against antigens? Give three examples.
4 What is an important distinction between the functions of primary lymphoid organs and secondary lymphoid organs?

HOT Challenge

5 Recent findings indicate that intestinal mononuclear phagocytes, comprising dendritic cells and macrophages, are crucial for maintaining intestinal homeostasis. There are also about 10^{14} commensal bacteria in the gut. Commensal bacteria derive food from the host without harming the host organism. Dendritic cells mediate inflammatory responses and can become antigen-presenting cells. Knowing that not all gut bacteria are pathogens, why do you think that dendritic cells and macrophages might be so vital?

Cells of the adaptive immune system

WB 7.1.2 CELLS OF THE ADAPTIVE IMMUNE SYSTEM PAGE 150

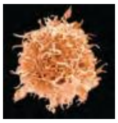
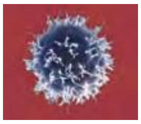
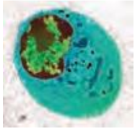
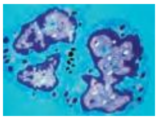
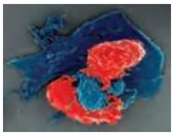
All cells of the immune system, including B and T cells of the adaptive immune system and the many cell types of the innate immune system, are produced in the bone marrow from blood stem cells. Collectively they are called white blood cells, or leukocytes. Some reside in the lymphoid organs while others circulate in the blood and lymph, acting like a mobile surveillance squad. As they move around the body, they detect invading pathogens and initiate an immune response to clear the infection.

Weblink
Types of immune responses

Online worksheet
Types of immune responses

Video
The T and B cells of the immune system

Table 7.2 **The cellular components of the immune system**

Cell	Function
Dendritic cell  Alamy Stock Photo/Science Photo Library	An antigen-presenting cell that acts as a messenger between the innate and adaptive immune systems Engulfs material and presents it to lymphocytes Produces cytokines that direct immune responses
Lymphocyte  Alamy Stock Photo/Science Photo Library	A general term for a range of specialised leukocytes (white blood cells) that respond to specific antigens in the process of adaptive immunity. This is an example of Cytotoxic T cell.
B lymphocyte  Alamy Stock Photo/Science Photo Library	A white blood cell that is produced and matured in bone marrow and travels to the spleen and lymph nodes Produces specialised proteins called antibodies, which bind to specific foreign material on the surface of pathogens, thereby labelling it for engulfment and destruction by other white blood cells such as macrophages
Plasma cell  Alamy Stock Photo/Science Photo Library	A specific B lymphocyte that is differentiated to secrete very large amounts of specific antibodies Contains many Golgi apparatus and large amounts of rough endoplasmic reticulum to assist with production and secretion of antibodies
Memory B cell  Alamy Stock Photo/Science Photo Library	A specific B lymphocyte with receptors that are specific to one type of antigen When stimulated, rapidly differentiates into plasma cells and more memory B cells
T lymphocyte  Science Photo Library/David M. Phillips	A white blood cell that originates in the bone marrow, then travels to the thymus where it matures Contributes to the adaptive immune system in a variety of ways
Cytotoxic T cell (also known as killer T cell) (T_C)  Science Photo Library/Edelmann	A T cell that produces lethal chemicals, such as perforin and cytoxins, which are injected into the infected cell and destroy it along with the virus by initiating apoptosis
Helper T cell (T_H)  Alamy Stock Photo/Science Photo Library	A T cell that activates other cells of the immune system
Regulatory T cell (also known as suppressor T cell) (T_{reg})  Alamy Stock Photo/Science Photo Library	A T cell that suppresses or turns off the activity of other cells once the threat has passed
Memory T cell  Science Photo Library/Steve Gschmeissner	A T cell that remembers a particular antigen to provide long-term immunity

Lymphocytes

Lymphocytes are cells of the adaptive immune system (Table 7.2). There are two major types: B and T lymphocytes (or B and T cells). Many B and T lymphocytes look so similar that scientists cannot tell them apart under the microscope. Special tests that measure surface proteins are required to distinguish between them.

B lymphocytes are a key cell type of the adaptive immune system. These cells go through a series of developmental stages in the bone marrow before they are released into the circulation, ready to respond to an infection. B lymphocytes (**B cells**) are responsible for the destruction of pathogens by producing specific proteins known as **antibodies** that bind to antigens and neutralise or opsonise them. Destroying virally infected and cancerous cells is the major role of **cytotoxic T cells (T_C cells)**. Helper T lymphocytes and regulatory T lymphocytes assist the other lymphocytes in performing their roles.

Helper T and regulatory T cells

Helper T cells (T_H cells) assist other cells of the immune system. They do this by secreting signalling molecules (including cytokines) that induce any activated B or T_C cell to divide and give rise to large numbers of clones that become the effector cells (which bring about the immune response) and memory cells. Cytokines can also stimulate macrophages to engulf invading cells more readily.

Regulatory T cells (T_{reg} cells) play an important role in modulating the action of lymphocytes. T_{reg} cells may enhance or suppress the actions of other lymphocytes. They are also capable of suppressing the action of phagocytes. In this way, they help prevent the immune system overreacting to a stimulus. A T_{reg} cell deficiency causes a very severe **autoimmune disease** resulting from overactive lymphocytes.

Lymphocytes of the adaptive immune system differ from cells of the innate immune system because they are *specific*. This means that individual lymphocytes can each detect a particular invader, attacking only those that contain the specific molecular pattern or **epitope** matching the receptors on their surface. An antigen will usually have several epitopes, and each epitope will only be detected by the lymphocytes with a complementary receptor for that specific epitope (Figure 7.6). Even a small section of a molecule, such as a toxin, can generate an immune response. As even a small peptide length may be potentially antigenic, most protein antigens have several epitopes, each of which is recognised by a different lymphocyte and induces the production of a different antibody. Each different epitope is a specific chemical group or structure.

Before a person is infected by a particular pathogen, they may only have a handful of lymphocytes that can detect the epitopes contained within the pathogen's antigenic components. However, once infected, an adaptive immune response is mounted, which results in cell division of those lymphocytes to produce an army of clones. These all have the same antigen receptor and collectively they can outnumber the pathogen and clear it from the system.

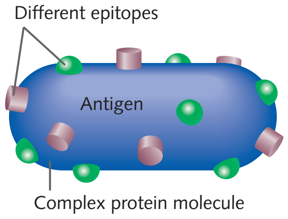


Figure 7.6 **The distinction between an antigen and an epitope. A large antigen, such as a bacterium or a large protein complex, may have several different antigenic determinants, called epitopes. The different epitopes are specific chemical groups or structures.**

Determining self from non-self

For the immune system to function properly, cells of the immune system must be able to distinguish between cells of the body and foreign antigens. Our body cells identify themselves to the immune system as 'self' by marker proteins on the surface of the plasma membrane. As is the case with all proteins, the amino acid sequence of these markers is determined by the information coded in genes. The group of genes that determines these protein markers is called the **major histocompatibility complex (MHC)**. Because these MHC markers are determined by the genotype of an individual, they are unique to that person. There are many different alleles in the MHC gene locus, resulting in great variability between individuals. It is as if each cell of a person's body is tagged with a message that is read as 'self'. Any cell not displaying that particular marker is 'non-self' and treated as an antigen.

MHC markers

WB
7.1.3
INTRACELLULAR
OR EXTRACELLULAR
PATHOGENS
PAGE 150

T cell receptors are present on the surface of T cells. These receptors do not bind directly with the antigen but rather with epitopes derived from the antigen protein that are displayed on the end of MHC marker molecules. MHC markers are the only molecules that can present the antigen to a T cell. The fact that the T cell receptor will only recognise the antigen when in association with the MHC marker molecule is termed **MHC restriction**.

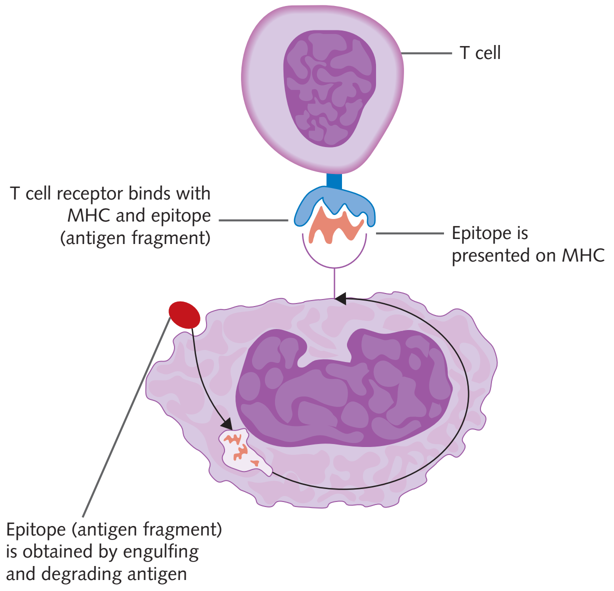


Figure 7.7 T cells must be activated to respond. T cell receptors will only recognise antigens presented by MHC markers.

There are two types of MHC proteins: MHC class I and MHC class II. Both types of MHC proteins contribute to the specific identity of the cell. MHC class I markers are found on all body cells that have a nucleus and act to identify the cells' self antigens from non-self antigens. MHC class II markers are found only on antigen-presenting cells (APCs): macrophages, dendritic cells and B lymphocytes and they present antigen fragments on their MHC class II markers to T lymphocytes and other immune cells.

MHC proteins contain a deep groove, which can hold a short peptide. Within a cell, antigens are broken down into small peptides. MHC proteins are synthesised inside the cell and pick up the antigen peptide lengths. These then sit inside the groove of the MHC. The MHC protein (bound to a peptide) travels to the cell surface where T cell receptors can then scan for their ability to bind to the MHC–antigen complex.

MHC class I and MHC class II proteins differ in the type of antigen that they can present, and the type of T cell that can recognise antigens bound to them (Figure 7.8a). MHC class I presents antigens that are found within the cell cytoplasm. These antigens are usually produced within the cell itself, and this method of antigen presentation allows the immune system to survey the intracellular activity of cells to detect virally infected or cancerous cells. Some pathogens are able to enter and divide within cells. Antigens from these pathogens are also presented this way. This process does not distinguish between antigens and normal proteins produced. Instead, a random sample of peptides from the breakdown of proteins within the cell is presented on the MHC. T cells can then bind to the MHC–antigen complex and trigger apoptosis of the cell if they recognise the presented peptide as non-self. As MHC class I proteins are found on all nucleated cells, this is how the immune system patrols the cells of the body to find any abnormal proteins within cells.

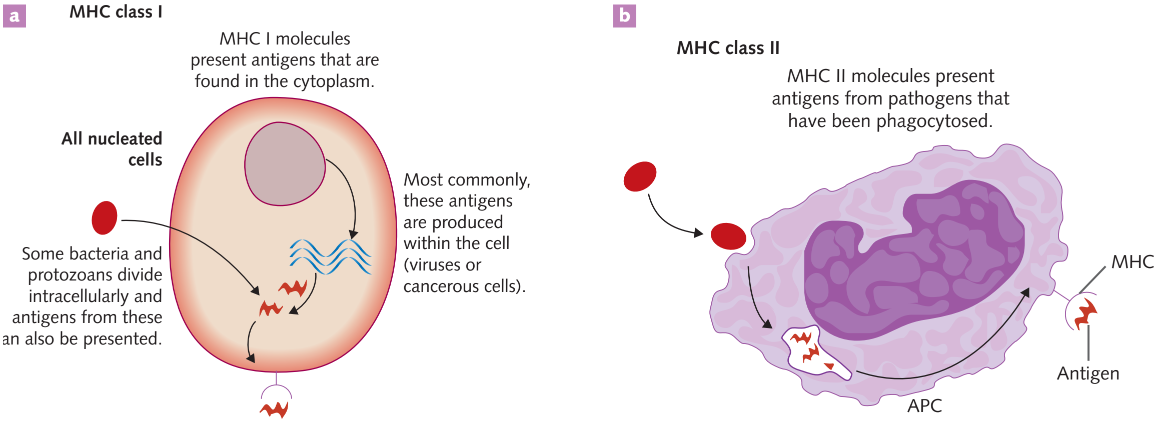


Figure 7.8 MHC class I and MHC class II proteins are cell-surface structures that present pieces of antigen to T lymphocytes. **a** Cytotoxic T lymphocytes recognise their specific antigens only if they are presented on MHC I molecules, and **b** helper T lymphocytes recognise their specific antigens only if presented on MHC class II molecules.

MHC class II proteins are only found on APCs and are used to present extracellular antigens (Figure 7.8b). APCs phagocytose pathogens following recognition by pattern recognition receptors or their opsonisation by complement or antibody, and then break them down in lysosomes. APCs then travel to the lymph nodes to present these antigens to T cells. Peptides that are derived from these antigens are presented on MHC class II proteins. APCs are usually macrophages and dendritic cells but B cells can also present antigens this way. APCs also express MHC class I molecules because they are nucleated cells.

Immune cell antigen receptors

Lymphocytes have surface receptors that distinguish self from non-self. **B cell receptors** and T cell receptors allow lymphocytes to identify foreign antigens. B cell receptors and T cell receptors recognise and bind to specific epitopes of the antigen. The binding of an antigen to a lymphocyte receptor is similar to that of a substrate binding to an enzyme. The molecules must have the correct shape (or conformation) and charge to be able to bind to each other.

Antibody molecules are glycoproteins whose function is to bind to antigens. Some forms are secreted into the circulation, and some contain hydrophobic protein sequences that anchor them to the plasma membrane. When antibodies are bound to the surface of B lymphocytes, they act as the B cell receptors. They have the ability to bind their specific antigens, and stimulate signal transduction pathways within the B cells. If paired with inflammatory cytokines or helped from helper T cells, this results in their activation and cell division. Antibodies also serve as effector molecules when secreted by B cells.

Receptor diversity

Each B or T cell carries a large number of identical copies of a receptor protein that will bind to a single, specific antigen. There are so many different receptors that about 10 million different epitopes can be recognised by all the B cell clones combined. This diversity means that, by chance, there will be a B and T cell receptor that is able to bind to almost any antigen that the body could encounter.

The genome does not encode for this number of different receptors. The particular type of receptor carried by a lymphocyte is determined during early embryonic development by random genetic recombination of the antibody or receptor genes. As a result of this genetic rearrangement, each B or T cell and all of its descendants will produce a unique receptor. This genetic lottery accounts for the huge diversity of lymphocyte receptors that are able to respond to the millions of different antigens that we experience in our lifetime. Whatever the antigen, there is a strong chance that there will be a lymphocyte receptor that can bind to it.

It is interesting to note that while receptor diversification is essentially a random process, the genetic pool from which each lymphocyte draws its receptor components has been shaped by the disease challenges our ancestors have faced during their evolution.

KEY CONCEPTS

- The adaptive immune response differs from the innate immune response because it has specific recognition of antigens and displays memory.
- B cells, T_H cells and T_C cells are the main players of the adaptive immune system. The adaptive immune response relies on these cells detecting foreign antigens and distinguishing them from self.
- T_{reg} cells are T cells that control the magnitude and duration of immune responses to limit damage to body tissues.
- Antigens are molecules that can generate an immune response. The particular molecular structures on antigens that are recognised by components of the immune system are called epitopes.
- The major histocompatibility complex (MHC) is an important way of distinguishing self from non-self.
- MHC restriction refers to the fact that T cells will only recognise epitopes when they are presented on an MHC molecule.
- MHC class I molecules present intracellular antigens and are present on all nucleated cells (self-markers).
- MHC class II molecules present extracellular antigens derived from phagocytosis and are present on antigen-presenting cells (macrophages, dendritic cells and B cells).
- Each B cell and T cell has an antigen receptor that is specific for a unique epitope, so the population of lymphocytes has the capacity to detect a vast range of antigens.

Concept questions 7.1b

1 Define 'major histocompatibility complex'.
2 What is the difference between an epitope and an antigen? What are epitopes made of chemically?
3 Describe the MHC class I and class II presentation pathways and identify the main differences between them.
4 List two mechanisms that prevent T cells from mounting an immune response against a normal body component in the absence of any true infection or injury.

HOT Challenge

5 Lymphocytes are responsible for both the induction and the expression of adaptive immunity. The lymphocytes in adaptive immunity are specific. There are two major classes of lymphocytes, B cells and T cells, which can be further divided into T_H, T_{reg}, T_C, memory T, plasma B and memory B cells.
  - **a** What are the specific functions of each lymphocyte?
  - **b** Compare the specific functions of B and T cells.

Avoiding self-recognition

The random generation of receptors results in some receptors that will bind to self-molecules. An important step in lymphocyte development is the killing off or inactivation of those self-reactive lymphocytes, to ultimately protect the individual from immune attack of their own body cells and tissues.

A group of cells in the thymus expresses a wide range of proteins that are usually found elsewhere in the body. These proteins are not expressed to perform their normal function, but rather so that T cells can develop self-tolerance. In effect, these cells serve as a 'showroom' of the proteins that the body is capable of producing. Any T cell bearing a T cell receptor that recognises a peptide presented in the thymus undergoes apoptosis and is deleted from the collection.

A similar selection process may occur for B cells as they develop in the bone marrow and also as they mature in the spleen. This negative selection of self-reactive lymphocytes continues when they are mature, and those clones that carry receptors for molecules that already exist in the body are either inactivated or self-destruct by apoptosis. This process provides the adaptive immune system with the capacity to distinguish self from non-self. The result is **self-tolerance**, which means that ideally there are no mature lymphocytes that will react against self-molecules. All the lymphocytes in the collection are tolerant to normal components of the body; that is, they don't mount an immune response against such normal components. However, the process is not perfect. A very small number of lymphocytes that react against self-molecules usually survive the process. If there is a strong innate inflammatory response, and if the individual has a collection of alleles that allow it, those self-reactive lymphocytes may become activated and attack the body. This can result in autoimmune diseases.

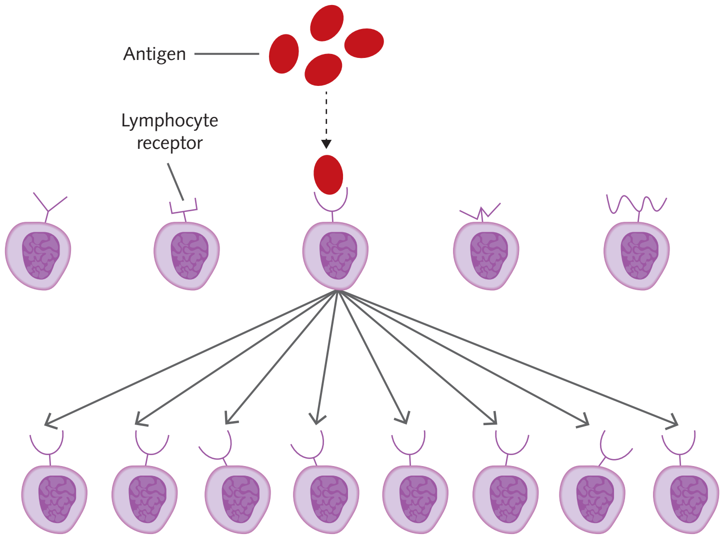
The interaction between APCs and T cells is another mechanism for preventing responses against self-antigens. T cells can only recognise an antigen if it is loaded onto an MHC protein, which means they must interact with an APC. A T cell that recognises a complementary antigen on an MHC protein must receive appropriate signals from the cell presenting that antigen to become activated. If that APC has recognised a pathogen-associated molecular pattern (PAMP) or a damage- or danger-associated molecular pattern (DAMP), indicating infection or tissue damage, it will signal to the T cell that it should mount a response against the peptide presented on its MHC. This signal is usually in the form of cytokines, such as **interleukins**, and contact-dependent signals. Without this danger signal, a T cell recognising a peptide bound to an MHC protein will not mount a response against the peptide. This provides an additional safeguard that prevents T cells from mounting an immune response against the body's own cells and tissues.

Clonal selection

B and T cells originate as stem cells in a process that starts when we are embryos. By the time we are born, we have a large number of different types of B and T cells, each with a small number of clones that can recognise a specific antigen circulating throughout our blood and lymphatic systems.

A young lymphocyte is released from its 'training ground' into the bloodstream, in which it may encounter an antigen it recognises. Recognition of a complementary antigen by a B lymphocyte (by direct binding) or T lymphocyte (via MHC) triggers an impressive response in the selected cell (clonal selection), causing it to divide rapidly, forming many copies or clones of itself and the specific antigen receptor it carries (clonal expansion). These clones can be one of two types of cells: effector cells (antibody-secreting B cells, or helper and cytotoxic T cells) or memory cells that confer long-term immunity (Figure 7.9).

Random genetic rearrangements allow for a diverse range of lymphocyte receptors to be generated. **Clonal selection** and clonal expansion are responsible for the proliferation of lymphocyte clones with receptors that have bound to antigens.



Clonal selection: There is a diverse range of lymphocyte receptors. By chance, one of these is the right shape to bind to the antigen.

Clonal expansion: Clonal population of lymphocytes; some of these will become effector cells and others memory cells.

Figure 7.9 The rapid division of a particular lymphocyte clone, once it has bound to an antigen, is called clonal selection. Thus, the antigen itself selects which of the millions of different B or T cell clones becomes active.

KEY CONCEPTS

- Lymphocytes with antigen receptors that could recognise self-components are deleted or inactivated during their development.
- During infection, only the lymphocytes bearing a receptor that can recognise epitopes on the invading pathogen are activated and proliferate, in the process of clonal selection.

Concept questions 7.1c

1 Define 'self-tolerance'.

2 Self-recognition can lead to inflammation and autoimmune disease. There are several ways in which the immune system avoids self-recognition. Outline two methods used to manage the preferred outcome of self-tolerance.

3 Define 'clone' in relation to clonal expansion.

4 Clonal selection theory explains the immune mechanism for generating a diversity of antibody specificity as part of adaptive immune system. The theory was postulated by Australian scientist Sir Macfarlane Burnett in 1957. The first experimental evidence was demonstrated by Gustav Nossal and Joshua Lederberg in 1958. The theory is still widely accepted. Both T cells and B cells are cloned to mount a response. Using a flow chart, describe the steps involved in clonal selection theory.

HOT Challenge

5 Azathioprine is a drug that blocks the production of purine nucleotides (adenine and guanine) in lymphocytes. Predict the effect that azathioprine would have on the process of clonal selection.

7.2 Humoral immunity

The **humoral immune response** is brought about by B cells, which produce an array of specific antibodies that tag foreign antigens for destruction. The word 'humoral' refers to the fact that the effects of this system are caused by the circulation of antibodies in 'humours', an antiquated concept that roughly means body fluids. You have already learned that antibodies bound to the surface of B cells act as the B cell receptor. Once activated, B cells divide rapidly (that is, they are clonally selected) and produce antibodies that circulate freely in the bloodstream and can lead to the destruction of pathogens.

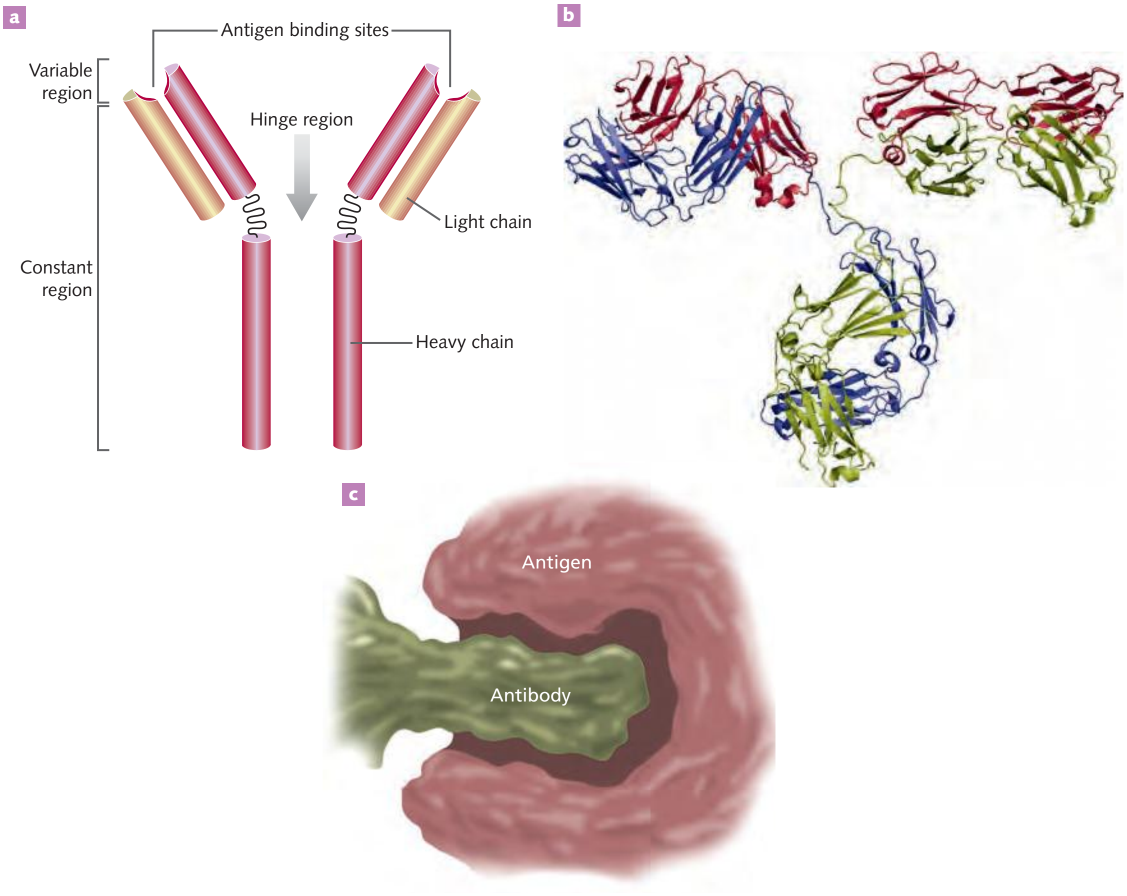
Antibodies

Antibodies are also known as **immunoglobulins (Ig)**. Antibodies have a quaternary structure. They consist of four polypeptide chains: two heavy chains and two light chains that are arranged in the shape of a Y (Figure 7.10a). All antibodies have a constant region (most of the Y shape) and variable regions at the two tips of the Y, where there are two identical binding sites that are complementary to a specific antigen. The variability in this region is based on the different amino acid sequences that allow for differential binding to various antigens. This is the part of the antibody that results from genetic recombination during development. The binding sites work with a lock-and-key system of identification, similar to that of enzymes binding with their substrate (Figure 7.10c).

EXAM TIP
Make sure you can draw and label an antibody.

WB
7.2.1
ANTIBODIES
PAGE 152

Weblink
Different levels of defence



Australian Synchrotron, PyMOL2 and Harris et al, Biochemistry 18;36 (7)(1997)

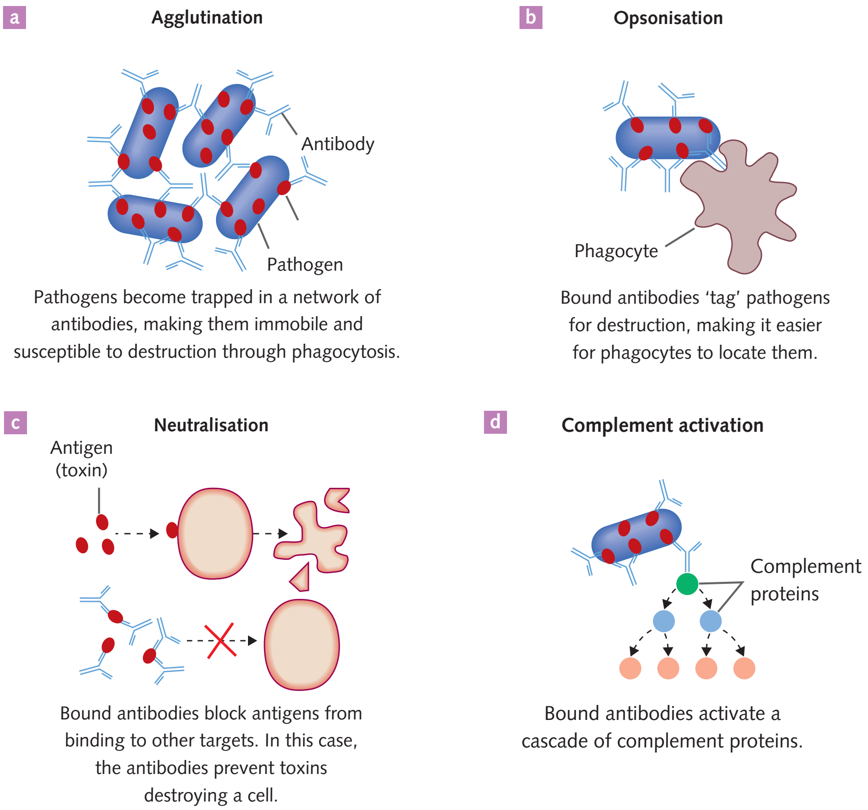
Figure 7.10 a The Y-shaped structure of an antibody. The hinge region gives antibodies great flexibility to improve binding to the antigen. b A ribbon diagram representation of the crystal structure of 1IGT, an antibody of the IgG family produced by plasma B cells. c The active sites on the antibody and antigen molecules are complementary; they fit together like a lock and key.

Once bound to an antigen, antibodies can lead to the destruction of pathogens in four ways, all of which may occur simultaneously (Figure 7.11).

- The binding of antibodies can cause **agglutination** of pathogens, meaning that they become stuck together in an antibody–pathogen net. In other words, the pathogens are immobilised and not able to spread. Being clumped together in one spot makes them more susceptible to destruction by phagocytosis.
- Bound antibodies are able to attract phagocytes, effectively 'tagging' pathogens for phagocytosis and destruction, a process known as opsonisation. Opsonisation by antibodies has similar results to opsonisation by complement but, in this case, opsonised antigens are recognised by receptors on cells that detect the constant region of the antibodies, called Fc receptors (FcR). FcR triggering can cause degranulation, phagocytosis, release of cytokines and chemokines and increased antigen presentation.
- Some antigens can act as toxins and cause cellular damage. In these cases, antibodies neutralise toxins by preventing them from binding to their target. This is known as **neutralisation**.
- Antibodies that are bound to antigens are potent activators of the complement cascade.

CONNECT

See Chapter 6, page 217 for details about the complement cascade and how complement, like antibodies, can also result in opsonisation.



Pathogens become trapped in a network of antibodies, making them immobile and susceptible to destruction through phagocytosis.

Bound antibodies 'tag' pathogens for destruction, making it easier for phagocytes to locate them.

Bound antibodies block antigens from binding to other targets. In this case, the antibodies prevent toxins destroying a cell.

Bound antibodies activate a cascade of complement proteins.

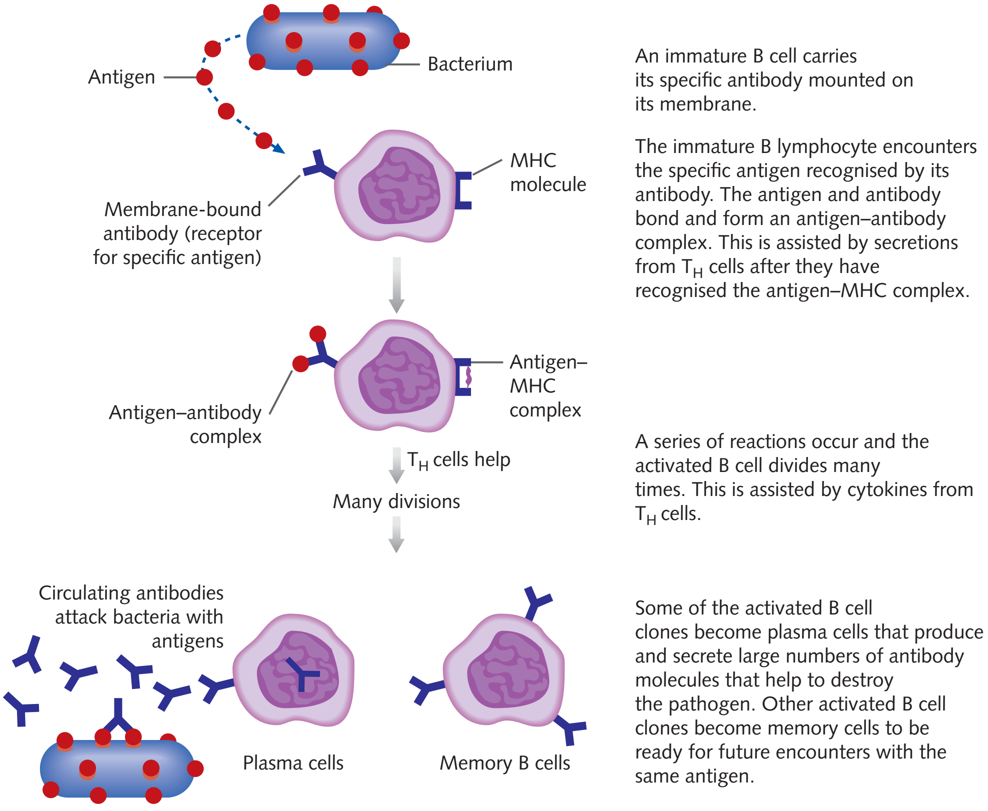
Australian Synchrotron, PyMOL2 and Harris et al, Biochemistry 18;36 (7)(1997)

Figure 7.11 Antibodies can cause the destruction of pathogens in four ways: **a** agglutination, **b** opsonisation, **c** neutralisation and **d** complement activation.

B cells and antibody production

As with phagocytosis activation, B cell activation involves the three-step process of reception and transduction followed by cellular response. The role of B cells is to produce antibodies, and a B cell needs to be activated by an antigen. When a pathogen enters the body, it encounters a large number of B cells. These cells circulate through the blood and lymphatic system and congregate in lymph nodes where they detect pathogens draining from the tissues or presented on APCs such as dendritic cells. B cells are also found in the spleen, where they can detect and respond to pathogens circulating in the bloodstream. Each B cell has a unique B cell receptor (antibody) on its surface that enables it to bind to a specific epitope. An invading pathogen will only activate B cells bearing a B cell receptor that is complementary to a particular epitope on the pathogen. In this way, the B cell is activated to produce an adaptive immune response. However, B cells are also APCs, and carry on their surface pattern recognition receptors that recognise foreign pathogens non-specifically. These cells engulf the pathogens they recognise and present pathogen components on MHC class II molecules for presentation to T_H cells. A T_H cell that bears a receptor that can specifically detect the same epitope, embedded in MHC class II, can become activated, and can provide direct assistance to the B cell in the form of cytokines (signalling molecules) and contact-dependent signals.

The result of this interaction between the T_H cells and the antigen-presenting B cells is that the B cell becomes activated and is clonally selected. It starts rapidly dividing to produce effector and memory B cells (Figure 7.12) in the process of clonal expansion, helped by the activated, antigen-specific T_H cell in this process. Following clonal selection and expansion, the B cell clones that have been activated will then be present in much greater numbers than others. This division occurs most effectively with the assistance of T_H cells that have been activated by the same antigen but can also proceed when the environment contains an abundance of inflammatory cytokines that indicate the presence of a foreign antigen.



An immature B cell carries its specific antibody mounted on its membrane.

The immature B lymphocyte encounters the specific antigen recognised by its antibody. The antigen and antibody bond and form an antigen–antibody complex. This is assisted by secretions from T_H cells after they have recognised the antigen–MHC complex.

A series of reactions occur and the activated B cell divides many times. This is assisted by cytokines from T_H cells.

Some of the activated B cell clones become plasma cells that produce and secrete large numbers of antibody molecules that help to destroy the pathogen. Other activated B cell clones become memory cells to be ready for future encounters with the same antigen.

Figure 7.12 Example of an antibody-mediated immune response to a bacterial pathogen, summarising the steps of B cell activation. This response usually occurs in a lymph node or in the spleen.

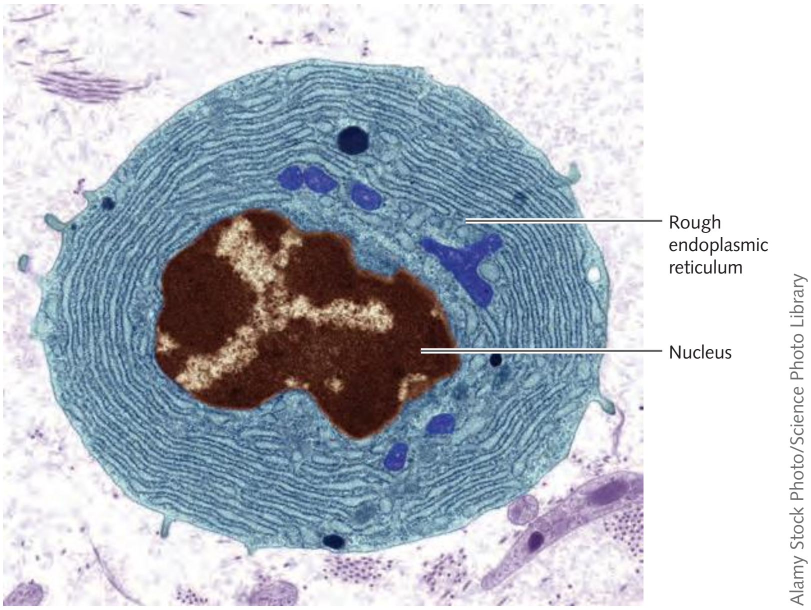


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Figure 7.13 A transmission electron micrograph of a plasma cell. There is extensive rough endoplasmic reticulum to allow for the production of antibodies.

The effector B cells that are produced after clonal selection of activated B cells are known as plasma cells. Plasma cells have differentiated to become highly specialised for antibody production (Figure 7.13), secreting up to 10 000 molecules of a specific antibody per second into the circulation. These antibodies generally provide protection for up to 28 days but the plasma cells that secrete them can last for years and even decades. Most antibodies will only attack one antigen, but a few will attack several different antigens if they are closely related and have similar structures, such as antigens located on the smallpox and cowpox viruses.

Memory B cells can persist within the body for months or even years, possibly almost a lifetime, not secreting antibodies but still carrying them on their plasma membrane. In this way, they can recognise the same antigen quickly should it reinvade the body of the host. Once activated by the specific antigen, the memory B cells rapidly divide and form plasma cells that produce large quantities of antibody, often attacking the pathogen before any symptoms of its presence arise. Memory B cells do not require T cell help to become activated and differentiate into plasma cells.

Figure 7.14 shows the speed of antibody production after initial and subsequent exposure to an antigen; for example, Rubulavirus, which causes mumps.

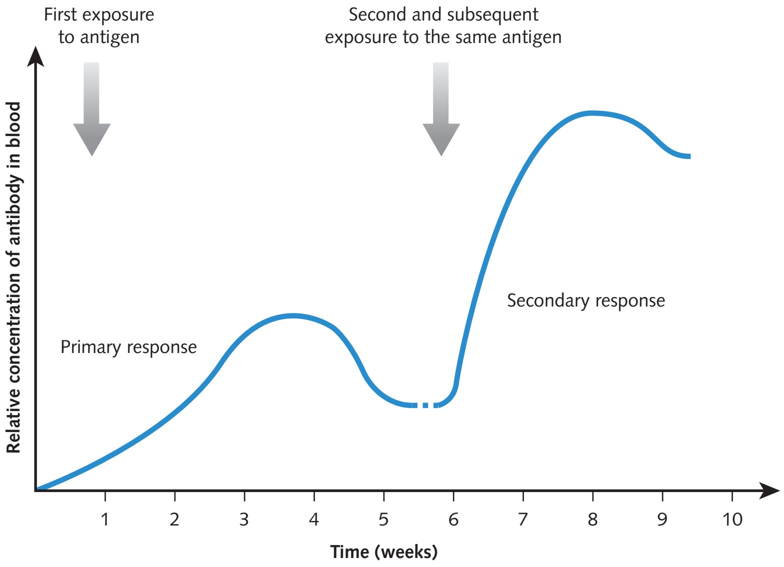

Figure 7.14 Antibody levels after an initial (primary) infection by an antigen and after a second exposure to the same antigen

When first exposed to the virus, the body produces antibodies, but there is a delay before enough are produced to neutralise the virus. This is why unvaccinated people develop symptoms of mumps when first exposed to the virus. When that person is later exposed to the virus again, memory cells recognising the virus quickly divide and form plasma cells, which produce antibodies that neutralise the virus while it is still in circulation before it can enter its target cells. You can see that the **secondary response** is faster (with a steeper response curve) and bigger than the **primary response** to that same antigen. This is why people are vaccinated against mumps. After an initial response to the vaccine, they become immune to future infections. Vaccination programs will be discussed further in Chapter 8.

KEY CONCEPTS

» Antibodies are present on the surface of B cells as the B cell receptors are secreted into circulation by differentiated B cells called plasma cells.

» Antibodies function by attaching to a specific antigen on the surface of a pathogen which is then destroyed through agglutination, opsonisation, neutralisation and complement activation.

» Antibodies exist in different forms that have different specialised functions.

» For B cell activation to occur, the same antigen must activate T_H cells, allowing them to provide contact-dependent signalling for B cells and secrete cytokines that stimulate B cell survival, activation and proliferation.

Concept questions 7.2

1. a What type of compound is an antibody?
   b Why might there be a constant region and a variable region in an antibody?
   c Identify the binding sites for antigens on antibodies.
2. Antigen–lymphocyte binding is often described as lock and key. What does this mean?
3. Complement cascade, opsonisation, neutralisation and agglutination are four modes of antibody action. Outline your understanding of each mode.
4. Plasma B cells have an extensive rough endoplasmic reticulum, and many Golgi apparatuses and mitochondria. Relate the structure of plasma cells to their function.
5. B cell activation involves three main steps before clonal selection. What are these, and what is involved in each step?
6. How do primary and secondary immune responses differ? Explain the difference in terms of antibodies.

HOT Challenge

7. Are plasma cells effector cells? If so, why and what do they do?

7.3 Cell-mediated immunity

WB
7.3.1
CELL-MEDIATED RESPONSE
PAGE 153

The response that results from the T_H cell detecting the antigen as presented on the MHC class 1 marker is **cell-mediated immunity**. The response involves the direct killing of virally infected and cancerous cells by T_C cells. Like B cells, T_C cells are able to distinguish self from non-self because of the various membrane-bound receptors, T cell receptors, that interact with antigens. You have already learned that T cells do not bind with antigens directly but bind with the antigens presented on the MHC proteins. MHC class I present antigens from inside the cell and thus flag virally infected or cancer cells.

Some viruses have evolved mechanisms to stop or reduce the expression of MHC class I on infected cells. This prevents T_C cells from recognising the infected cells as virally infected, allowing the virus to avoid destruction and continue to divide. In response, natural killer cells have evolved to destroy cells that have low levels of MHC class I on the surface. This is a clear example of how the immune system has influenced the evolution of pathogens and vice versa.

Like B cells, activated T_C cells (with the help of contact-dependent signals and cytokines from T_H cells) proliferate by dividing many times to form an army of clones. Some of these clones become effector cells, while others remain as memory T_C cells and migrate in the lymph fluid and to the lymph nodes where they can be activated quickly upon a second encounter with the same pathogen.

T_C cells are highly effective killers; they can eliminate infected body cells or tumour cells by releasing powerful cytotoxins directly into the cell when they contact a cell that carries an unrecognised antigen (Figures 7.15 and 7.16). The cytotoxins, just as in natural killer cells, include the proteins perforin and granzymes, which work together to induce apoptosis in the target cell. Perforins can form pores in the target plasma membrane, and granzymes can enter through these pores and directly activate the process of apoptosis.

EXAM TIP

If you are asked to compare, you need to discuss both similarities and differences. For example, humoral and cell-mediated responses both involve lymphocytes, but a humoral response involves B cells producing antibodies, whereas a cell-mediated response involves cytotoxic T cells killing infected cells.

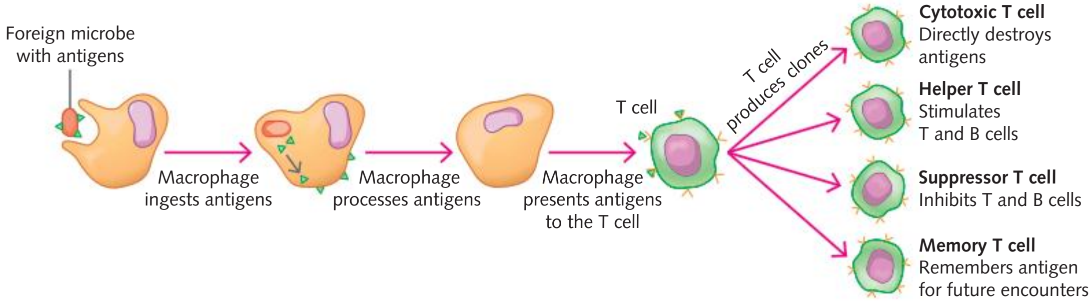

Figure 7.15 **A cell-mediated response to a viral pathogen. This response will occur at the site of the infected cell.**

The memory of cell-mediated immune responses can be demonstrated in experiments that use skin transplants (known as grafts) in mice. If a mouse is given a skin graft from a non-identical mouse, the graft will be rejected after about 14 days. If that same mouse later receives a second graft from the same donor mouse, the rejection only takes 4–5 days (Figure 7.17). This is because memory T_C cells formed after the first graft respond more rapidly when they encounter the foreign graft a second time.

T_C cells are the primary cause of transplant tissue rejection because they destroy the transplanted cells directly. Thus, patients receiving transplants must take high levels of immunosuppressant drugs to help counteract this response so that the new organ is not destroyed by the immune system. This is also the reason for HLA matching of organ transplant donors to recipients: HLA is the name of the human MHC markers, and the more closely related the donor's HLA markers are to the recipient's markers, the less likely it is that the T_C cells from each of the donor and recipient's immune systems will recognise the cells of the other as being foreign.

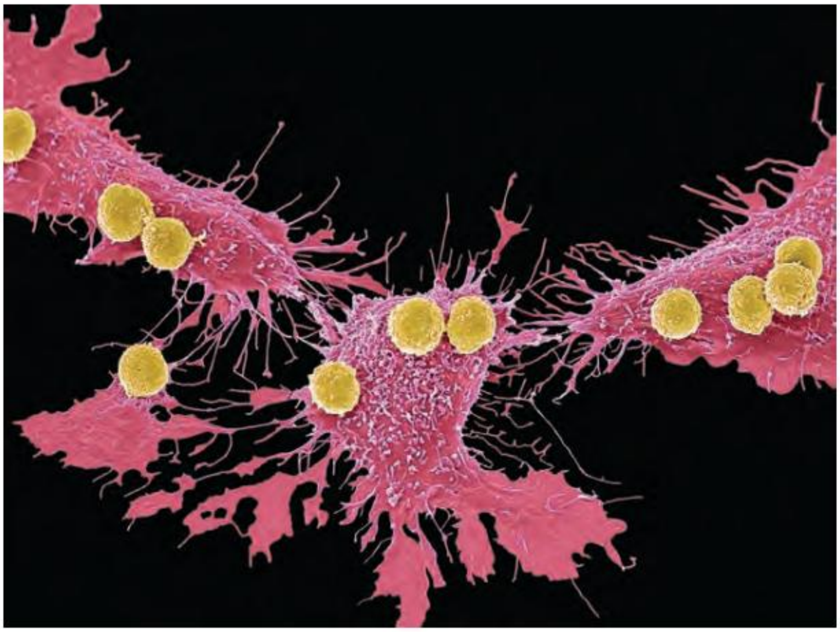

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Figure 7.16 **A scanning electron micrograph showing T_C cells (red) attacking a cancer cells (yellow)**

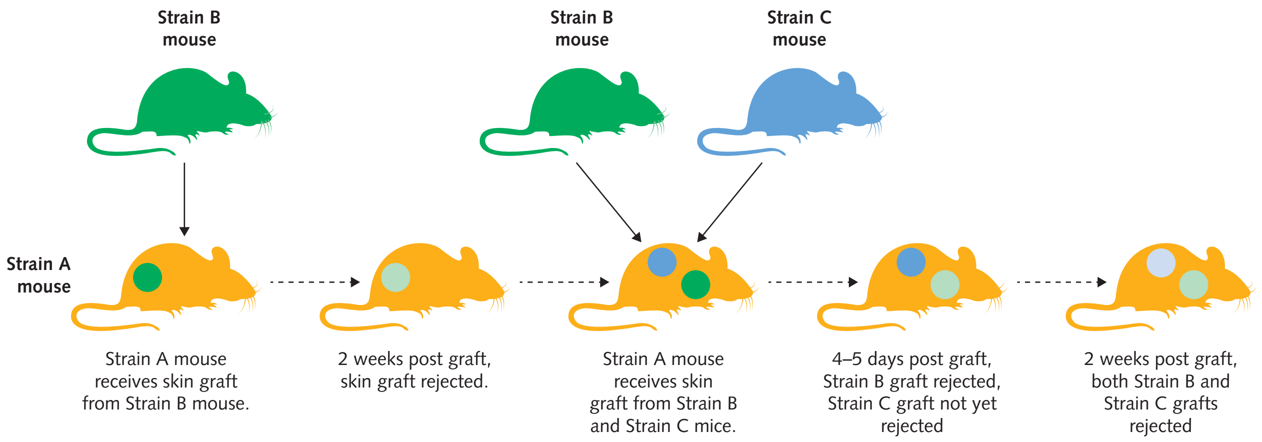

Figure 7.17 A graft-rejection experiment demonstrating that cell-mediated immunity displays memory

EXAM TIP

It is not a case of one type of pathogen causing a cytotoxic T cell response and another causing a B cell response. In most situations, both responses are initiated. A useful way of distinguishing their actions is that the humoral immune response is most effective against bloodborne antigens, whereas the cell-mediated response is most effective when the antigen is in the cell. Activation and participation by T_H cells ensures that each type of response is successful and carefully managed.

WB 7.3.2 ADAPTIVE IMMUNE RESPONSES PAGE 154

Humoral and cell-mediated responses work together

The immune system is a complex network of cells that rely on one another to function properly.

B and T cells share a number of features, which are summarised in Table 7.3. They both have a system for generating a diverse range of receptors for different antigens, and they rely on clonal selection to allow for proliferation of relevant clones. Both B and T cells form effector and memory cells.

Table 7.3 Three major groups of lymphocytes: B cells, T_H cells and T_C cells

	B cells	T_H cells	T_C cells
Development of self-tolerance	Occur in bone marrow	Occur in thymus	Occur in thymus
Antigen recognition	Recognise antigens not presented in MHC	Recognise antigen in MHC class II	Recognise antigen in MHC class I
Undergo clonal selection	Yes	Yes	Yes
Effector functions	Plasma cells produce antibodies, which attach to the antigen and mark it for destruction	Production of cytokines to aid B cell, T_C cell and macrophage functions	Releases cytotoxins directly into infected cell which induces apoptosis destroying infected cell and infecting virus
Formation of memory cells	Yes	Yes	Yes

The adaptive immune functions of lymphocytes are distinguished from the cells of the innate immune system by specific recognition of antigens and the ability to exhibit memory. The adaptive and innate systems are closely interlinked and do not operate in isolation. Communication between the cells of these systems is critical for the functioning of both. The following list and Figure 7.18 summarise some of the major connections.

» Antigen presentation by macrophages and dendritic cells allows T cells and B cells to recognise antigens.
» Full activity of T cells and B cells requires cytokine production by APCs that have recognised a PAMP or a DAMP.

- The binding of antibodies to pathogens can activate complement directly and promote phagocytosis by cells of the innate immune system.
- Phagocytosis is also promoted by cytokines produced by T_H cells.
- Following the destruction of cells by T_C cells, phagocytes play a role in 'cleaning up' the cell fragments produced.
- T_C cells release cytokines that promote destruction of phagocytosed antigens.

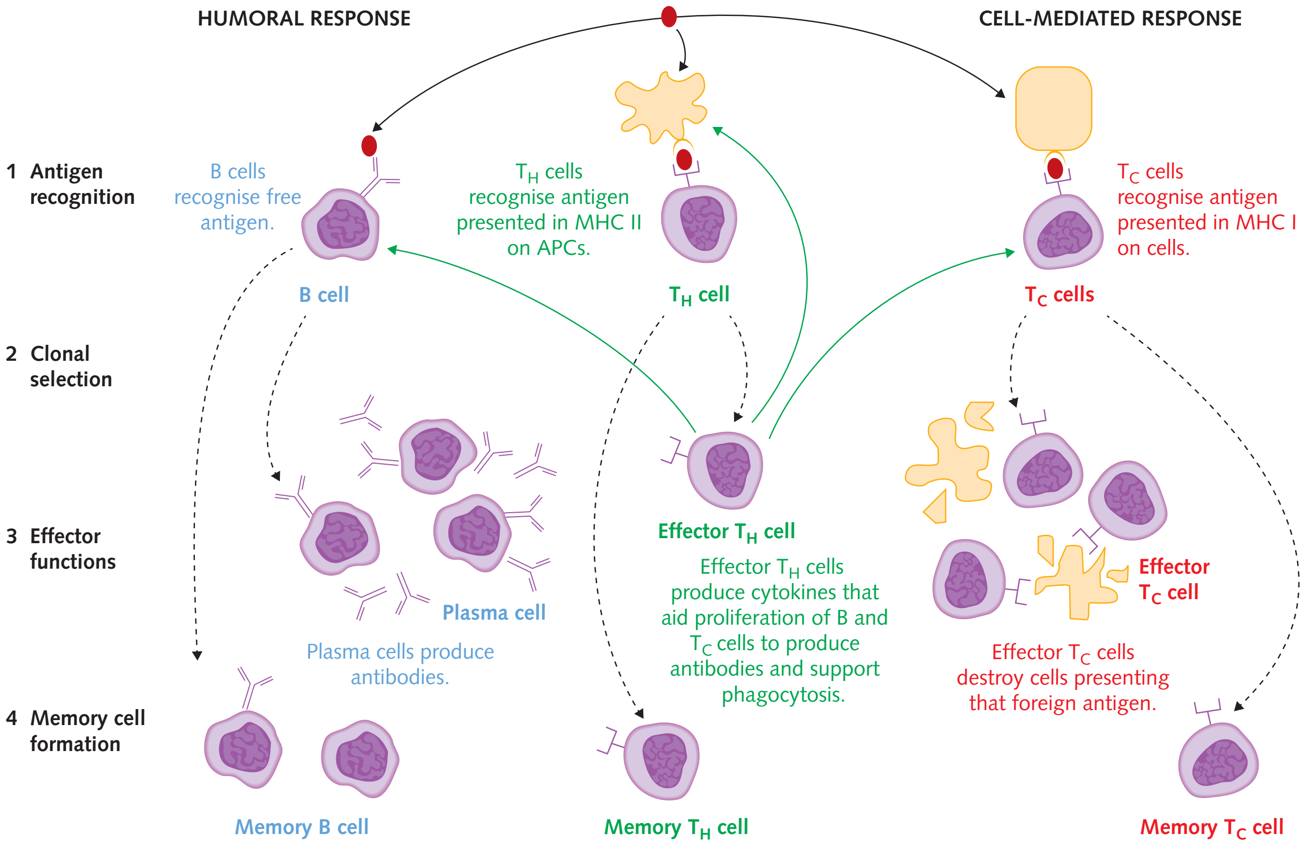

Figure 7.18 A summary of the actions and functions of the cells of the adaptive immune system

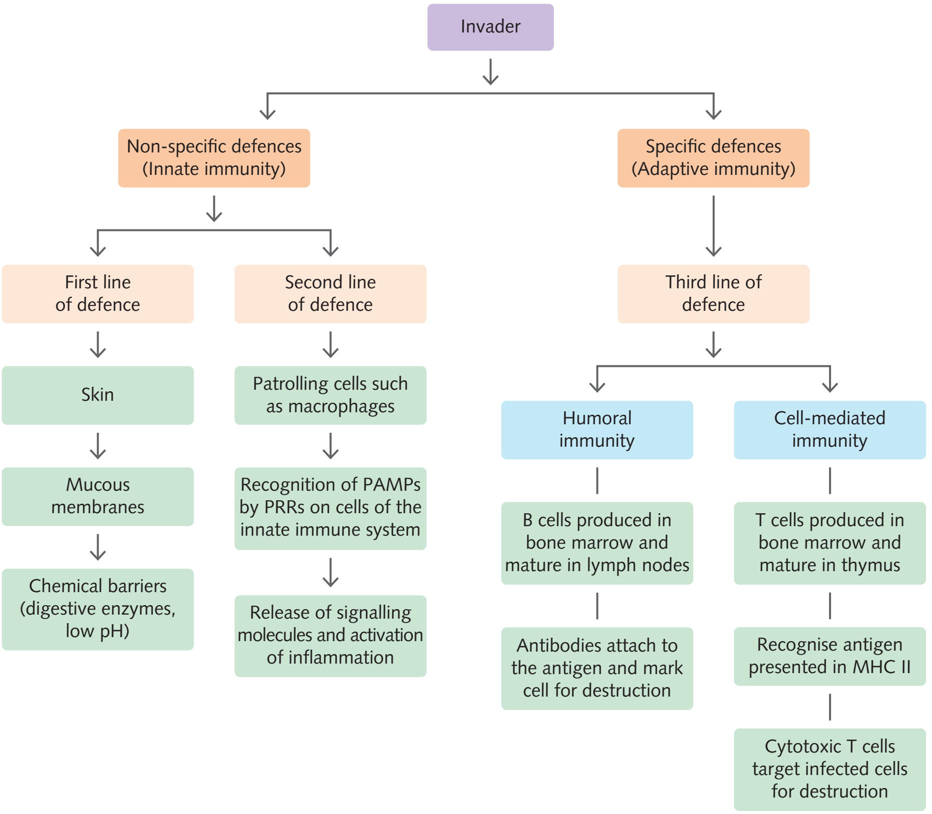

Figure 7.19 The three lines of defence in the human immune system

ACTIVITY 7.1

Adaptive immune analogies

Aim

To develop a set of analogies for different parts of the immune system

You will need

- Pen
- Paper

What to do

1 Working with a partner, brainstorm an analogy for each of the parts of the immune system listed below. Be creative and try to think of unusual ideas. For example, an analogy for 'vaccine' is: 'A vaccine is like a trial exam. In a trial exam, exposure to questions trains a student to perform better on the real exam. Similarly, exposure to an antigen in a vaccine trains the body to respond more rapidly and effectively to the real antigen.'

- Antibody
- APC
- T_H cell
- T_C cell
- MHC class I molecule
- MHC class II molecule
- Phagocyte
- Vaccine
- Plasma cell
- Cytokine
- Lymphocyte receptor

2 Discuss your lists in groups of three or four. How well does each analogy work? Are there limitations? Decide among the group on the analogy that best fits each term.

3 Present your group's list to the rest of the class. You could vote and have a prize for the best analogy.

What did you discover?

Reflect on whether these analogies have helped your understanding of the adaptive immune system. Make a list of things that have become clearer as a result of this exercise.

KEY CONCEPTS

- T_C cells scan peptides presented on MHC class I molecules. When they detect foreign peptides, with the help of signals from T_H cells, they secrete granzymes and perforin to kill the affected target cell.
- T_H cells scan peptides presented on MHC class II molecules. They express contact-dependent signalling molecules and cytokines to help activate T_C cells and B cells.
- The innate and adaptive immune systems are interlinked with many connections that are essential for proper immune responses.

Concept questions 7.3

1 Which of the following statements are true for T_H cells and which are true for T_C cells?

  a Recognise antigens presented by MHC class I molecules

  b Undergo clonal selection

  c Destroy cells by producing cytotoxic proteins

2 Describe how the roles of natural killer cells and T_C cells are different.

3 The adaptive immune system is often described as having memory. Explain what this means, using T_C cells as an example.

4 List three ways in which the innate and adaptive immune systems communicate.

5 Explain how T_H cells help many other cells of the immune system fight off invaders and mount an immune response.

HOT Challenge

6 T_{reg} cells initiate responses within the immune response to maintain homeostasis. T_{reg} cells used to be known as suppressor cells. The mechanism of action is not well understood and is an area of active research, especially in the fight against cancer. But the outcome of their action controls the magnitude and duration of the immune response. The following list is of some of the known functions of T_{reg} cells. Choose two and determine why these functions might be useful in homeostasis and at what stages of the immune response they might act.

- Production of inhibitory cytokines
- Induction of various cell types to synthesise interleukin-10
- Production of granzyme B, which induces apoptosis of effector cells
- Reverse signalling through direct contact with dendritic cells
- Signalling through the production of immunosuppressive adenosine

7.4 Active and passive immunity

WB
7.4 ACTIVE AND PASSIVE IMMUNITY
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When the body is infected by a pathogen or stimulated with a vaccination, the memory T and B cells produced will be activated rapidly if that antigen is encountered again. This kind of immunity, which produces memory cells, is known as **active immunity** and generally lasts many years, although the immune system may need booster shots periodically to enhance its army of memory cells.

Passive immunity occurs when antibodies are provided from an external source (Table 7.4). These externally sourced antibodies will provide protection from the pathogen, but only for as long as those antibodies last. Because there are no plasma cells or memory B or T cells, the person will not be immune if they encounter the pathogen again.

Table 7.4 Examples of active and passive immunity

	Active immunity	Passive immunity
Natural	Exposure to a pathogen, resulting in production of antibodies and B and T memory cells by the individual's own immune system.	Transfer of antibodies from mother to foetus through the placenta Transfer of antibodies from mother to baby through breast milk
Artificial	Vaccination, resulting in the production of antibodies and B and T memory cells by the individual's own immune system.	Anti-venom containing antibodies Antibodies against particular pathogens (e.g. rabies) Mix of antibodies for immunodeficiency

CONNECT

Vaccination is discussed in more detail in Chapter 8.

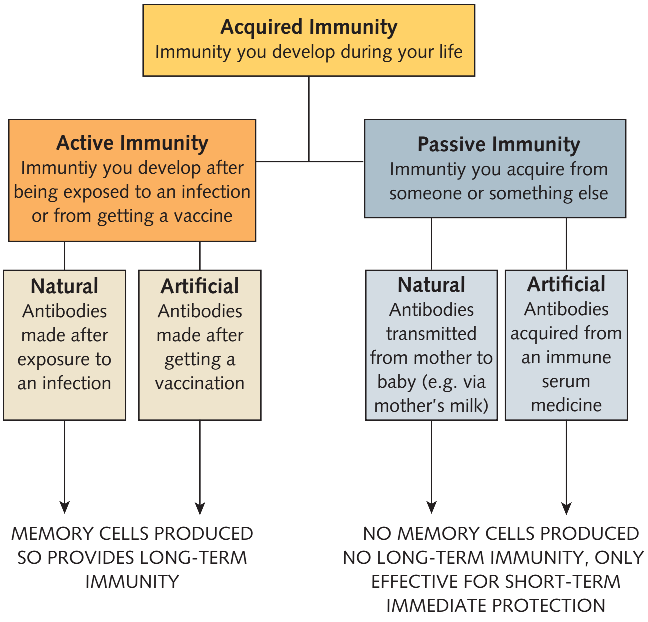

Figure 7.20 Summary diagram of acquired immunity

Natural and artificial active immunity

Natural active immunity develops when an organism comes into contact with a pathogen and develops memory B and memory T cells to the antigens of that pathogen or its products. In the development of natural immunity, the contact with the pathogen occurs naturally. This is the typical way humans gain immunity to pathogenic organisms. Antibodies are made after exposure to infection, giving long-term immunity.

Artificial active immunity develops after a vaccination. Vaccinations introduce antigens (weakened or dead microbes or their fragments). The body produces specialised lymphocytes (B plasma cells) and antibodies. Memory B cells are produced. This type of immunity is long term because of the production of memory B cells.

Natural and artificial passive immunity

With natural passive immunity, the person does not produce their own antibodies, but receives them from another source. This means they have no memory B cells; hence, it is only short-term immunity. Natural passive immunity occurs when antibodies pass from a mother to the foetus through the placenta and to the baby during breastfeeding. These antibodies, which are mainly IgA antibodies, are essential for protecting a newborn or very young baby from pathogens. A baby is most vulnerable to infection two or three months after birth because its own immune system is not yet fully developed and the antibodies it received from its mother through the placenta have disappeared.

Artificial passive immunity also occurs when antibodies come from another source. In some cases, there is insufficient time for antibodies to be produced actively by the patient before death or serious injury occurs. In such instances, a dose of antibodies targeted to a specific antigen is administered directly to the patient. For example, the anti-venom given after a snake or spider bite is a solution of antibodies against the venom.

Solutions of antibodies can also be used to prevent the development of disease in someone who has been exposed to a pathogen. Rabies is a viral disease that is spread in the saliva of infected animals. Untreated, rabies is always fatal once symptoms start because the immune system cannot produce a response quickly enough. However, the development of symptoms can be prevented by quickly administering antibodies against the rabies virus if somebody has been bitten by an infected animal.

Rarely, people are born with or develop a condition in which they cannot produce enough of their own antibodies. As a result, these people are highly susceptible to infections. A condition where the immune system does not function properly is called an immunodeficiency. A way of treating this type of immunodeficiency is to give the patient a mix of antibodies from healthy donors. This treatment is called IVIG (intravenous immunoglobulin). This will only provide protection for a short time, so these patients will need antibody infusions every month or so. This type of immunity is short term.

Preparing purified antibodies

Despite having many of the world's most venomous snakes, Australia has few deaths from snake bite. This is partly due to the availability of anti-venom treatment. Anti-venom is a solution of antibodies that are targeted against the venom.

In order to use antibodies for anti-venom or to protect against disease, solutions of antibodies need to be produced. One way to prepare a pure sample is to initially inject the specific antigen into a host such as a rabbit or a horse. This induces the animal to produce antibodies, which are secreted into their bloodstream. These are then extracted for use (Figure 7.21). This process is costly and time-consuming, and the purification of the sample is difficult.

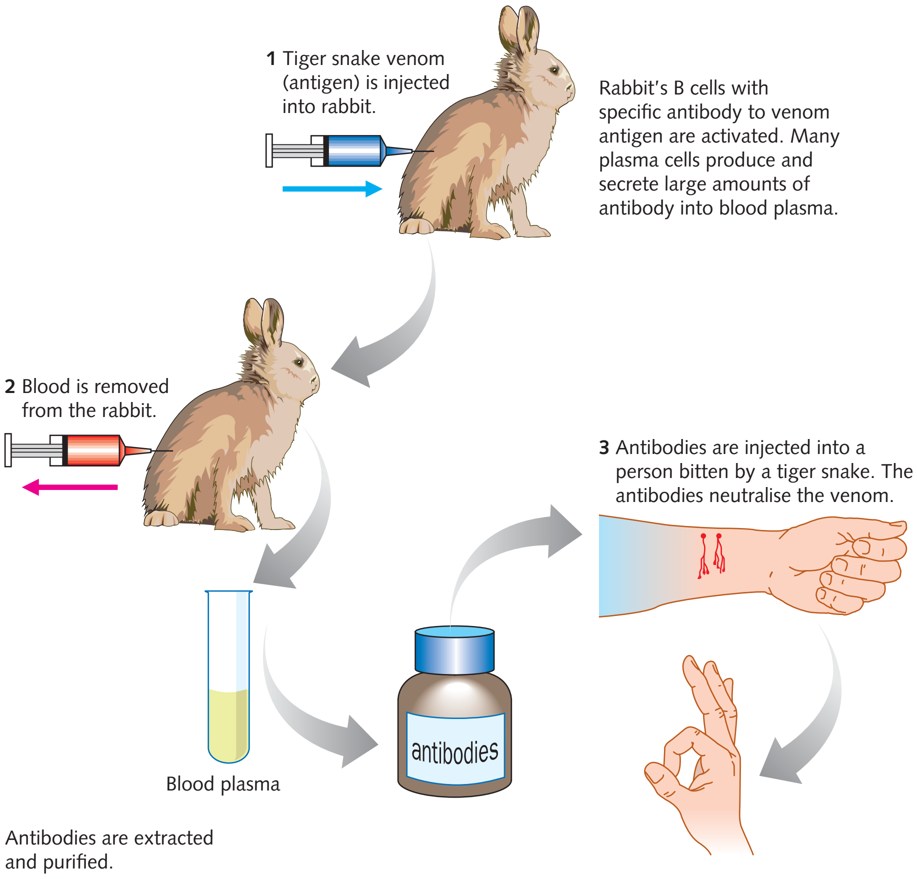

Figure 7.21 Anti-venom for a tiger snake bite can be produced by collecting antibodies from a rabbit that has been injected with small amounts of venom.

KEY CONCEPTS

- The protection provided by antibodies may be passive or active. Only active immunity provides long-term protection against pathogens.
- Examples of passive immunity include transfer of antibodies from mother to baby and anti-venom to treat snakebite.
- Natural immunity is when the body produces its own antibodies or they are passed to a baby through the placenta or breast milk.
- Artificial immunity is when vaccination occurs or antibodies created in another animal are injected into the body.

Concept questions 7.4

1 Describe how active immunity is acquired through vaccination.
2 Explain why breast milk can confer advantages to a baby's immune system that baby formula cannot.
3 Discuss why the production of anti-venom is usually costly.
4 Outline why passive immunity lasts only about 28 days.
5 Why do infections such as rabies need treating with antibodies from another source rather than relying solely on natural active immune responses?

HOT Challenge

6 IgA antibodies in adult mammals protect the internal surfaces of the digestive system, including the mouth, stomach and intestines, and the surfaces of the lungs. IgA antibodies are passed from mother to child.
   a How are they passed?
   b What type of immunity is this?
   c What aspects of this process protect newborns?
   d Does this process confer lifelong immunity?
   e After birth, maintenance of homeostasis in the gut depends on healthy interactions between commensal gut microbiota and the immune system of the individual. Research whether IgA antibodies in breastmilk from the mother initiate lifelong intestinal homeostasis.

BRANCHING OUT

The following press release was released on 29 January 2020.

Doherty Institute scientists first to grow and share Wuhan coronavirus

Scientists from The Peter Doherty Institute for Infection and Immunity (Doherty Institute) in Melbourne have successfully grown the Wuhan coronavirus from a patient sample, which will provide expert international laboratories with crucial information to help combat the virus.

This is the first time the virus has been grown in cell culture outside of China.

The Royal Melbourne Hospital's Dr Julian Druce, Virus Identification Laboratory Head at the Doherty Institute, said this was a significant breakthrough as it will allow accurate investigation and diagnosis of the virus globally.

'Chinese officials released the genome sequence of this novel coronavirus, which is helpful for diagnosis; however, having the real virus means we now have the ability to actually validate and verify all test methods, and compare their sensitivities and specificities – it will be a game changer for diagnosis,' Dr Druce said.

'The virus will be used as positive control material for the Australian network of public health laboratories, and also shipped to expert laboratories working closely with the World Health Organization (WHO) in Europe.'

Dr Mike Catton, Deputy Director of the Doherty Institute, said that possession of a virus isolate extended what could be achieved with molecular technology in the fight against this virus.

The Doherty Institute-grown virus is expected to be used to generate an antibody test, which allows detection of the virus in patients who haven't displayed symptoms and were therefore unaware they had the virus.

'An antibody test will enable us to retrospectively test suspected patients so we can gather a more accurate picture of how widespread the virus is, and consequently, among other things, the true mortality rate,' said Dr Catton.

'It will also assist in the assessment of effectiveness of trial vaccines.'

The virus was grown from a patient sample that arrived at the Royal Melbourne Hospital's Victorian Infectious Diseases Reference Laboratory (VIDRL) at the Doherty Institute on Friday, 24 January.

'We've planned for an incident like this for many, many years and that's really why we were able to get an answer so quickly,' said Dr Catton.

Dr Catton also credited the success to Australia's network of laboratories and public health authorities effectively working together.

'We are very pleased at how it has come together and are glad we were able to respond quickly, which we will continue to do.'

The Peter Doherty Institute for Infection and Immunity

Questions

1 Using this press release as an example, comment on the international cooperation that is necessary in science.
2 Explain why the virus had to be grown in cell culture. You may need to refer to Chapter 6 to help you answer.
3 What did Dr Druce mean when he said '… having the real virus means we now have the ability to actually validate and verify all test methods, and compare their sensitivities and specificities …'?
4 'The virus will be used as positive control material …' What does this statement mean?
5 The grown virus will be used to generate an antibody test. Using your knowledge of viral infection and antibodies, explain what this means.

Online key concepts
Chapter 7: Summary of key concepts

7 Summary of key concepts

7.1 Adaptive immune response – third line of defence

KEY CONCEPTS

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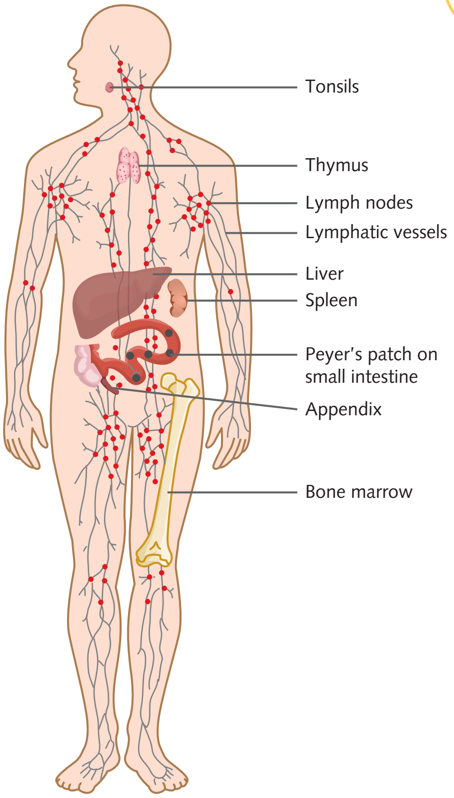


Figure 7.3 The location of organs and tissues involved in the lymphatic system in the human body

- All blood cells are produced from stem cells in the bone marrow. Some leukocytes reside in lymphoid organs and others circulate in the blood and lymphatic system. Others are resident in the tissues.
- Primary lymphoid organs include the bone marrow and thymus, where cells of the immune system are produced and mature.
- Secondary lymphoid organs include lymph nodes, spleen and mucosal-associated lymphoid tissue (MALT), where immune responses are initiated and carried out.
- The lymphoid system, including the secondary lymphoid tissues, is a transport system linking innate and adaptive immune responses.
- The adaptive immune response differs from the innate immune response because it has specific recognition of antigens and displays memory.
- B cells, T_H cells and T_C cells are the main players of the adaptive immune system. The adaptive immune response relies on these cells detecting foreign antigens and distinguishing them from self.
- T_{reg} cells are T cells that control the magnitude and duration of immune responses to limit damage to body tissues.
- Antigens are molecules that can generate an immune response. The particular molecular structures on antigens that are recognised by components of the immune system are called epitopes.
- The major histocompatibility complex (MHC) is an important way of distinguishing self from non-self.
- MHC restriction refers to the fact that T cells will only recognise epitopes when they are presented on an MHC molecule.
- MHC class I molecules present intracellular antigens and are present on all nucleated cells (self-markers).
- MHC class II molecules present extracellular antigens derived from phagocytosis and are present on antigen-presenting cells (macrophages, dendritic cells and B cells).
- Each B cell and T cell has an antigen receptor that is specific for a unique epitope, so the population of lymphocytes has the capacity to detect a vast range of antigens.
- Lymphocytes with antigen receptors that could recognise self-components are deleted or inactivated during their development.
- During infection, only the lymphocytes bearing a receptor that can recognise epitopes on the invading pathogen are activated and proliferate, in the process of clonal selection.

7.2 Humoral immunity

KEY CONCEPTS

p. 260

- Antibodies are present on the surface of B cells as the B cell receptors are secreted into circulation by differentiated B cells called plasma cells.
- Antibodies function through agglutination, opsonisation, neutralisation and complement activation.
- Antibodies exist in different forms that have different specialised functions.
- For B cell activation to occur, the same antigen must activate T_H cells, allowing them to provide contact-dependent signaling for B cells and secrete cytokines that stimulates B cell survival, activation and proliferation.

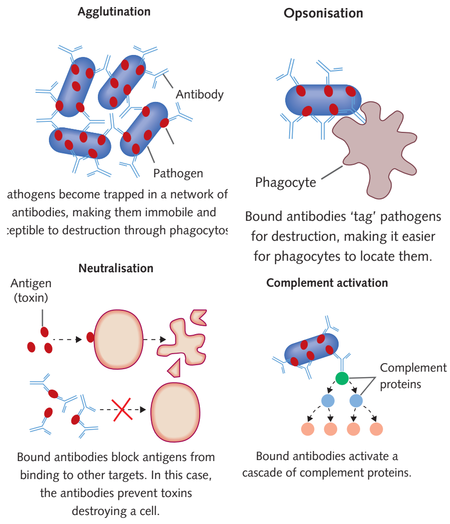


Figure 7.11 Antibodies can cause the destruction of pathogens in four ways: **a** agglutination, **b** opsonisation, **c** neutralisation and **d** complement activation.

7.3 Cell-mediated immunity

KEY CONCEPTS

p. 264

- T_C cells scan peptides presented on MHC class I molecules. When they detect foreign peptides, with the help of signals from T_H cells, they secrete granzymes and perforin to kill the affected target cell.
- T_H cells scan peptides presented on MHC class II molecules. They express contact-dependent signaling molecules and cytokines to help activate T_C cells and B cells.
- The innate and adaptive immune systems are interlinked with many connections that are essential for proper immune responses.

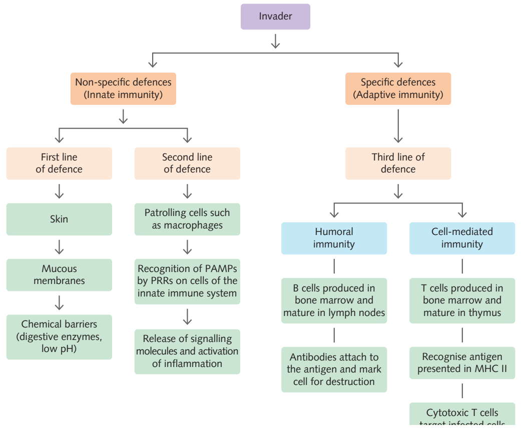


Figure 7.19 The three lines of defence in the human immune system

7.4 Active and passive immunity

KEY CONCEPTS

p. 270

- The protection provided by antibodies may be passive or active. Only active immunity provides long-term protection against pathogens.
- Examples of passive immunity include transfer of antibodies from mother to baby and anti-venom to treat snakebite.
- Natural immunity is when the body produces its own antibodies or they are passed to a baby through the placenta or breast milk.
- Artificial immunity is when vaccination occurs or antibodies created in another animal are injected into the body.

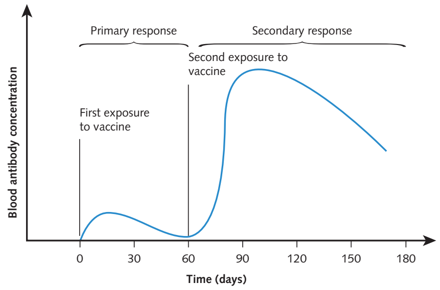


Figure 7.21 Response to vaccination against tetanus

WB
7.5.1
KEY TERMS
PAGE 158

7 Chapter glossary

active immunity when, after vaccination, memory cells are created that provide immunity against further exposure to antigens

agglutination when antigens or pathogens become stuck together because of antibody binding

antibody a Y-shaped protein that binds to foreign substances that invade the body; also called immunoglobulin

antigen-presenting cell (APC) a cell that displays peptides derived from processed antigens on major histocompatibility complex class II molecules for presentation to T_H cells; can be B cells, macrophages and dendritic cells

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

B cell a class of lymphocyte that, once activated, produces antibodies; also called a B lymphocyte

B cell receptor a surface-bound antibody that serves as a receptor so that B cells can detect antigens

B plasma cell a cell that originates in the bone marrow and produces large quantities of antibodies

bone marrow soft tissue found inside some bones that contains stem cells that produce cells of the immune system

cell-mediated immunity an immune response initiated by cells, which does not involve antibodies

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

cytotoxic T cell (T_C cell) a class of lymphocyte that destroys virally infected or cancerous cells by secreting proteins that result in the extrinsic pathway of apoptosis; also called a cytotoxic T lymphocyte

epitope a small part of a larger molecule that binds to a receptor site such as B cell receptors and T cell receptors

helper T cell (T_H cell) a lymphocyte that assists cytotoxic T cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling; also called a helper T lymphocyte

humoral immune response an adaptive immune response mediated by antibodies

immune having resistance to infection by a specific pathogen

immunoglobulin (Ig) a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called antibody

interleukin a subset of cytokines that assists with the coordination of cells involved in the immune response

interstitial fluid a fluid that lies between cells; also known as tissue fluid or extracellular fluid

lymph a colourless fluid that originates from tissue fluid

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes to present to lymphocytes and initiate an adaptive immune response

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and removing foreign matter

major histocompatibility complex (MHC) protein markers found on cell surfaces that are important in distinguishing self from non-self; MHC class I is found on all cells and MHC class II is found only on antigen-presenting cells

memory cell a long-lived lymphocyte capable of responding to a particular antigen when it is reintroduced; made from B cells and T cells

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

mucosal-associated lymphoid tissue (MALT) an extensive system of lymphoid tissue deposited all over the body; initiates immune responses along mucosal areas such as gastrointestinal tract, eyes and lungs

neutralisation the process by which antibodies prevent toxins from acting by binding to them and blocking them from binding to their targets

passive immunity immunity characterised by the transfer of antibodies from one individual to another; does not generate immunological memory

primary lymphoid organ the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

regulatory T cell (T_{reg} cell) a class of lymphocyte that helps to negatively regulate the immune response; also called a regulatory T lymphocyte

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and mucosal-associated lymphoid tissue

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self-antigens to prevent an immune response to these antigens

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

spleen an abdominal organ that stores white blood cells

T cell receptor a protein receptor found on the surface of T cells; binds to antigens presented on major histocompatibility complex proteins

thymus the gland in the upper chest where T cells mature

WB
7.5.2
EXAM PRACTICE
PAGE 159

7 Chapter review

Remembering

1 **a** Identify two functions of MHC proteins.
  b What is the name of MHC in humans?
  c Why is it important for T_C cells to recognise MHC I?
2 Recall where the following cell types undergo their development.
  a T cells
  b B cells
3 The ability to distinguish between self and non-self antigens is crucial to the functioning of the immune system.
  a Define 'self-antigen'.
  b Outline how leukocytes learn to distinguish between self and non-self antigens.
  c Discuss the problem that can occur if the immune system responds to self-antigens.
4 Draw a diagram comparing the amount and speed of antibody production in response to an antigen after the first and second exposures.
5 List the different ways that antibody binding can inhibit pathogens.
6 **a** Describe the role of T_H cells.
  b Identify how they are able to perform this function.
7 List three ways that dysfunction of the immune system can cause disease, giving specific examples.
8 Identify whether the following statements are true or false.
  a Immunodeficiency can be inherited or acquired.
  b An autoimmune disease is one where the immune system attacks the body's own cells.
  c People born without B cells can mount a fully functional adaptive immune response against a virus.
  d Vaccination against measles is an example of natural active immunity.

Understanding

9 Describe what is meant by clonal selection, using B cells as an example.
10 Millions of different antibodies can be made by our B cells, even though our genome has only about 30 000 genes. Explain how this can occur.
11 Present an argument as to why passive immunity does not display memory.
12 Draw a diagram to illustrate one way that antibodies can be produced for commercial uses.
13 Distinguish between:
  a natural and artificial active immunity
  b natural and artificial passive immunity.

Applying

14 Liver, heart and kidney transplants are fairly common surgical procedures. However, recipients of these transplants face the problem of rejection of these organs.
  a Explain why the immune system rejects these organs.
  b Transplant patients are usually prescribed immunosuppressant drugs to prevent transplant rejection. Many immunosuppressant drugs work by interfering with DNA synthesis.

    - i Suggest a negative effect that these drugs may have on the health of the patient.
    - ii Explain how a drug that interferes with DNA synthesis can prevent transplant rejection.
    - iii A patient with kidney failure was successfully 'cured' with a kidney transplant from his identical twin brother. He was concerned that the doctor did not prescribe immunosuppressant drugs. Are the patient's fears warranted? Justify your response.

15 The Australian death adder (*Acanthophis antarcticus*) has one of the most dangerous bites in the world. The active component of the venom is an alpha-neurotoxin that binds to the receptor sites for acetylcholine (a neurotransmitter molecule). Paralysis of muscles results and death can occur when the muscles of the diaphragm become paralysed and breathing is prevented.

- a What type of substance is acetylcholine?
- b Describe the function of acetylcholine at a cellular level.
- c Explain how the alpha-neurotoxin prevents acetylcholine from working.
- d Fortunately, an anti-venom is available that, if injected quickly enough, prevents the paralysis. Anti-venom is prepared by injecting tiny amounts of snake venom into a horse over a long period of time. The amounts of venom injected are so small that the horse is unaffected; however, there is a response by the horse's immune system.
    - i Name the substances the horse would produce to counteract the snake venom in its body.
    - ii Name the cells in the horse that would be responsible for the formation of this substance.
    - iii Explain why small amounts of venom are injected into the horse over a long period of time.
    - iv Outline the steps involved in the formation of these substances.
    - v After 10–12 months, blood is extracted from the horse and the plasma can be injected into snakebite victims. Identify the term given to the use of horse plasma as a treatment for snakebite.
    - vi Explain how this is effective in treating the snakebite victim.

16 Immune thrombocytopenic purpura (ITP) is an autoimmune disease in which platelet counts drop very low. Patients may develop bruising, rashes and, in extreme cases, severe internal bleeding. Antibodies against platelet surface markers can often be found in the bloodstream of patients with ITP.

- a Recall the role of platelets.
- b Explain how the formation of anti-platelet antibodies may lead to the symptoms described.
- c Platelets from blood donations can be given to patients as a transfusion. Predict whether or not these would be effective at preventing symptoms in patients with ITP. Justify your response.

17 Explain how the body's ability to distinguish between self and non-self is important in the development of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

18 HIV infection is caused by a viral pathogen.

- a What are the initial symptoms in the primary stage?
- b If left alone, a person can continue to be infectious as they move into a secondary stage. T_H cells are deleted from the immune response. What effect does this have on the adaptive T cell and B cell responses?
- c T_C cells remain robust in their response, and it was found that up to 19% of T_C cells in infected patients were specific for HIV. Yet, the virus continues to spread in the bloodstream. Why does the viral progression continue if there is a good response from cytotoxic T cells?
- d Sometimes a person might not realise they have the disease until it has progressed to AIDS. What does AIDS mean and how are opportunistic pathogens often involved that lead to the death of the individual rather than the HIV?

19 Figure 7.21 shows the response to two different doses of a vaccine against tetanus.

- **a** Explain the body's primary response.
- **b** Explain the trend shown following the second dose of antigen.
- **c** Copy the graph and add a second line that shows the expected response if the same person was exposed to a first dose of a vaccine against diphtheria at 60 days.

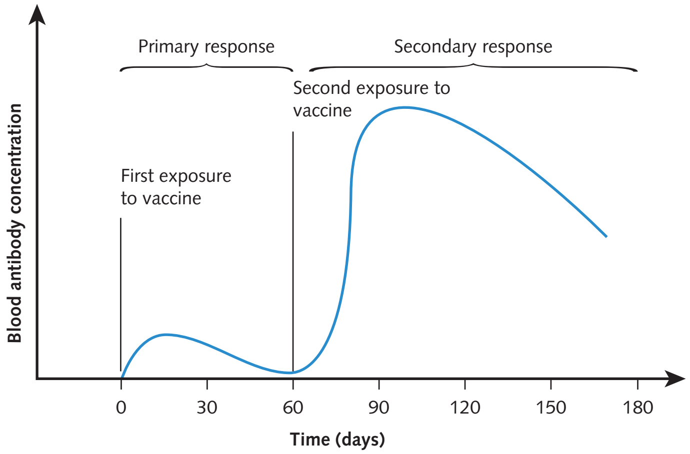


Figure 7.21 Response to vaccination against tetanus

Analysing

20 Compare the roles of the humoral and cell-mediated immune responses with regard to the type of pathogen targeted and how pathogen destruction is brought about.

21 Compare and contrast the MHC class I and MHC class II molecules.

22 Figure 7.22 is a graft-rejection experiment that builds on the one in Figure 7.17 on page 266. The aim of this experiment is to determine if the memory that the immune system exhibits with regard to graft rejection can be transferred between individuals.

- **a** Name the components of the immune system that are responsible for graft rejection.
- **b** Identify which part(s) of the blood (plasma, lymphocytes or both) would be expected to contain these components.

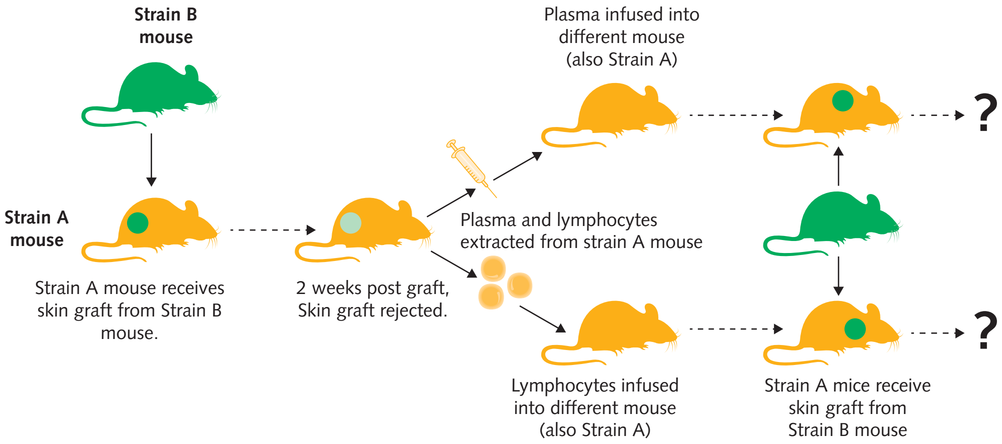


Figure 7.22 Experimental set-up to see if immune memory can be transferred between individuals

- **c** Predict how long it will take each mouse (the one that has received the plasma and the one that has received the lymphocyte infusions) to reject the skin graft. Explain your reasoning.
- **d** In this experiment, the infused lymphocytes are not rejected by the recipient's immune system. Explain why this is the case.

23 A mutation in a single gene on the X chromosome can prevent B cells from maturing. This causes the condition known as X-linked agammaglobulinaemia (XLA) in which patients produce extremely low levels of antibodies.

- **a** Predict whether XLA is an immunodeficiency or an autoimmune condition.
- **b** Draw a line graph to show the normal response to first and second exposure to a vaccine. Add a line to show the response to the same vaccine you would expect in somebody with XLA.

Evaluating

24 The adaptive immune system is sometimes described as more 'sophisticated' or 'important' than the innate immune system. Evaluate whether either or both of these adjectives is appropriate.

Creating

25 Draw a diagram that shows all the different defences encountered by an antigen, such as that associated with the influenza virus, when it enters the body. Indicate how these different defences communicate.

Disease challenges and strategies

8

By the end of this chapter you will have covered the following material.

Key knowledge

Disease challenges and strategies

- the emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples pp. 285–290
- scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission and measures to control transmission pp. 290–302
- vaccination programs and their role in maintaining herd immunity for a specific disease in a human population pp. 302–305
- the development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer pp. 306–310

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify independent, dependent and controlled variables in controlled experiments pp. 298–299; 300–301
- predict possible outcomes pp. 298–299; 300–301

Plan and conduct investigations

- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 298–299; 300–301

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 298–299; 300–301
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 298–299; 300–301
- demonstrate ethical conduct when undertaking and reporting investigations pp. 298–299; 300–301

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 298–299; 300–301
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 298–299; 300–301
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 298–299; 300–301

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 298–299; 300–301
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 298–299; 300–301
- identify outliers, and contradictory or provisional data pp. 300–301

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 300–301
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 300–301
- analyse and explain how models and theories are used to organise and understand observed phenomena and concepts related to biology, identifying limitations of selected models/theories p. 296

Online Chapter Map
Chapter 8 map

8

Disease challenges and strategies

Sometimes, new pathogens such as SARS-CoV-2 emerge and old pathogens re-emerge to challenge living things. These pathogens can travel around the world so fast that they can quickly become in pandemic proportions.

8.1 Emerging and re-emerging pathogens

p. 285

Sometimes the global connectedness works against the human population. Human mobility carries new and re-emerging pathogens around the world in just one plane journey. In this day and age, we can learn from history, particularly the effects of white colonisation and the diseases such as smallpox, scarlet fever and measles that colonists introduced to Aboriginal and Torres Strait Islander Peoples in Australia.

8.2 Strategies employed to control pathogen transmission

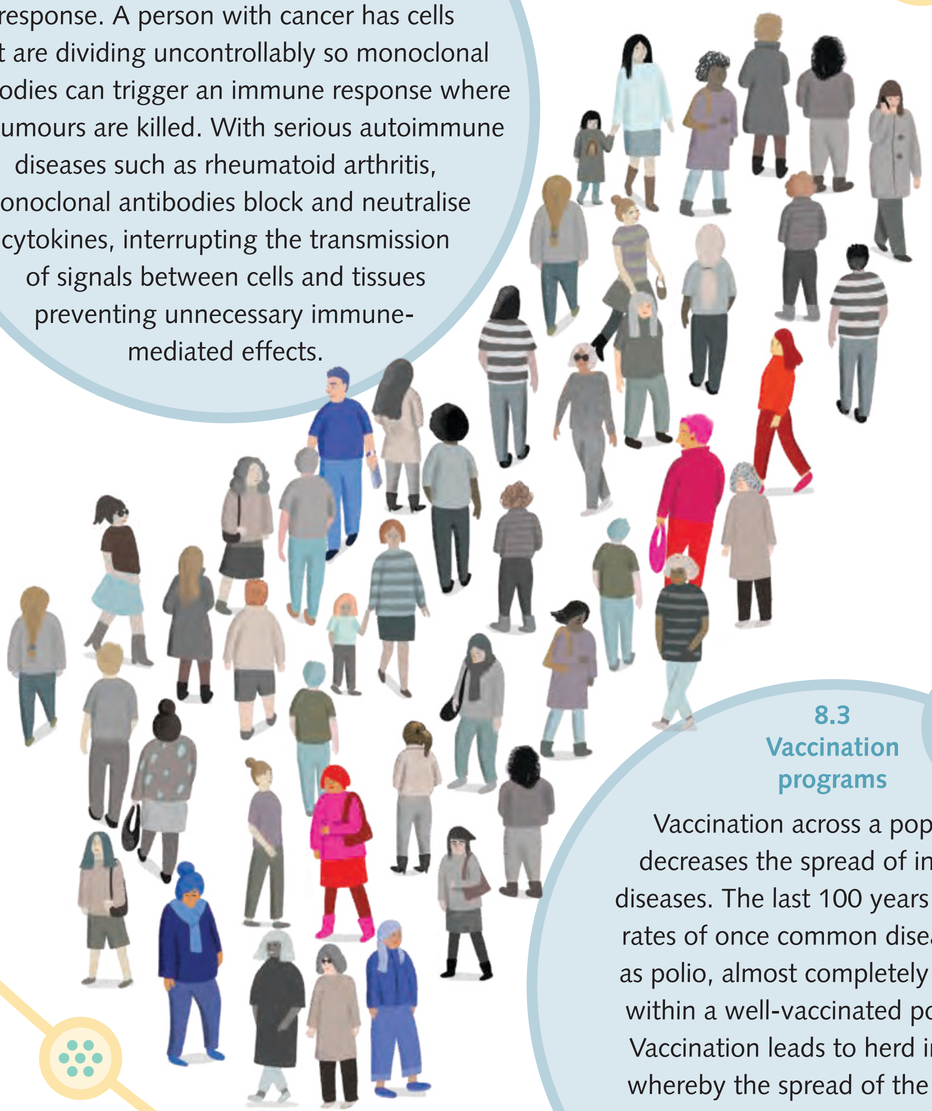
p. 290

In order to control a pathogen, we must understand how it is transmitted. Governments and health departments work together to control spread through a population and between populations. Cancelling public events, shutting schools and enforcing social distancing and quarantine are some of the ways to mitigate spread. Vigilant hygiene and health education are also key in changing behaviours to aid infection control.

8.4 Immunotherapy strategies

p. 306

Immunotherapy is designed to stimulate an individual's immune response. A person with cancer has cells that are dividing uncontrollably so monoclonal antibodies can trigger an immune response where the tumours are killed. With serious autoimmune diseases such as rheumatoid arthritis, monoclonal antibodies block and neutralise cytokines, interrupting the transmission of signals between cells and tissues preventing unnecessary immune-mediated effects.



8.3 Vaccination programs

p. 302

Vaccination across a population decreases the spread of infectious diseases. The last 100 years have seen rates of once common diseases, such as polio, almost completely disappear within a well-vaccinated population. Vaccination leads to herd immunity, whereby the spread of the infection is markedly reduced as since most people in the population have been vaccinated.

Understanding of new pathogens is increasing rapidly; so is our ability to react to them both on a social and scientific level. Communication with, and education of, the community concerned plays a large part in combatting new and re-emerging pathogens.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 8 map (p. 282)

Online Key Terms:
- Play and say (p. 284)

Weblinks:
- Use the interactive lab simulator to investigate the spread of disease (p. 296)
- The flu vaccine explained (p. 302)
- How does herd immunity work? (p. 303)
- Watch a video of T cells killing cancer cells (p. 306)

Online Worksheets:
- The flu vaccine explained (p. 302)
- How does herd immunity work? (p. 303)

Online Key Concepts:
- Chapter 8: Summary of key concepts (p. 314)

Online Key Terms
Chapter 8 Flashcards

Know your key terms

autoantibody
autoimmune disease
cancer
carrier
endemic
epidemic
herd immunity
hybridoma
immunisation
immunotherapy
infectivity
Koch's postulates
latent
monoclonal antibody
pandemic
primary host
quarantine
secondary host
sporadic
T-cell transfer therapy
tumour
vaccination
virulence
zoonotic

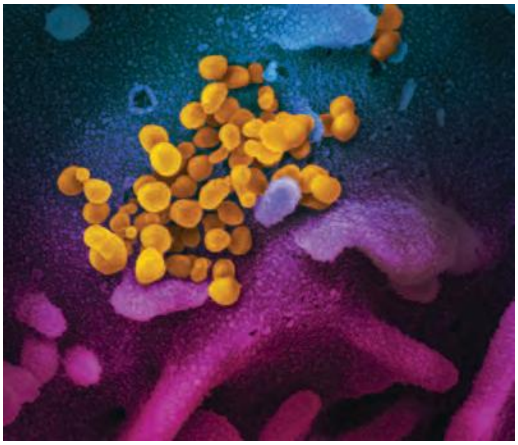
Remember

WB
REMEMBER
PAGE 162

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1. Diseases can be infectious or non-infectious.
2. Infectious diseases are caused by pathogens, which include viruses, bacteria and protozoa.
3. Antigens stimulate an immune reaction. Antibodies are produced in response to antigens.
4. Cytokines are cell signalling molecules that stimulate immune cells to move towards the site of infection or inflammation.

The term ‘coronavirus’ encompasses a large family of viruses that cause diseases, from the common cold to much more severe diseases such as SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome) and COVID-19 (coronavirus disease). Coronaviruses can be transmitted from animals to humans (**zoonotic**). SARS was found to be transmitted from bats to civet cats to humans, and MERS from dromedary camels to humans. It was later discovered that the viruses could also be transmitted from humans to humans. In December 2019, a novel coronavirus outbreak was detected in people who had been in a fish market in Wuhan, Hubei Province, China. It spread rapidly and led to shutdowns of whole cities, factories and airports, and travel bans from China in an effort to restrict contact with infected individuals. By the end of January 2020, deaths from coronavirus were recorded in the USA, Thailand and Japan, and cases were reported in the US, Nepal, France, Australia, Malaysia, Singapore, South Korea, Vietnam and Taiwan. By mid-February 2020, it had infected 45 171 people worldwide with more than 1360 deaths on mainland China alone. By end of February 2021, it had infected more than 114 million people, killed more than 2.5 million, and was continuing to spread uncontrollably around the world.



NIAID - National Institute of Allergy and Infectious Diseases https://www.niaid.nih.gov/news-events/novel-coronavirus-sarscov2-images CC BY-2.0 https://creativecommons.org/licenses/by/2.0/

Figure 8.1 Researchers at Rocky Mountains Laboratories in the United States imaged samples of the virus and cells taken from a US patient infected with SARS-CoV-2, the pathogen that causes the disease COVID-19, using two different kinds of high-resolution microscopes – a scanning electron microscope and a transmission electron microscope. The virus has been artificially coloured yellow, and patient cells are purple and blue.

8.1 Emerging and re-emerging pathogens

Diseases that are caused by pathogens are:

- **sporadic** if they are only seen infrequently and in a small number of people; for example, the occasional cases of rabies that occur in western USA when people are in contact with infected rats
- **endemic** if they are consistently found in certain regions; for example, malaria, which is endemic to tropical regions
- **epidemic** if there is a rapid spread of the disease across a number of countries and a large number of people are affected; for example, the Ebola outbreak in 2018, which spread across the Democratic Republic of the Congo and Uganda
- **pandemic** if pathogens become worldwide threats to health; for example, the spread of Spanish flu in 1918 with deaths estimated at around 50 million people (1% of the world’s population) and the COVID-19 pandemic starting in late 2019.

EXAM TIP
Make sure you know the difference between sporadic, endemic, epidemic and pandemic.

Emerging pathogens

Emerging pathogens are pathogens that were previously undetected, unrecognised or unknown; or they could be known pathogens that have spread to new locations. Emerging pathogens can be viruses, bacteria or protozoa. They cause infectious diseases in human populations and cause significant public health issues. Recently, emerging viral pathogens have caused significant deaths from diseases such as SARS, MERS, Ebola, chikungunya, swine influenza, avian influenza and COVID-19.

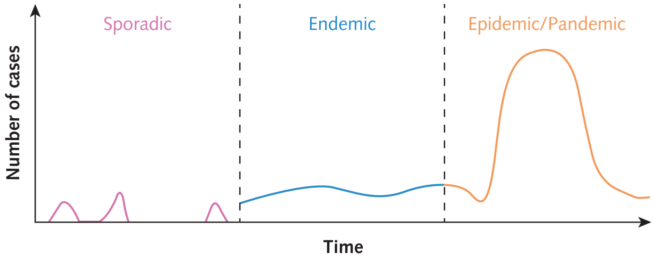
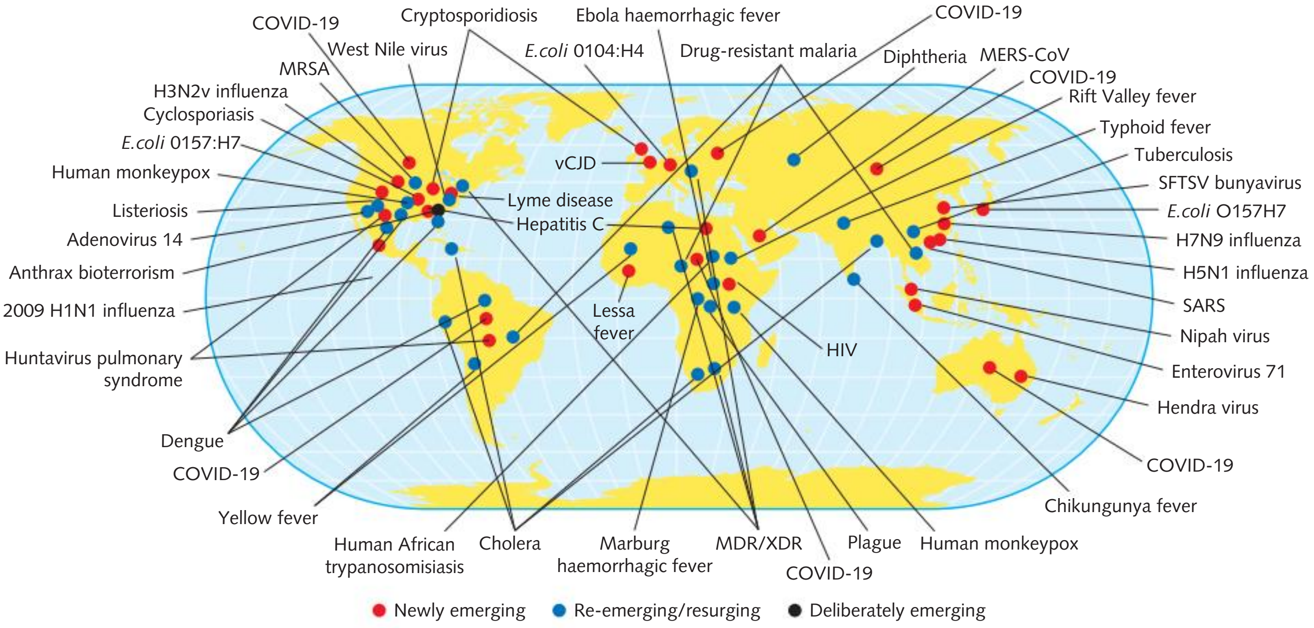


Figure 8.2 Within a population a disease may occur sporadically, at an endemic level, or at an epidemic/pandemic level.

Re-emerging pathogens

Re-emerging pathogens are pathogens that have caused disease in the past and are now reappearing. Since the number of infections has dropped, the impact of the disease on public health has diminished. The pathogen reappears and spreads into a population without immunity (naïve) and increases to epidemic proportions. Re-emerging pathogens cause diseases such as measles, pertussis, cholera, tuberculosis, malaria, gonorrhoea, syphilis, pneumococcal disease and influenza.



Courtesy Dr Anthony Fauci, NIH/NIAID

Figure 8.3 Examples of emerging and re-emerging pathogens causing infectious diseases around the world

Spread of pathogens in a globally connected world

WB
8.1.1
SPREAD OF PATHOGENS IN A GLOBALLY CONNECTED WORLD
PAGE 163

On 31 December 2019, Chinese authorities alerted the World Health Organization (WHO) to a number of cases of pneumonia. The cause of the pneumonia was unknown. All 44 patients were connected in some way to the Huanan seafood market in Wuhan, China. On 1 January 2020, the seafood market was closed down for environmental sanitation and disinfection. On 9 January 2020, the first death was reported in China. On 13 January 2020, the first case of COVID-19 was reported in Thailand. The Thai patient had no connection with the Huanan seafood market. A similar story was reported in Japan on 16 January 2020. By mid-February, the coronavirus SARS-CoV-2 had spread across the world (Figure 8.4).

Pathogens can be spread by close contact of animals with humans, or humans with humans. In an era of rapid global movement and increasing population numbers, it is inevitable that the number of such contacts will increase. People are far more mobile than they were even 50 years ago, with interstate and international travel possible in under 24 hours. Planes, trains, ships and cars now move people between towns, cities and even continents in a matter of hours. When the incubation period of many infectious diseases is longer than 24 hours, a person can travel between Melbourne and London before they start showing any symptoms. In an effort to curb the import of highly infectious pathogens, the Australian *Biosecurity Act 2015* compels the operator of an international aircraft or vessel to report any unwell passenger who shows signs and symptoms of an infectious disease.

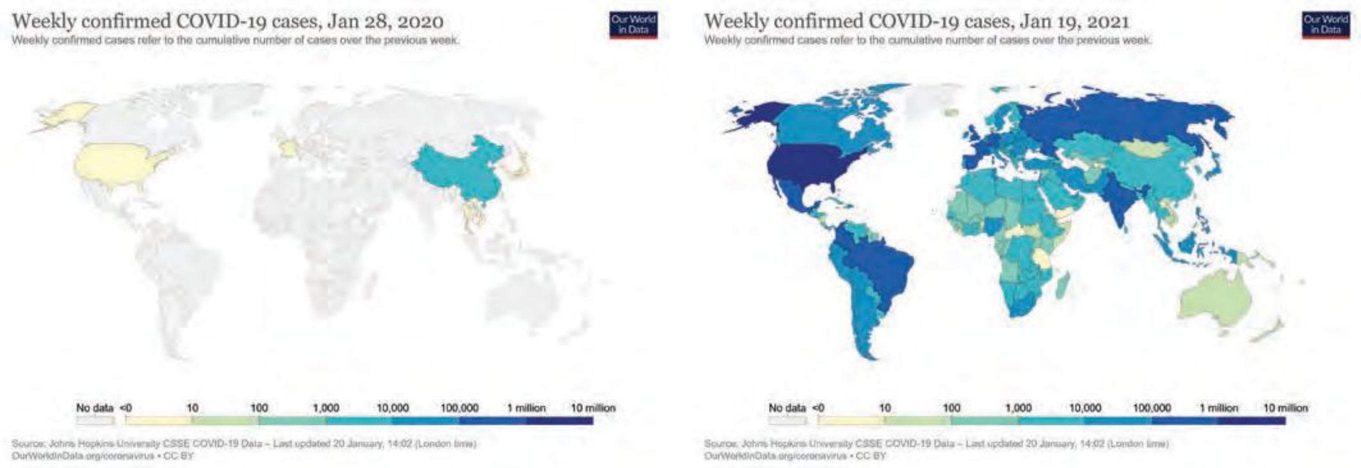


Figure 8.4 a **The number of reported cases of coronavirus (SARS-CoV-2) from Wuhan Province in China from 28 January 2020 to b across the world by 19 January 2021**

Our World in Data. World Map Jan 28, 2020 CC BY 4.0 https://creativecommons.org/licenses/by/4.0/;
Our World in Data. World Map Jan 19, 2021 CC BY 4.0 https://creativecommons.org/licenses/by/4.0/

The rapid spread of SARS-CoV-2, the pathogen that causes the disease COVID-19, was a major priority for decision makers during the outbreak in 2020. Countries such as Australia shut their borders initally to non-Australian citizens returning from China. This prevented hundreds of thousands of international students returning to Australia for the start of the academic year. Following this, Australia only allowed Australian citizens and a few exceptions to return, and also to undergo quarantine for two weeks upon arrival. The cruise ship *Diamond Princess* spent many weeks held under quarantine off the coast of Yokohama, Japan, after a passenger from Hong Kong travelled for five days on the ship before disembarking in Hong Kong and presenting with COVID-19 six days later. All on board the *Diamond Princess* were immediately tested for SARS-CoV-2 and 10 were found to be positive for the virus. Two days later, 41 new cases were reported, then after three more days, 66 new cases were confirmed. Three thousand passengers and crew aboard the *Diamond Princess* were forbidden to leave the ship and were mainly confined to their cabins in an effort to halt the spread of the disease among those on board and at the port where the ship would eventually dock. However, the effectiveness of the quarantine measures on board was questionable because almost 300 people on board had contracted the virus by mid-February 2020.

Alamy Stock Photo/EFE News Agency

Figure 8.5 **Efforts have been made to screen passengers arriving from international ports for symptoms of infectious disease.**

Although those on board were held in a situation of high risk, the virus was prevented from infecting people on land in Yokohoma. Arrival of a new infectious agent into a naïve population can have devastating consequences and is a pattern that has been repeated throughout the course of human history.

WB

8.1.2
IMPACT OF EUROPEAN ARRIVAL ON ABORIGINAL AND TORRES STRAIT ISLANDER PEOPLES
PAGE 167

Impact of European arrival on Aboriginal and Torres Strait Islander peoples

For tens of thousands of years before Europeans arrived in Australia, Aboriginal and Torres Strait Islander peoples had a healthy lifestyle, a well-balanced diet with varied food sources, and an intricate knowledge and use of natural medicines. They collected and stored water and cultivated native plants by agricultural methods that produced bountiful amounts of food to sustain populations through the seasons. Indigenous people developed sophisticated means of herding and harvesting kangaroos and other native fauna, including eels (Figure 8.6) to be captured for food. Well-respected cultural traditions and spiritual rules meant that clans did not have to settle in one site to protect crops and they could move to plant and tend crops with the rotation of the seasons. There was no overcrowding for prolonged periods, which reduced accumulation and exposure to natural waste and prevented transmission of infectious diseases by the faecal–oral route.

Aboriginal and Torres Strait Islander peoples wore body coverings made from animal products and woven materials. The clothing only covered part of their body, which allowed their skin to receive ample sunlight, which helped keep surface bacteria in check. In such a geographically isolated country as Australia, the diseases present among Indigenous populations before the arrival of the Europeans were chronic diseases, such as heart and liver diseases, rather than acute infectious diseases capable of resulting in epidemic outbreaks.


Fairfaxphotos/Justin McManus

Figure 8.6 UNESCO World Heritage Listed Budj Bim eel traps

The First Fleet arrived in Sydney in 1788, bringing with it up to 1500 convicts, marines, seamen, civil officers and free settlers. More ships arrived in the following months and years, bringing British settlers, supplies and more convicts. Also on these ships was a multitude of infectious pathogens that had not previously been present in the land now known as New South Wales.

These pathogens caused diseases that devastated the Indigenous populations. Diseases that were common among the early European settlers were measles, smallpox, influenza, scarlet fever, chicken pox, bronchitis and the common cold, and they were deadly for Indigenous people. Respiratory and sexually transmitted diseases spread rapidly, as the lands and waters used by Aboriginal people became inundated with European settlers and animals carrying these diseases. With no immunity developed from previous exposure to these diseases, local populations were decimated.

Within 14 months of settlement, Governor Arthur Phillip estimated that 50% of the local Indigenous population had been affected. The Wurundjeri people of the Yarra region, which is now modern Melbourne, were also severely affected by the new diseases, which caused about 60% of the deaths of Aboriginal people across the Port Phillip area. Aboriginal clans around the Melbourne area felt the severe impact of European colonisation even before Europeans reached Melbourne. An earlier smallpox epidemic had spread south from Sydney and killed up to a third of the population of the eastern Australian tribes.

Traditional food sources became restricted as the settlers arrived, with land being fenced off and vigorously defended by settlers. Settlers claimed and defended water wells and killed native animals for sport. Indigenous crops and gardens were ravaged and destroyed by introduced animals such as sheep, cows, rabbits, foxes, cats, dogs and birds. These crops had always been managed sustainably with a 'take one, leave one approach' combined with regenerative fire stick burning. The crops could not cope with the European style of grazing and were unable to regenerate. They also could not compete with noxious weeds, which grew from seeds carried into the country on animals and ships.

Native animals fell victim to diseases that came with the introduced species and were also preyed upon by exotic animals and birds, thus reducing their populations and availability as a food source for Aboriginal people. Many Aboriginal people starved, and others were forced to adopt a European diet. Aboriginal people started living much closer together in communities in smaller areas to have access to the available food. With poor sanitation and overcrowding, dysentery spread through the populations. The European diet was also heavily based on flour, refined sugar, offal and poor-quality grains and meats, quite different from the traditional high protein diet, making Indigenous populations more susceptible to diseases.

In the winter of 1847, an influenza infection swept through an Aboriginal camp at the place where the Merri Creek and Yarra River met. This epidemic devastated the camp inhabitants, causing many deaths and much grief, which led to despair and sadness among the survivors. There was little desire to live in the camp anymore, adding a further force driving Indigenous people from their homes. Indigenous birth rates plummeted, partly due to the introduction of syphilis, and partly because Aboriginal and Torres Strait Islander peoples did not want to have babies if the babies could not be fed and kept safe.


Alamy Stock Photo/Davo Blair

Alamy Stock Photo/Christine Osborne Pictures

Figure 8.7 The Indigenous diet was well balanced with varied food sources.

KEY CONCEPTS

- Diseases that are caused by pathogens can be sporadic, endemic, epidemic or pandemic.
- Emerging pathogens are previously unrecognised pathogens or pathogens that have spread to new locations.
- Re-emerging pathogens are pathogens that have caused disease in the past and are now reappearing.
- Rapid global movement of humans, animals and other biological material means it is inevitable that the spread of pathogens has increased.
- Pathogens brought into Australia by European settlers, such as the viruses that caused measles, smallpox and the common cold, were deadly for Indigenous peoples who had not been exposed to these pathogens before.

Concept questions 8.1

1 Tetanus is a sporadic disease. Malaria is usually classified as an endemic disease in Africa. In 2003, SARS (severe acute respiratory syndrome) was classified as an epidemic in Asia. The Spanish influenza outbreak of 1918 was classified as a pandemic. What is the difference between each classification?

2 Over the last 50 years, about 40 pathogens have been classified as causing emerging diseases. The diseases include COVID-19, Zika virus disease, MERS, Ebola and swine flu. State two main criteria that may cause WHO to classify a pathogen as causing emerging disease.

»

3 Re-emerging diseases are those that have previously been classified as controlled, eradicated or sporadic and therefore not a public health issue. How do the following factors contribute to the re-emergence of disease?
   - **a** Antibiotic resistance
   - **b** Travel
   - **c** Destruction of habitat
   - **d** Mutations in pathogens

4 SARS, avian flu, swine flu and COVID-19 (as of December 2020) are believed to have undergone cross-speciation from animals to humans. What is cross-speciation when considering disease?

5 In the mainstream media, the following expressions are used when discussing disease.
   - **a** Ravage Indigenous populations
   - **b** Communicable disease
   - **c** Flatten the curve
   - **d** Schools are a Petri dish

   What does mainstream media really mean in each case?

HOT Challenge

6 List three impacts that Europeans had on Aboriginal and Torres Strait Islander peoples. Discuss how each of these impacts contributed to the spread of emerging diseases amongst the Aboriginal and Torres Strait Islander populations.

WB
8.2 STRATEGIES FOR CONTROLLING PATHOGEN TRANSMISSION PAGE 170

8.2 Strategies for controlling pathogen transmission

As the world was reeling from World War I, many nations faced a new serious threat. Soldiers in crowded quarters at the major staging and hospital camp in Étapes, France, and at least a dozen military camps in the USA, were succumbing to a deadly infectious respiratory disease, which physicians recognised as the flu. Reports of the infections were suppressed to maintain morale, but this allowed time for the outbreaks to spread. Once it reached the neutral country of Spain, the press could report on its devastation of populations, giving the perception that the infection began there. With military personnel travelling to new areas and returning home from war zones, and with more modern systems for civilian transportation, the 1918–19 Spanish influenza outbreak spread rapidly throughout the world. Strikingly, this strain killed healthy, young people along with the frail, and it killed up to 100 million people, many more people than the war itself. Despite intensive efforts to stop the disease spreading to Australia, the Spanish influenza outbreak killed about 12 000 Australians.

Newspix/News Ltd

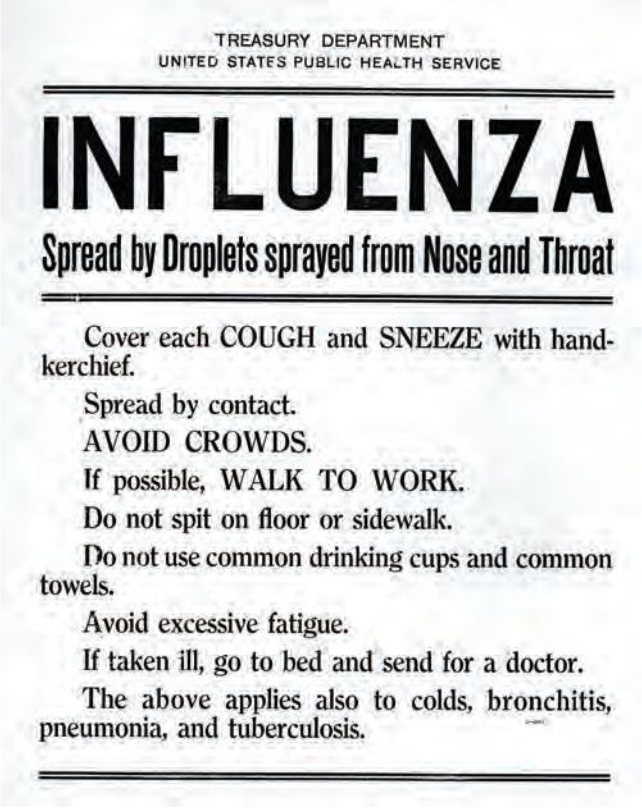
Figure 8.8 **Military personnel returning home in cramped conditions from World War I spread the Spanish influenza among themselves and to naïve populations.**

Several factors contributed to the rapid spread and made the outbreak so devastating. People were exhausted, stressed and poorly nourished as a result of the war, making them more susceptible to infection. The transportation of soldiers to many different countries around the world helped the rapid spread of the disease. War had disrupted normal healthcare programs, leaving countries unprepared and unable to effectively quarantine those infected. Additionally, Spanish influenza was deadlier than regular seasonal influenza, primarily killing otherwise healthy adults 20-40 years old. Even people with strong immune systems were susceptible. Many patients infected with influenza succumbed to the secondary deadly bacterial infection pneumonia, and many experienced a 'cytokine storm' – a massive systemic flood of inflammatory cytokines that overwhelmed the body and proved to be fatal.

Spanish influenza is an excellent example of how factors relating to the host organism, the pathogen, modes of transmission and the environment must all be considered when implementing measures to control the transmission of the pathogen. The hosts in which the Spanish flu strain flourished were highly susceptible adults affected by war. The pathogen, an avian-derived influenza strain, likely mutated to produce a deadly second and third wave of infections, following a relatively mild early first wave. The high concentration of individuals housed in training and staging camps and hospitals, and the global displacement and mobilisation of civilians and troops, produced ideal conditions for disease spread. The wartime environment was directly to blame for the extent of this pandemic.

Many important lessons were learned from the Spanish influenza pandemic. Today, major investments are made throughout the world to monitor and investigate new disease outbreaks. The primary goal of investigating a disease outbreak is to limit its spread and to prevent any more outbreaks (Figure 8.9). Once a country has identified a new outbreak, they must initiate immediate measures to control its spread, and begin to investigate its source, the nature of the infectious agent, and its pattern of transmission. The results of these investigations determine the implementation of longer-term control measures.

Figure 8.10 shows just how difficult it is to control viral spread in large populations. This figure shows the timeline



TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

INFLUENZA

Spread by Droplets sprayed from Nose and Throat

Cover each COUGH and SNEEZE with handkerchief.

Spread by contact.

AVOID CROWDS.

If possible, WALK TO WORK.

Do not spit on floor or sidewalk.

Do not use common drinking cups and common towels.

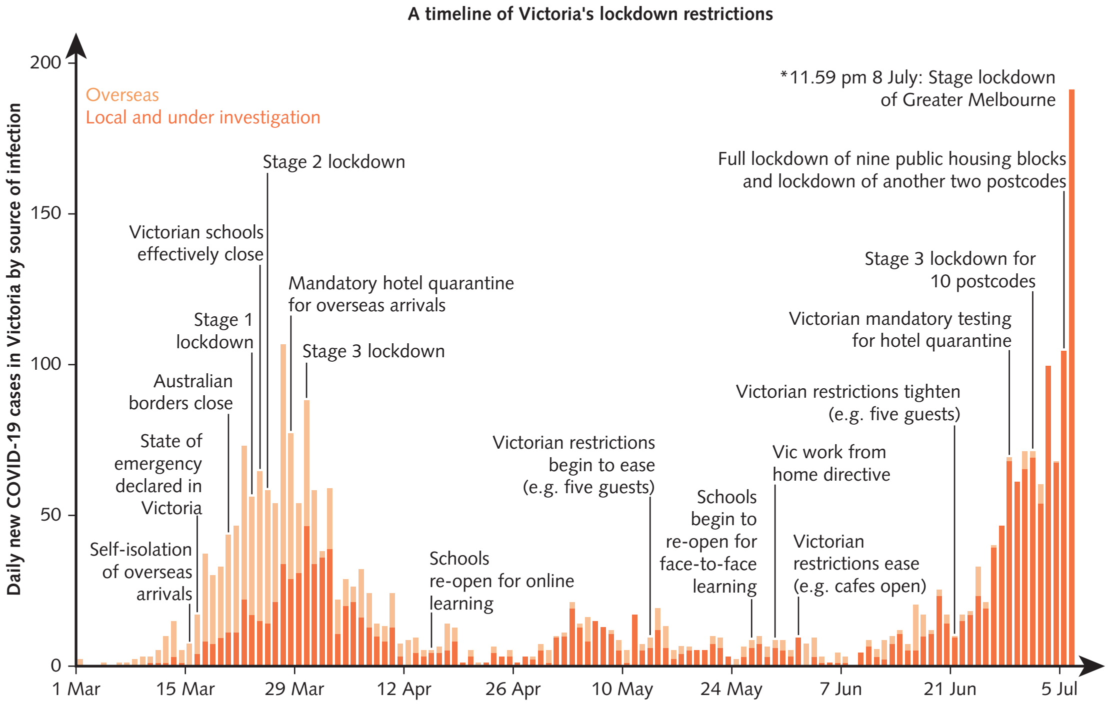
Avoid excessive fatigue.

If taken ill, go to bed and send for a doctor.

The above applies also to colds, bronchitis, pneumonia, and tuberculosis.

Science Photo Library/Library of Congress

Figure 8.9 Control measures used to contain the spread of the 1918 influenza pandemic in the USA



Note: Only major events are indicated. Data current as at 6 July 2020.

Grattan Institute. CC BY-SA 4.0 https://creativecommons.org/licenses/by-sa/4.0/

Figure 8.10 A timeline of Victoria's lockdown restrictions to manage COVID-19, March–July 2020 of new daily cases of COVID-19 in Victoria from March to July 2020 by source of infection as well as the lockdown restrictions imposed by the Victorian government. In the initial stages of infection, most of the cases were brought in from overseas. All arrivals into Victoria were quarantined in hotels. Victorian schools were closed in mid-March and Stage 3 lockdown was imposed on 30 March.

From mid-May, case numbers fell and restrictions began to ease, schools reopened for Term 2, cafes opened with restricted numbers and five guests were allowed to visit homes. Community transmission rose from this point to be the major mode of transmission of the virus. From mid-June, it was apparent that the daily number of new cases was increasing, particularly in 10 hotspot postcodes. These postcodes were locked down in early July along with nine public housing blocks. By 8 July, new cases went into triple digits each day and Victoria was well into a second wave of the virus. All metropolitan Melbourne locked down along with one regional shire on 8 July 2020 and came out of lockdown on 28 October 2020 after 112 days.

Scientific strategies to control spread and eliminate infectious diseases

Scientific strategies act to reduce or stop transmission of and infection by a particular pathogen and potentially develop treatments to control or eliminate the pathogen.

Initially, it is essential to identify the pathogen and the host or hosts involved in the infectious disease, followed by determination of the modes of transmission of the pathogen, in order to determine strategies to reduce or stop its spread. Treatments may include medications such as antibiotics, antivirals, and fungicides.

Identify the host

A host is the organism in which the disease-causing pathogen resides to gain nutrition and shelter, and a place for reproduction. Some pathogens can have many different hosts.

Ebola is a fatal haemorrhagic disease caused by viruses in the genus *Ebolavirus* (EBOV). An outbreak of Ebola virus occurred in West Africa in 2014 and there were more than 28 600 reported cases and 11 325 deaths. Death is due to multiple organ failure and tissue death. It is thought that the Ebola virus' natural **primary host** was the fruit bat and that the virus spread to **secondary hosts** such as gorillas, chimpanzees, antelopes and pigs when they either ate or were bitten by infected bats. Humans in close contact with the bodies (blood, organs and body fluids) of infected animals can become infected with the virus. It is thought that the 2014 outbreak started when a young boy from Guinea was infected by bats. Human-to-human transmission occurs with close contact with infected humans through broken skin, mucus or other bodily fluids. Corpses of Ebola victims have high viral loads and the local custom of funerals and burials accompanied by ceremonial washing and touching of deceased persons was a large factor in the spread of the virus.

Identify the pathogen

Researchers must be able to decide whether a pathogen is the cause of a disease. They do this by applying a set of criteria known as **Koch's postulates**. Koch's postulates enable researchers to isolate a suspected pathogen and demonstrate that this pathogen causes the disease under investigation. The postulates are as follows.

1. The suspected pathogen must be present in large numbers in all organisms suffering from the disease, but not in healthy organisms.
2. The suspected pathogen must be isolated from the infected organism and grown in pure culture.
3. The suspected pathogen should cause the disease when introduced into a healthy susceptible host.
4. The suspected pathogen must be recoverable from the inoculated organism from step 3, re-cultured and compared to the pathogen from step 2. If they are the same, then it is concluded that this pathogen has caused the disease.

Koch's postulates need to be modified when investigating viral diseases. Viruses cannot reproduce outside a living cell so they need to be grown in a cell culture (step 2). This can cause unintentional genetic alterations that change the way the disease presents in a new host (step 3). More modern techniques have now replaced Koch's postulates.

Ebola virus is usually diagnosed by its early symptoms of fever and headache, which can take up to three days before they are at detectable levels. These symptoms could be attributed to other diseases such as malaria. By the time the person develops more definitive symptoms, they may have spread the virus to other people.

It is important to give a diagnostic test for the early stages of Ebola infection as soon as possible. Polymerase chain reaction (PCR) (Chapter 3, p. 93) is the most commonly used diagnostic method. PCR can detect the presence of low levels of the virus in small amounts of blood. The ability to detect the virus increases as the amount of virus increases during an active infection.

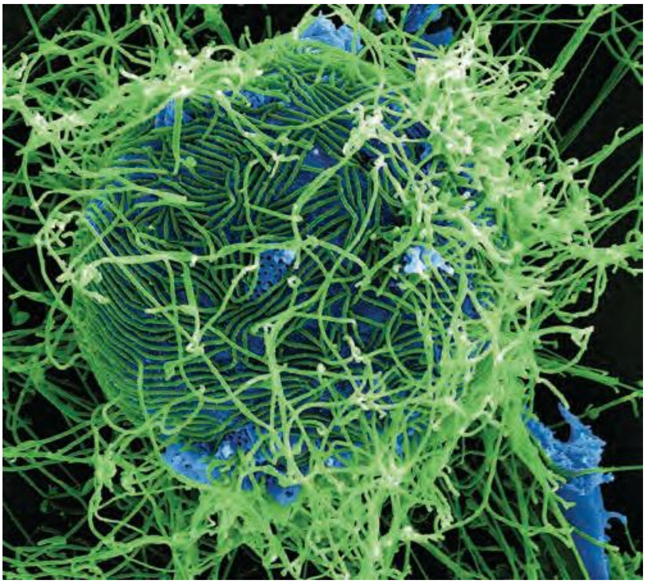
Another diagnostic method is based on detecting antibodies that the body produces as a result of Ebola infection (Chapter 7, p. 260). This can be used to confirm a patient's exposure to the virus and then measures can be taken to reduce their risk of infecting others.

Modes of disease transmission

The mode of transmission of a pathogen strongly affects its ability to spread within a population. Transmission may be direct including via air, droplets, or via bodily fluids; or it may be indirect including via vectors, food, water, or host animals. To limit the spread of infectious diseases, it is crucial to understand how the pathogen is transmitted.

Geographic area

Some of the transmission methods restrict the spread of the disease to certain climates or geographic areas. Malaria is a disease caused by protists from the *Plasmodium* genus and transmitted between human hosts by the *Anopheles* mosquito, which acts as a vector or **carrier** of the pathogen. Malaria is found only in areas of South America, Africa and Asia that are near to the equator, which are areas where the *Anopheles* mosquito can live. By contrast, influenza can spread through most populations of the world because its mechanism of spread by droplet transmission (coughs and sneezes) does not depend on a vector or specific environmental conditions.



Alamy Stock Photo/Image Source

Figure 8.11 **A cell (blue) infected with the Ebola virus (green)**

Groups within a population

Some modes of transmission mean that infections are more likely to spread in certain groups within a population. These are groups that have behaviours or risk factors that promote the spread of infection. Hepatitis C is a chronic viral infection that can cause cirrhosis (scarring) of the liver or liver cancer. It is spread by body fluid contact, particularly through contact with infected blood. Consequently, a high risk group is people who share needles and syringes to inject drugs. Historically, another group at risk of acquiring hepatitis C infection were people who require regular transfusions of blood products, such as those with haemophilia. Before 1990, blood donated for transfusion was not screened for viruses and hepatitis C transmission occurred when some patients received transfusions from infected individuals. Today, extensive screening means that the risk of acquiring infections this way is very low.

Infectivity

The transmission of disease is also influenced by a pathogen's **infectivity**, which is its ability to spread from one host to another host. Diseases with high infectivity, such as influenza, spread readily through a population. Infectivity is different from **virulence**, which is the capacity of a pathogen to cause severe disease within its host. For example, rabies kills all people who are infected with the virus once symptoms begin, making it an extremely virulent disease. Some pathogens with a high level of virulence may have low infectivity, and vice versa.

Once an individual has been infected with a pathogen, there are several possible outcomes (Figure 8.12). An infected host may be able to transmit the infection at several stages, including before they develop symptoms and when they are symptomatic. The different stages of infection can vary with each pathogen and can influence how an infection spreads within a population.

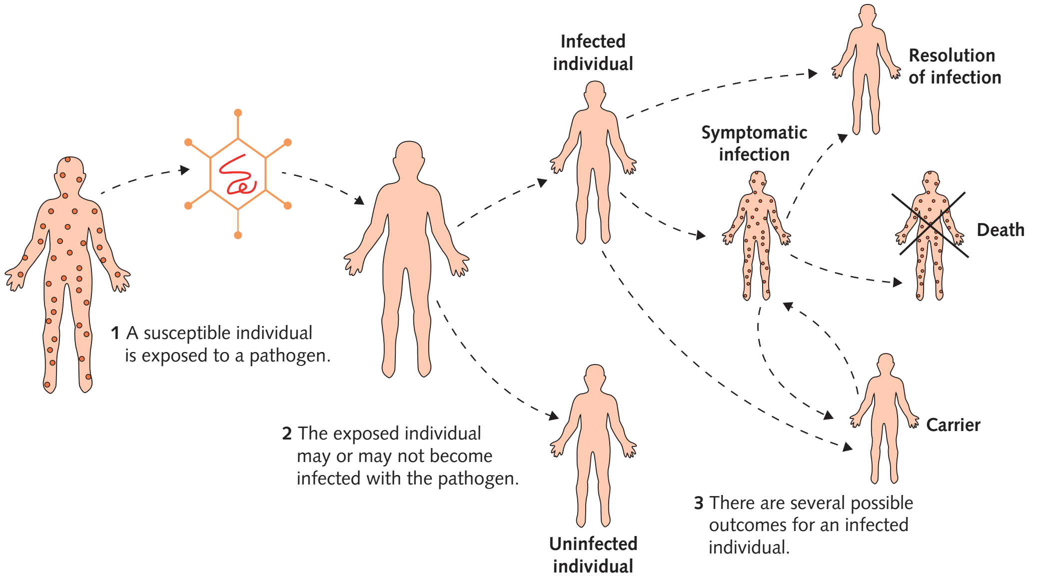


Figure 8.12 Exposure to a pathogen can have several different outcomes for an individual.

Latent infection

The persistence of a pathogen within its hosts can also contribute to the spread of the disease. Some pathogens may persist in carriers who can transmit the infection to others, but do not show symptoms themselves. This is the case with people who are HIV positive because their white blood cells are infected with HIV, but they show no symptoms. In some other diseases, such as tuberculosis (TB), the pathogen can survive within its host for a long time before causing symptoms. TB is caused by the bacterium *Mycobacterium tuberculosis* and is spread by droplet infection. Individuals become infected by inhaling the bacterium, which settles in their lungs. At this stage, most patients enter a **latent** phase of infection during which they are asymptomatic (not showing symptoms) and not contagious. In about 10% of those infected, the disease can reactivate years or even decades later and cause symptoms. A period of latent infection such as this may be advantageous for a pathogen, allowing for its spread within a population over a longer time or into new populations as individuals move to new areas.

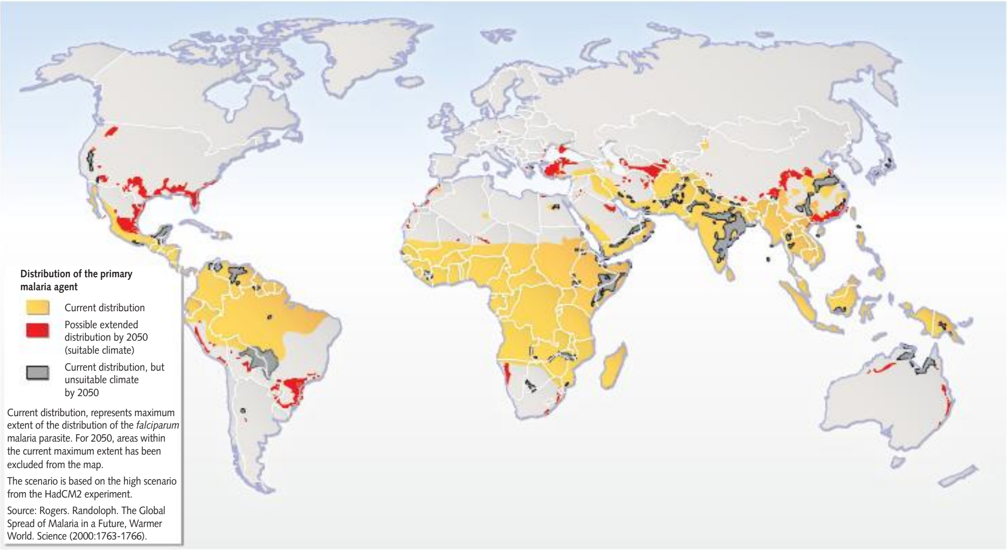
Environmental factors

A wide variety of environmental factors, including infrastructure and climate, can affect the spread of disease. The design and quality of infrastructure, such as water supply, roads and sewerage systems, can have a profound impact on disease transmission.

Communication and collaboration between scientists, doctors and politicians within and between regions, countries and continents are vital to help reduce spread, encourage and provide vaccination programs and aid in development of new drugs. Effective treatment of TB can prevent the spread of the disease but requires therapy with many drugs over a long time. In turn, this requires collaboration between drug manufacturers, supply networks, public health officials and healthcare workers. The spread of TB after the fragmentation of the Union of Soviet Socialist Republics (USSR) is an example of how infrastructure breakdown can result in the spread of infectious disease. Previously well-coordinated treatment programs became fragmented and countries of the former USSR could no longer ensure regular, constant supply of antitubercular medications. The fact that many patients received partial, but not complete, courses of treatment, led to very high levels of TB strains that are resistant to conventional drugs in countries of the former Soviet Union.

Some diseases, such as dengue fever, are particularly prone to transmission in urban environments. Dengue fever is a mosquito-borne viral illness that can cause fevers, muscle aches, headaches and rashes, and, in severe cases, death. The vector for dengue fever, the mosquito *Aedes aegypti*, is well adapted to living in urban environments and breeds in artificial water pools such as water tanks or buckets. This, along with a high population density in urban areas, contributes to the spread of this disease.

Changes in global temperature are predicted to have profound effects on ecosystems worldwide and to impact on human health in several ways. Models are used to make predictions about the possible spread of diseases under new conditions. Models help to predict changes within the already complex set of factors that influence disease transmission. As global climates change, the geographic distribution of pathogens and vectors, and the diseases they carry, can change. Increases in temperature and changes in rainfall are likely to result in the spread of disease-carrying vectors, such as malaria-harbouring mosquitoes, into previously uninhabitable regions. Figure 8.13 shows the predicted changes in the distribution of malaria as a result of climate change.



Current distribution, represents maximum extent of the distribution of the *falciparum* malaria parasite. For 2050, areas within the current maximum extent has been excluded from the map.

The scenario is based on the high scenario from the HadCM2 experiment.

Source: Rogers. Randoloph. The Global Spread of Malaria in a Future, Warmer World. Science (2000:1763-1766).

UNEP/GRID-Arendal/Hugo Ahlenius

Figure 8.13 **This map shows the predicted change in distribution by 2050 of *Plasmodium falciparum* malaria, as a result of climate change, based on modelling data.**

Extreme climate events, such as tsunamis, floods and droughts, can also promote the spread of disease. In these situations, displacement of populations and the breakdown of sanitation, food and health infrastructures are major contributing factors. For example, outbreaks of diarrhoeal illnesses such as cholera, typhoid and hepatitis A can occur with both floods and droughts (Figure 8.14). During flooding, water supplies can become contaminated with human waste containing bacteria or eggs of parasites. In drought, the lack of fresh water can lead to breakdown in hygiene practices and promote disease spread.

Getty Images/Casmir Oduor

Figure 8.14 **An increase in diarrhoeal illness in Kenya was observed after extensive flooding in 1998.**

Hosts

It is not only the characteristics of the pathogen and the environment that affect the spread of disease. The

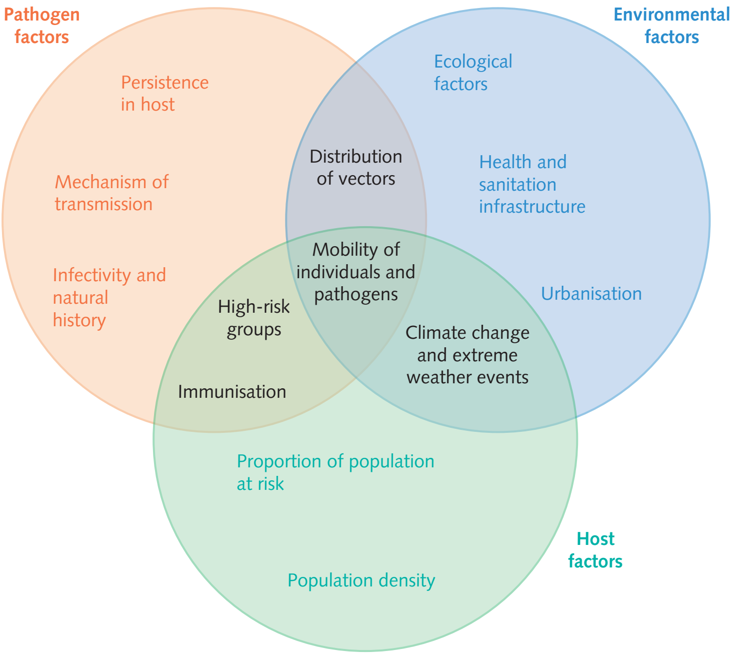


Figure 8.15 The transmission of disease is affected by a wide range of factors, many of which are interrelated.

characteristics of the infected population and individual hosts are important too. A person's behaviour may increase their risk of becoming infected. For example, the risk of becoming infected by a blood-borne virus is increased by injecting drugs and getting tattooed. Risk of infection may also vary with age, sex or socioeconomic status. Once exposed to a pathogen, some hosts, such as the very young, elderly or chronically ill, may be more susceptible to contracting infections because their immune system may be less able to combat the pathogen.

The transmission of disease is complex and can be affected by a wide range of different factors. The large number of factors, as well as their interrelatedness, can make understanding the transmission of a particular disease difficult. Figure 8.15 shows how these factors are connected. Other factors also may affect transmission of each specific disease.

ACTIVITY 8.1

Modelling disease spread

Models are tools employed by epidemiologists to predict the impact of different factors on disease spread. The disease laboratory simulator allows you to modify various disease characteristics (infectivity, mortality rate and duration of infection), population density and vaccination status, and observe their impact on the spread of disease.

Use the interactive lab simulator to investigate the spread of disease.

Aim

To explore the impact of several variables on disease transmission

What to do

1. Access the weblink and carry out your own investigation. Choose a disease and the simulation parameters, and then run the disease. What parameters lead to a quick spread of disease? How could you change some parameters to slow the spread of disease?
2. The risk of another pandemic spreading to Australia is very real. Using what you have learned in this activity, discuss how this risk is different from 100 years ago and identify the pathogen, host and environmental factors that contribute to this risk.

Social strategies to control transmission of pathogens

Social strategies are strategies that support the scientific effort to reduce and control the spread of a pathogen in a population. Government organisations play a key role in controlling the transmission of pathogens. Transmission can be prevented by improving access to vaccinations (p. 302) and contraceptives, and by enabling access to health care, screening, counselling and education. In the case of epidemics or pandemics, governments can also limit freedom of movement in an attempt to control the outbreak.

Scientists who study disease transmission (epidemiologists) can design interventions to halt disease spread. The implementation of several interventions, such as hand hygiene and vaccination programs, has had an enormous impact on our control of communicable diseases.

Improved public education

Childcare centres and kindergartens have been educating young children about their health for many years. They have handwashing policies that enable young children to learn the technique and value of handwashing. Primary and secondary school children learn about the hazards of sharing drink bottles, the importance of healthy eating practices, drug education and sexual health.

The Australian Department of Health has devised the National Framework for Communicable Disease Control. Part of this framework is public education. In a country as diverse as Australia, this framework takes into account peoples' different sets of beliefs, values and attitudes, along with social and cultural norms and perceptions. Any health communication aimed at educating the public needs to tailor its message to maximise its success in raising awareness of health risks, improving health literacy and adopting good health behaviours.

Public health care

Public health legislation mandates that anyone suffering from a notifiable disease must report it to their state health agency. The notifiable disease list is extensive and includes bloodborne diseases (e.g. hepatitis), gastrointestinal diseases (e.g. cholera), sexually transmitted diseases (e.g. chlamydia) and vaccine preventable diseases (e.g. diphtheria). In the event of an infectious disease outbreak, it is important to screen people to determine whether they have been exposed to the pathogen. This enables early intervention and quarantine to prevent further infection. Screening can be coupled with counselling, ensuring test results remain confidential or protecting people suffering from certain diseases from discrimination.

Figure 8.16 A public health message on COVID-19

© Commonwealth of Australia. Department of Health

Physical distancing

A key strategy in the fight against COVID-19 has been the roll-out of public education campaigns promoting social distancing, which means maintaining a distance of 1.5 metres between individuals and a maximum of one person per 4 m^2 inside a building. Other strategies include working from home where possible, cancelling mass gatherings, and enforcing lockdowns in which people's movements are tightly restricted.

Face masks

The outbreak of COVID-19 in 2019 saw the sale of P2-grade face masks spike to the point where it was nearly impossible to source one. In July 2020, the wearing of face masks became mandatory for Victorians as part of further restrictions brought into play to reduce the spread of COVID-19 within the community.

Hand hygiene

Before the mid-19th century, the transmission of infection was not well understood. In hospitals, surgeons did not wash their hands and death rates from post-operative infection were extremely high. In fact, the contamination of a surgeon's clothes with bodily fluids was considered a sign of experience. A British surgeon, Joseph Lister, having read Louis Pasteur's theory that micro-organisms cause disease, hypothesised that preventing their entry may stop disease. Lister experimented with the use of carbolic acid to clean wounds and instruments, as well as handwashing, as a way of maintaining a sterile environment and preventing infection. These strategies proved successful in lowering post-operative infection rates and Lister's practices gained favour with other surgeons. Today, regular handwashing and the use of sterile equipment are considered key elements in effective health care.

Stress-tested by Southern Biological

INVESTIGATION 8.1

Effectiveness of alcohol-based antiseptic

Alcohol-based hand rub is widely used in hospitals as an alternative to soap and water. To use the hand rub, squirt a small amount into the palm of your hand and rub your hands together so that the liquid covers your hands. The alcohol rapidly evaporates, leaving your hands dry.

Aim

To determine whether handwashing with alcohol-based hand rub or handwashing with soap and water is more effective in reducing the number of bacteria on hands

Materials

- Alcohol-based hand rub
- Liquid soap (not antibacterial handwash)
- Sink with water
- Paper towel
- 2 sterile agar plates
- Clear tape or Parafilm

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Liquid soap or alcohol-based hand rub may be irritating to people with sensitive skin.	If you know you cannot use one of these products, inform your teacher or arrange to use the alternative.

Method

1. Write a research question and a hypothesis before beginning the investigation (Chapter 1, pp. 6 & 8).
2. You will conduct this investigation in pairs. One person will use an alcohol-based hand rub and the other will use soap and water to wash their hands.
3. Label your agar plates on the underside with your name, the date and your treatment. Label one plate ‘Before washing’ and the other ‘After washing’.
4. Remove the lid from the plate labelled ‘Before washing’ and press the palm of one hand firmly on the agar, covering as much of the plate as possible. Replace the lid.
5. Following the guidelines, wash your hands with either alcohol-based hand rub or soap and water.
6. Without touching anything, repeat step 4 using the opposite hand on the plate labelled ‘After washing’.
7. Place the plates upside down (agar layer on top), seal with clear tape or Parafilm and incubate them at 25°C for 24 hours.

Results

1. Count the number of colonies on each agar plate before and after handwashing. Copy the results table into your logbook, extending the table to show the number of pairs in your class. Record your results in the table. Combine all class data to increase the sample size.

Results of investigation comparing alcohol-based hand rub to soap and water

Pair	Alcohol-based hand rub			Soap and water		
	Number of colonies before washing	Number of colonies after washing	Percentage reduction in number of colonies	Number of colonies before washing	Number of colonies after washing	Percentage reduction in number of colonies
1						
2						

»

Analysis of results

1 Calculate the mean percentage reduction in number of colonies for each treatment.

Discussion

1 Compare the mean percentage reduction between the two treatments. Is there any difference? Do your results support your hypothesis?
2 Identify some potential sources of error in the experimental design.
3 Explain why you have calculated the percentage reduction in number of colonies, rather than comparing the number of colonies remaining for each treatment.
4 Do you think that it would be better to use the same hand or the opposite hand for the control plate? Justify your response.
5 In hospitals, it is not just the ability of the treatment to reduce the number of bacteria on hands that influences the transmission of infection. Make a list of other factors that might influence whether alcohol-based hand rubs or soap and water are more effective in reducing hospital-acquired infections.

Conclusion

Draw a conclusion that is consistent with the evidence you obtained during this investigation and is relevant to the question under investigation.

Quarantine

You have already seen how the mobility of individuals can facilitate the rapid spread of infections such as SARS and COVID-19 around the world. **Quarantine** is a practice used to stop individuals who have been exposed to infectious diseases from carrying that disease into healthy populations. Potentially exposed individuals are kept from entering a healthy population until the incubation period of the disease has passed, and they can demonstrate that they are not infected.

From the mid-19th century, people arriving on ships to Australia from ports where certain infections were present were subject to quarantine. Luggage was fumigated and non-immune individuals were detained at quarantine stations, such as at Point Nepean in Victoria (Figure 8.17). People who contracted the disease were given appropriate treatment.

Shutterstock.com/Neil Cox

Figure 8.17 At one time, all immigrants to Australia had to pass through the Point Nepean Quarantine facility.

Australia has quarantine laws that give the government the power to exclude, detain and segregate people to prevent or control the spread of disease. The Australian government used these laws in 2020 to detain more than 200 Australian nationals who had recently been evacuated from Hubei province in China, the epicentre of the COVID-19 outbreak. They were flown to the Christmas Island detention centre, 2700 km off the coast of Western Australia, where they were placed in quarantine for 14 days, the incubation period of the virus. No cases of disease were reported during the quarantine period and everyone returned to their homes after two weeks.

Developed by Southern Biological

INVESTIGATION 8.2

Infectious disease transmission

Infectious diseases can spread through several ways, such as direct contact with an infected person, indirect contact via surfaces or objects, and airborne droplets when infected people sneeze, cough or laugh. The transmission of disease through droplets depends on how close the infected person and potential host are because the droplets disperse and settle quickly. The pathogens responsible for the common cold, influenza and COVID-19 are typically transmitted through droplets in the air.

Local health departments, WHO and the US Centers for Disease Control and Prevention are responsible for monitoring infectious disease outbreaks. One of their responsibilities is to identify the first person in a population who has the disease (patient zero) and then track routes of transmission. Over the past 100 years, these organisations, along with vaccine development and sanitation improvement, have effectively fought the spread of disease. Many of the infectious diseases that have historically been responsible for devastating epidemics have now been reduced or even eradicated.

Aim

To simulate infectious disease transmission and identify patient zero

Time requirement

45 minutes

Materials

Per student:

- Plastic pipette
- Screw-cap vial containing a solution representing a body fluid
- Permanent marker
- Index card
- Disposable gloves

Per class:

- Phenol red indicator
- 4 × 96 well-plates labelled 0, 1, 2 or 3

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
The vials may contain sodium hydroxide, which can cause severe skin burns and eye damage.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

1. Collect an index card, plastic pipette and screw-cap vial containing solution.
2. There are four well plates labelled 0, 1, 2 and 3. Locate your individual wells on the class well plates. These will be labelled with the number corresponding to your vial.
3. Using a plastic pipette, transfer 5 drops of the fluid from your vial into your well on Plate 0.
4. Select a partner for your first exchange. Record their name and vial number on your index card.
5. Using your plastic pipette, transfer 5 drops from your vial to your partner's vial. Return any remaining liquid in your pipette to your vial. Replace the vial cap and mix the solution by inverting it several times.
6. Using a plastic pipette, transfer 5 drops of liquid from your vial into your corresponding well on Plate 1.
7. Repeat steps 3–6 for the second and third exchanges and deposit your liquid into your wells on Plates 2 and 3, respectively. Select a different partner for each round and complete each step before proceeding to the next exchange.
8. After all exchanges have been made, your teacher will add one drop of phenol red to your vial, which is an indicator solution that will determine if your vial has become 'infected'. Vials that turn red or pink are positive for the pathogen (infection). Vials that turn yellow are negative, which indicates that your vial did not become infected.
9. Report whether your vial tested positive. If so, share the names of the partners you exchanged fluids with.

10 Based on your individual results and the data from your classmates, try and identify which vial the infection spread from. Your teacher will add a drop of phenol red to each of the wells in the well plates. You may be able to trace the spread of infection to the original source by observing which samples are positive in each round.

11 Copy the results table into your logbook and complete the table to help you identify the source of infection. Once you have listed the positive vials and who they exchanged with, circle the numbers of the partners whose vials tested positive.

Results

Positive vials and exchange partners

Positive vial numbers	1st exchange partner number	2nd exchange partner number	3rd exchange partner number

1 Who was patient zero?

2 After the three rounds of exchanges, how many vials tested positive? Calculate what percentage of your class this represents.

3 Construct a graph showing how many students were infected after each round.

Discussion

1 If the class were divided into three groups of 10 at the start of this investigation and allowed to exchange only within their group, what would the transmission of the disease look like?

2 Did you know which vials were infected during the procedure?

3 Do you think an individual who does not show any signs of a disease is capable of transmitting the disease to others?

4 What is the importance of identifying patient zero in epidemics?

5 How does this simulation differ from the spread of disease in the real world; for example, the spread of COVID-19? Explain.

6 List the appropriate measure that individuals should take to limit the spread of diseases.

Taking it further

Research past infectious disease epidemics. Your research should include:

- origins of the disease
- how the disease is transmitted
- typical incubation period
- symptoms of the disease
- impact (i.e. death toll, cultural shifts, historical context)
- possible vaccines and treatments
- preventative measures.

KEY CONCEPTS

- Factors relating to the host, pathogen, mode of transmission and environment all contribute to the measures taken to control the transmission of the pathogen.
- In an outbreak, it is important to identify the pathogen causing the disease.
- The transmission of disease-causing pathogens is complex and can be affected by many factors relating to pathogens, the environment and the host.
- Measures to control transmission include public health, education, good hygiene, social distancing and quarantine.

Concept questions 8.2

1 Robert Koch developed his postulates in 1884 in relation to tuberculosis and cholera, two infectious diseases that were rampant in the human population. Although still used, Koch's postulates are not useful for studying diseases caused by viruses or in asymptomatic carriers. Explain why.

2 Other ways of studying infectious disease epidemiology is to record symptoms, causative agent, mode of transmission, infectivity, incubation rates and presence of antibody to the causative agent in the patient. Summarise the value of investigating two of these in studying disease.

3 How is virulence different from infectivity of a pathogen?

4 Tyhpoid Mary was a domestic helper who worked in many households during the late 19th and early 20th centuries. She infected 53 people with typhoid fever but never showed symptoms herself. How would Mary be classified in relation to this disease, and what does this classification mean?

5 When health departments and epidemiologists study the transmission of infectious diseases through communities, they consider many factors so that they can track and control the disease. List 8 of these factors and provide a short description of each.

6 Consider the government's response to the COVID-19 case numbers in Victoria (Figure 8.10) and account for the second wave that started in June 2020.

HOT Challenge

7 Research and write a brief explanation of the quarantine measures used for two of the following.
- **a** Bubonic plague
- **b** Tuberculosis
- **c** Leprosy
- **d** Yellow fever
- **e** Ebola
- **f** COVID-19

8.3 Vaccination programs

Weblink
The flu vaccine explained

Online Worksheet
The flu vaccine explained

Vaccination programs have great potential for limiting or even eliminating human infection, as exemplified by vaccination programs targeting the *Variola major* virus, which causes smallpox. Smallpox is estimated to have been a major problem to human health as far back as 12 000 years ago, and probably even earlier than this. Evidence of smallpox has been found in Egyptian mummies dating back to 3000 BCE. As civilisations and trade expanded across the globe, so did smallpox. Smallpox was recorded in China in the fourth century, and in India and Asia from the seventh century. The Crusaders brought it to Europe on their return from the holy wars in the 11th century. As European colonisation spread through Africa and the Americas, smallpox arrived in Australia in the 18th century with the colonists from the United Kingdom.

Smallpox is spread by direct and prolonged face-to-face contact. It has also been known to spread through contact with body fluids, clothes and bedding from infected people. Smallpox has an incubation period of 7–17 days, causes high fever, aches and pains, and culminates in a rash. The raised, pus-filled blisters can take 3–5 weeks to subside. After the infection, large pitted scars can form on the face and body. Smallpox can also lead to blindness if blisters form near the eyes.

Research into protecting people from the ravages of smallpox began with Edward Jenner and his studies on cowpox, which led to the development of what is now widely accepted as **vaccination**. In the 1790s, Jenner observed that people who had been infected with cowpox did not develop smallpox if exposed to the *Variola* virus. Within his farming community, cowpox was prevalent among those who worked with animals. Jenner speculated that a bout of cowpox would produce immunity to smallpox. To test this, he inserted pus from an infected milkmaid into a cut on the arm of a local boy. A little while later, Jenner exposed the boy to smallpox and the young boy was found to be immune. He called his method vaccination, but it wasn't until 30 years after Jenner's death in 1823 that smallpox vaccination was made compulsory in parts of the United Kingdom.

The Global Smallpox Eradication Program was initiated by the WHO in 1959. The aim of the program was to eradicate smallpox from all humans in the world. Initially, travellers from various countries were vaccinated, as well as local populations. By the mid-1960s, the program was still not particularly effective at eliminating the disease and, in some countries, containment and monitoring those infected proved to be a more effective strategy. Increasing availability of international air travel hindered efforts to keep countries free of the disease, even though it had previously been eradicated. Despite a range of setbacks, including scepticism about the feasibility of the vaccination, as well as inadequate technical and material resources (qualified staff to administer the vaccine and quantity of vaccine required per population), the disease was officially declared eradicated by the WHO in 1980.


Science Source/NMHM

Figure 8.18 In 1901, one of these boys was vaccinated against smallpox and one was not vaccinated.

Vaccination leading to **immunisation** is a highly effective public health intervention that has substantially reduced worldwide morbidity and mortality from infectious diseases. In Australia, children are routinely vaccinated against many infectious diseases, including hepatitis B, diphtheria, pertussis (whooping cough), tetanus, measles, mumps, rubella and poliomyelitis. Groups that are at high risk of infection, such as the elderly or chronically ill, may also need additional vaccinations. As new vaccines are developed, immunisation programs against more diseases are being introduced. Figure 8.19 shows the rates of infection of *Haemophilus influenzae* and meningococcal C after the introduction of vaccines against these pathogens.

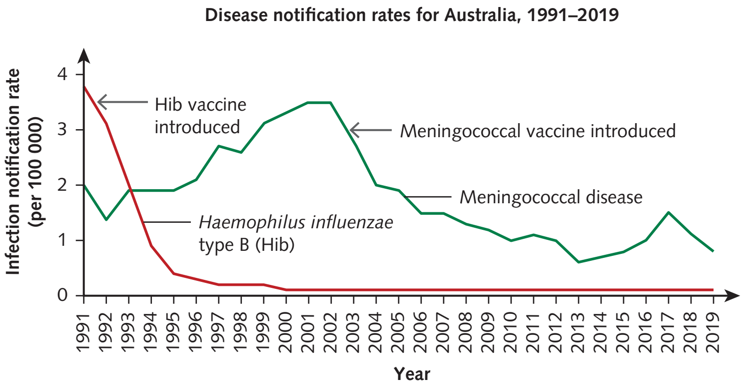


Figure 8.19 Rates of *Haemophilus influenzae* type B (Hib) and meningococcal infection since the introduction of vaccines against these organisms.

EXAM TIP

When you are presented with a graph, take the time to interpret the graph and work out what it is telling you. Once you are confident that you know what the graph is telling you, then answer the question.

WB

8.3.1
HERD IMMUNITY
PAGE 171

Herd immunity

Not all individuals within a population need to be vaccinated to prevent the spread of a disease. If a large enough proportion of a population are vaccinated and achieve artificial active immunity to a disease, then there are too few susceptible individuals to sustain disease spread. This effect is known as **herd immunity** and is demonstrated in Figure 8.20. Only infected individuals (orange) can spread the disease to those they have contact with. When there are enough immune individuals (blue), the chance of an infected individual contacting a susceptible individual (black) is so low that the disease cannot spread. For herd immunity to prevent the spread of disease, often a high proportion of the population

Weblink
How does herd immunity work?

Online Worksheet
How does herd immunity work?

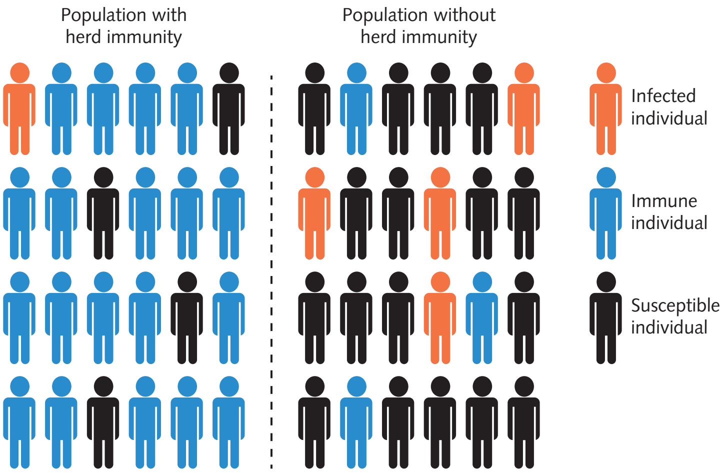


Figure 8.20 **Herd immunity occurs when a large enough proportion of a population is immune to a disease. Disease cannot spread because there are too few susceptible individuals.**

needs to be immune, however this can vary widely for different diseases. The exact proportion depends on the virulence and infectivity of a specific disease. For measles, 95% of people must be vaccinated for herd immunity, but for polio, it is 80%. Some people have health conditions that mean that they cannot be immunised and so they rely on herd immunity for protection from infection.

Some people object to immunisation of children. The reasons for this vary, but a major concern raised by these groups is the safety of vaccines. While vaccines can have some side effects, these are usually mild (such as pain or swelling at the site of injection) and serious reactions are very rare. Overall, vaccines are far safer than the diseases they protect against.

One of the conditions erroneously linked to vaccination is autism. In 1998, a small, unsubstantiated report suggested that the measles, mumps and rubella (MMR) vaccine could cause autism. The claim was later discredited, and further research has not shown any link. Despite the lack of scientific evidence, vaccination rates dropped markedly, and measles infections rose after publication of this report.

WB
8.3.2
VACCINES
PAGE 173

In some cases, levels of immunity within the population have dropped so low that herd immunity is no longer sustained. In 2013, several measles outbreaks in the United Kingdom were linked to low vaccination rates after the MMR scaremongering. The risk of potentially devastating infectious diseases re-emerging is substantial if high vaccination rates are not maintained. To combat this risk, several states in Australia have legislation requiring children to be vaccinated before they can be enrolled in childcare or school.

The Victorian government implemented a ‘No Jab, No Play’ policy in 2017, which requires parents to supply the childcare provider, kindergarten or school with a current immunisation record as well as a statement to show their child is up to date with their immunisations. The introduction of this policy was coupled with extensive media and social media coverage, as well as online support through the Department of Education website.

Samoa experienced a measles epidemic in 2019. In a country with a population of 200 000, there were 5700 reported cases and 83 deaths. Thirty-five of those deaths were children under 4 years of age. In 2015, the vaccination rate for measles was at 84%. In 2018, only 31% of children under five had been vaccinated. The decrease in rates was put down to the deaths of two infants following vaccination. Further investigation ruled out any negative effects from the vaccine, with the deaths thought to be due to other medications that were incorrectly administered.

The Samoan government declared a state of emergency on 17 November 2019, closing schools and cancelling all Christmas celebrations. Unvaccinated families were ordered to display a red flag at the front of their house to alert others and to enable mass vaccinations to occur. On 7 December 2019, the state of emergency was lifted, and the government declared that 90% of the population was now vaccinated.

KEY CONCEPTS

- Vaccination is a highly effective public health intervention that has substantially reduced worldwide morbidity and mortality from infectious diseases.
- Herd immunity occurs when a large enough proportion of the population is immune to a disease (usually due to a successful vaccination program), and there are too few susceptible individuals to sustain disease spread.

Concept questions 8.3

1 Distinguish between vaccination and immunisation.

2 Measles is a notifiable disease. It can lead to severe medical problems, including encephalitis, brain damage and death. In Australia, measles devasted the Indigenous population when it was introduced during European settlement. A measles vaccine was developed in 1963 and is given in Australia to young babies and children in the MMR vaccine as part of a nationwide vaccination program. Worldwide, there are about 20 million cases every year. Why is measles re-emerging?

3 Before there was a chicken pox vaccination, parents would hold chicken pox parties for their children.
   a What were the parents doing?
   b Why were the parents doing this?
   c What did the discovery of a vaccination do to these parties?

4 Herd immunity for measles requires 95% of people to be vaccinated, but for polio it is 80%. Explain what is meant by herd immunity and why the percentage of the population that needs to be vaccinated for specific diseases is different.

5 The *Vaccinia* virus that is the active ingredient of the smallpox vaccine is a very close relative of, and may be the same species as, the cowpox virus that Jenner used for his first smallpox vaccine. However, it is not the *Variola* virus that causes smallpox. How could the *Vaccinia* vaccine produce a lifelong immunity in humans against smallpox?

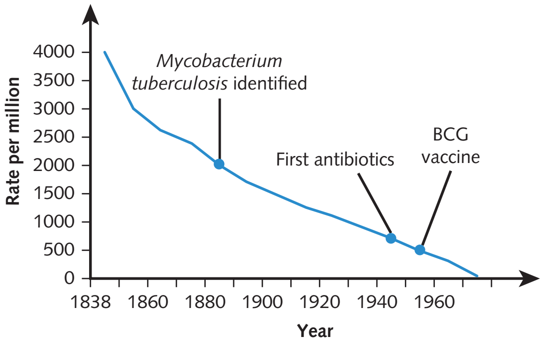


Figure 8.21 The decline in respiratory tuberculosis rates in England and Wales since 1838

6 What is the 'No Jab, No Play' policy? What is your opinion about this policy?

HOT Challenge

7 a Interpret the epidemiology of TB that is shown in Figure 8.21.
   b Compare the data in Figures 8.21 and 8.22.
      i The data in Figure 8.21 implies that TB rates dropped to 0 per million in about 1975 in England and Wales. Does the data in Figure 8.22 correlate with that inference?
      ii If the BCG vaccination has been available since about 1920, why does Figure 8.21 show it to be in use since 1954?
      iii Has TB been eradicated as of 2016 in England and Wales?
      iv BCG is not part of the regular vaccination program for minors in Australia. Explain why.
      v In Australia, 1440 new cases of TB were recorded in 2018. It is a notifiable disease. What does this mean?
      vi In Australia, the incidence of TB before 2018 was extremely rare because of a 27-year eradication program. Where are the new cases coming from?

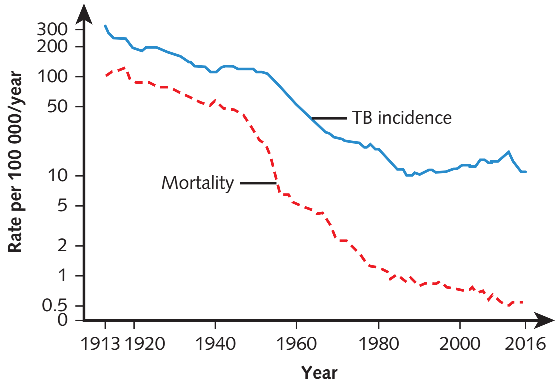


Figure 8.22 TB incidence and mortality rates per 100 000 people per year in England and Wales, 1913–2016

8.4 Immunotherapy strategies

Immunotherapy is a treatment given to a person suffering from cancer or an autoimmune disease to boost or suppress their immune system. Boosting or suppressing the immune system has to be undertaken with extreme care. It can have unwanted side effects, such as fungal infections and lymphomas, and trigger life-threatening complications such as autoimmune damage to normal tissue.

Cancer

Cancer is a disease that is caused by uncontrolled cell division. Cancer arises when a cell escapes the normal immune response that limits its growth and survival. This is due to mutations in the cell's genome that change the expression or function of genes. Because apoptosis is the main way of removing new potentially cancer-causing mutations, the most important factor in the initiation of cancer is the ability of mutated cells to evade apoptosis and continue to survive and divide.

Cancerous cells arise from normal body cells, and only a relatively small number of molecular changes are needed to cause disease, so cancers can be hard to treat specifically without inducing harmful side effects for other cells or tissues. Some small molecules can target specific gene mutations that commonly occur in cancers or can reduce the activity or expression of signalling pathways that cancers rely on to grow and spread.

Immune checkpoint inhibitors

T cells are one of the immune cells that our immune system uses to fight cancers. T cells have proteins on their surface that activate the cells to start their immune response. These cells also have other proteins that turn off the immune response when the threat is over. These proteins are called checkpoints. Cancer cells can produce their own proteins that can turn T cells off so they do not attack cancer cells. Checkpoint inhibitors are drugs that stop the proteins on the cancer cells from turning off T cells, thereby allowing the T cells to attack the cancer cells.

T-cell transfer therapy and cancer treatment

n.

Weblink
Watch a video of T cells killing cancer cells.

T-cell transfer therapy is a technique that boosts the natural ability of a person's T cells (Chapter 7, p. 255) to fight cancer. Scientists remove T cells from a cancerous **tumour**. They select the most active against the cancer, alter them in the laboratory to make them more effective and then grow them in large batches *in vitro* (in glass). These super-powered T cells are reintroduced into the patient by intravenous injection and then target and kill cancerous cells. (See weblink.)

Immunotherapy using monoclonal antibodies

WB

8.4.1
MONOCLONAL ANTIBODIES AND THE TREATMENT OF CANCER
PAGE 175

Monoclonal antibodies are artificially produced antibodies, each type being identical to each other and specific to a particular antigen. They bind to and neutralise the antigen in the same way as naturally produced antibodies in the body. They are useful as they are (1) identical ie. clones; (2) can be produced in large quantities; (3) are highly specific. They have many uses in diagnostic testing, such as pregnancy tests, and are used extensively in therapies, such as the treatment of cancers and autoimmune diseases.

There are different methods of producing monoclonal antibodies, however most are produced using mice. When a mouse is injected with a specific antigen foreign to its body, its immune system will be stimulated to produce specific antibodies against it. After several days, some of the B lymphocytes that produced these antibodies are removed from the spleen of the mouse. The problem with these B cells is that like most mammalian cells, they do not live for very long in culture and so do not mass produce the specific antibody for any length of time. This can be overcome by fusing the B cell clone that produces the antibody of interest with cells extracted from a plasma cell tumour, thus providing a source of cells that do not stop dividing. Some of the mouse B cells fuse with the tumour cells to create a **hybridoma**. A selection process can be used by growing the cells on a culture medium to select only those that have formed hybridomas. The hybridoma can produce antibodies coupled with the property of tumour cells to divide repeatedly *in vitro* (the cells have now become 'immortalised'). Each hybrid cell produces many clones of itself, and each clone produces the same antibody. Hybridomas have revolutionised the production of antibodies. When used in therapeutic treatment, monoclonal antibodies can act in two main ways:

1. Activation immunotherapy which aims to induce or amplify an immune response
2. Suppression immunotherapy which aims to prevent or reduce an immune response

Sometimes they may have no effect on the immune response.

Monoclonal antibodies and cancer treatment

Antibodies that detect antigens unique to cancer cells can be used as anticancer drugs. The body can be stimulated to produce these antibodies or they can be produced in a laboratory.

Monoclonal antibodies can be designed to trigger an immune response to attack cancer cells (Figure 8.23), block signals that cause cancer cells to divide, or transport toxic molecules or radioisotopes to cancer cells (examples are shown in Table 8.1). An example is the medication trastuzumab, which is a preparation of monoclonal antibodies that can bind to cells of some types of breast cancer, blocking their growth and promoting their destruction by the immune system. Ideally, the monoclonal antibody will bind to an antigen that is unique to the cancer and not present on normal body cells. This means there are no unwanted side effects of the monoclonal antibody. However, sometimes antibodies bind to antigens that are also present on other cells of the body, resulting in various side effects depending on the target cells.

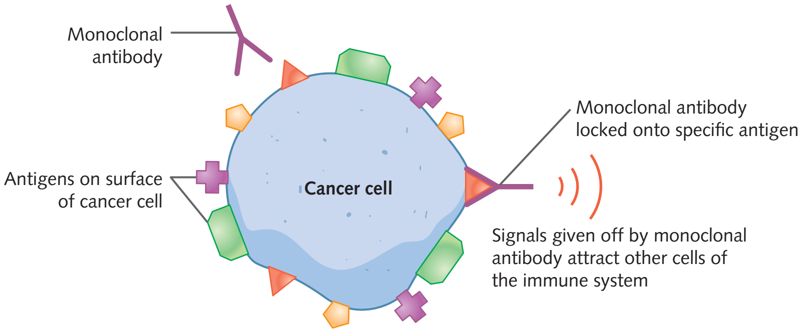


Figure 8.23 Monoclonal antibodies lock onto the antigens on the surface of the cancer cell. This can signal other immune system cells to attack the cancer cell.

Table 8.1 Monoclonal antibodies and specific cancer treatments

Cancer type	Mechanism of action
Non-Hodgkin lymphoma	Binds to the protein CD20 on the surface of cancer cells and activates the immune system to destroy them
Advanced melanoma	Binds to and blocks CTLA4, a negative regulator of the immune system, to keep immune cells stimulated
Breast cancer	Binds to the growth factor receptor HER2 to block the signal and stop cancer cells growing
Bowel, breast and some other cancers	Binds to the growth factor VEGF to inhibit binding to its receptor

Autoimmune disease

The human immune system is a complex mechanism with many regulatory checks in place, but it can malfunction. **Autoimmune diseases** can occur when the immune system recognises a normal component of the body as a foreign antigen and mounts a response. This results when cells of the immune system fail to distinguish between self and non-self or when the immune system overreacts to substances that are not pathogenic. The immune system is carefully tuned to remove or suppress any B or T cells that may respond to antigens in the person's own body, but sometimes this system of self-tolerance fails, and an autoimmune disease develops. There are many different types of autoimmune diseases and almost any part of the body can be affected. The effects the autoimmune disease has on the body depends on which self-antigen (known as an auto-antigen) the body is reacting to.

Autoimmune diseases caused by autoantibodies

Some autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE, or lupus) are the result of the action of **autoantibodies**. The production of autoantibodies is a malfunction of the adaptive humoral response. Autoantibodies are produced by the immune system and target the body's own components, including proteins, nucleic acids, lipids and carbohydrates. The autoantigen is usually specific at the onset of disease, but as more tissues are damaged by the uncontrolled inflammation, more antigens are released, activating other clones of auto-reactive B cells. In rheumatoid arthritis, autoantibodies target components in cartilage and bone in the joints, causing inflammation, bone destruction, pain and joint malformation. The autoantibodies bind to the tissue lining the joints and work to destroy the cells, much in the same way that antibodies work to neutralise the effects of pathogens by targeting specific antigens (Figure 8.24). In lupus, autoantigens are varied and can include

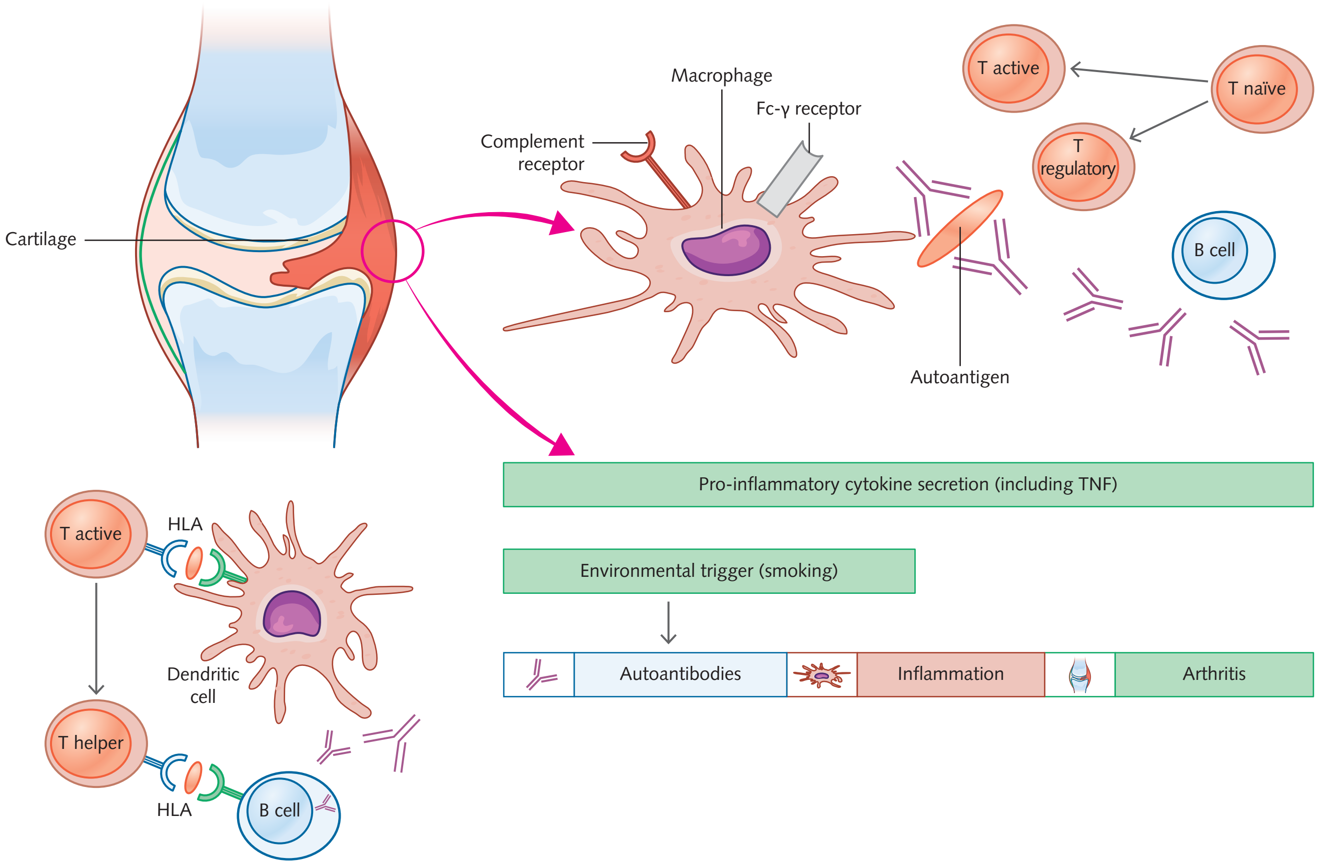


Figure 8.24 The action of autoantibodies in rheumatoid arthritis of the knee joint

ubiquitous (universally utilised) components such as DNA, which means that any organ or tissue can potentially come under antibody-mediated autoimmune attack.

Autoimmune diseases caused by T cells

Autoimmune conditions also arise when T cells target the body's own cells and mark them for attack. Conditions such as Crohn's disease and multiple sclerosis develop in this manner. Multiple sclerosis results when reactive T cells attack the myelin sheath of neurons and form plaques that lead to neurological decline. It presents as less or spasmodic control of movement. Crohn's disease is the result of T cells targeting cells in the bowel. Inflammation in the bowel leads to blockages and extreme pain, and sometimes resection (removal) of entire segments of the bowel is required to prevent sepsis, or widely disseminated bacterial infection.

Type 1 diabetes is the result of a coordinated autoimmune attack on beta cells (insulin-producing cells) in the pancreas. Both helper T cells and cytotoxic T cells mistakenly target beta cells for destruction, so the person cannot produce insulin. The action of the T cells is often the result of a person's genetic predisposition and becomes apparent when the person is young. This is the reason type 1 diabetes is also known as juvenile diabetes.

Monoclonal antibodies and treatment of autoimmune diseases

WB

8.4.2
CANCER AND AUTOIMMUNE DISEASES
PAGE 177

Monoclonal antibodies are effective against some autoimmune diseases. Cytokines are signalling proteins that coordinate immune responses (Chapter 7). The expression and release of cytokines usually occurs as a cellular response to a signal, such as the presence of a foreign invader during infection or injury. Because cytokines have such powerful effects on cells of the immune system, and therefore the whole organism, several signals are often needed before a cytokine can be secreted or released. There are also many natural mechanisms to degrade cytokines and counteract their functions so that immune responses last only as long as needed to clear the danger. Other types of cytokines are anti-inflammatory, and these often start acting at the same time as the pro-inflammatory cytokines, to limit the extent of the inflammatory response. These factors ensure that immune cell activity only occurs when it is really needed, preventing unnecessary immune-mediated effects such as asthma, allergy and autoimmune diseases.

Monoclonal antibodies can be made that block and neutralise cytokines, interrupting the transmission of signals between cells and tissues. Tumour necrosis factor (TNF) is a cytokine that is involved in the very early phase of inflammation. However, TNF is a strong mediator of harmful inflammation in many conditions, such as inflammatory bowel disease and rheumatoid arthritis. Monoclonal antibodies that neutralise TNF are used in patients with these diseases, limiting the actions of this pro-inflammatory cytokine. A clinical trial in patients with the autoimmune disease lupus showed effectiveness of a monoclonal antibody that binds to the interferon-alpha receptor, preventing the whole family of interferons from binding to it, reducing inflammation in this disease.

KEY CONCEPTS

- Cancer arises when a cell starts dividing uncontrollably and escapes detection by the immune system.
- An autoimmune condition is when the immune system starts attacking self-cells.
- Immunotherapy is a treatment that works to stimulate the immune system to either suppress or enhance its response in those suffering from either autoimmune diseases or cancer.
- Antibodies specific to an antigen can be produced through stimulation of B cells or they can be produced in a laboratory and are known as monoclonal antibodies.
- Monoclonal antibodies lock onto the antigen on the surface of the cancer cell, causing the immune system to attack it.
- Monoclonal antibodies block and neutralise cytokines, interrupting the transmission of signals between cells and tissues, limiting the actions of pro-inflammatory cytokines.

Concept questions 8.4

1 What two types of conditions can immunotherapy be used to treat?

2 Cytokines are involved in the immune response. They can be anti-inflammatory and pro-inflammatory. What does this mean?

3 Monoclonal antibodies are commonly manufactured using a hybridoma.

  a Explain how a hybridoma is made.

  b Describe two situations in which monoclonal antibodies may help save a person's life.

  c Explain what property of tumour cells makes them useful for fusing to B cells for monoclonal antibody production.

4 What has gone wrong in the body when an autoimmune disease develops?

5 What faulty steps of the adaptive humoral response are involved in the production of autoantibodies in diseases such as rheumatoid arthritis?

HOT Challenge

6 Figure 8.25 summarises an inflammatory response to cancer cells.

  a Would the cytokines in Figure 8.25 be anti-inflammatory or pro-inflammatory? Explain.

  b Effector T cells are signalled by cytokines to mount a response to an antigen.

    i List three possible types of effector T cells involved.

    ii What is the role of the dendritic cell in this example?

    iii What is the role of the macrophage?

    iv The final cell is labelled as an exhausted T cell. What do you think might be the next step in progression of disease?

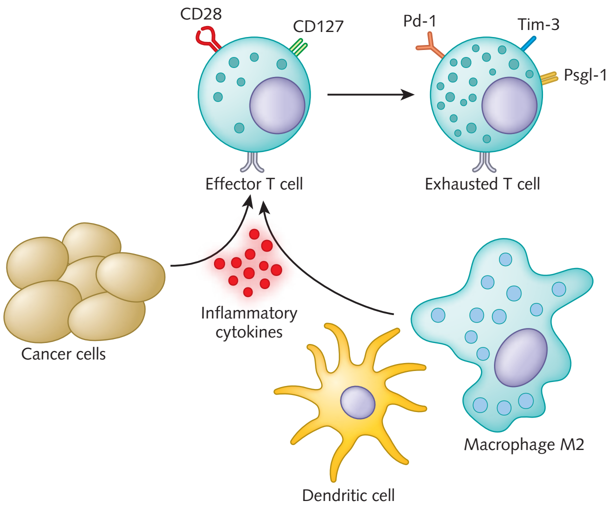


Figure 8.25 A cytokine pathway showing the outcome of T-cell exhaustion

BRANCHING OUT

Tracking influenza to prevent a pandemic

Influenza (the flu) is a contagious disease of the respiratory tract caused by infection with influenza virus. Worldwide, approximately 500 million people suffer with the flu each year. In Australia, 1500 deaths are associated with flu infections each year. Vulnerable people include the very young, the elderly and those with underlying health issues. Influenza symptoms include fever and muscle ache, which require bed rest.

Human influenza virus infects cells of the respiratory system. Once inside the host, a protein on the virus surface called haemagglutinin attaches to sialic acid residues located on the tip of glycoproteins that protrude from the host cell's plasma membrane (Figure 8.26). Haemagglutinin is the key required to gain entry into the host cell. Once the virus has attached, receptor-mediated endocytosis is triggered so the human cell engulfs the virus. Inside the human cell, the virus tricks the cell into replicating viral components. Virus capsids assemble, enclosing a set of virus genes. Hundreds of viruses start to bud from the host cell. But as they try to leave, their haemagglutinin proteins get stuck to the host cell's plasma membrane. A second protein on the surface of the virus, an enzyme called neuraminidase, acts like scissors to cut the virus free from the cell. The virus then infects another cell in the respiratory system or leaves the host in mucus drops as the infected person coughs, sneezes or talks. If a susceptible host is infected, they will become sick.

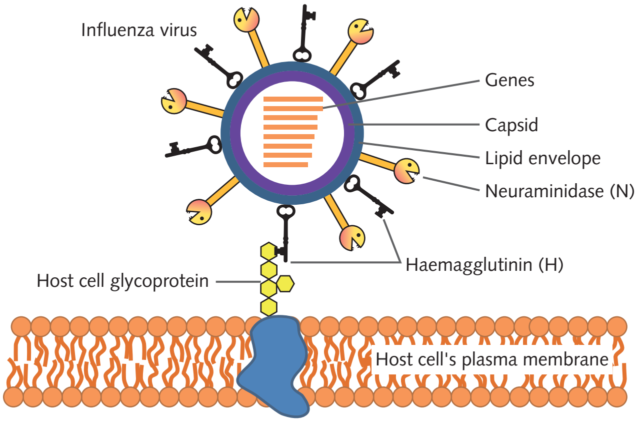


Figure 8.26 **Influenza virus uses haemagglutinin (H) protein to enter a host cell. A second protein, an enzyme called neuraminidase (N), is required to release budding viruses from the host cell's plasma membrane.**

Three types of influenza can infect humans. Type A and type B can cause epidemics, while type C only causes mild infections. Type A viruses can infect a number of different species and are further divided into subtypes based on their haemagglutinin and neuraminidase surface proteins. There are 18 different haemagglutinins and 11 different neuraminidases. These proteins vary slightly in structure as a result of mutations that have arisen in the haemagglutinin and neuraminidase genes. Haemagglutinin and neuraminidase proteins are the antigens that trigger an immune response to the virus. If these surface proteins change, the new flu virus may not be recognised by our immune system

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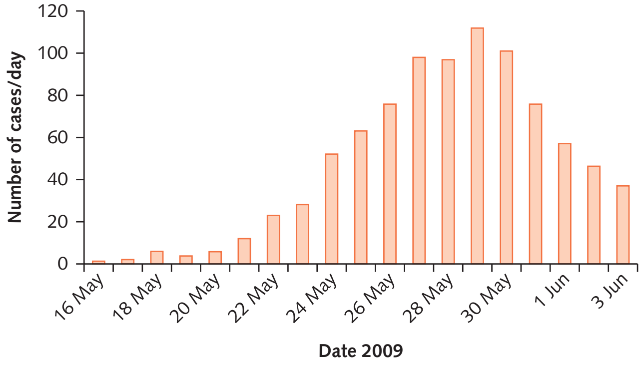
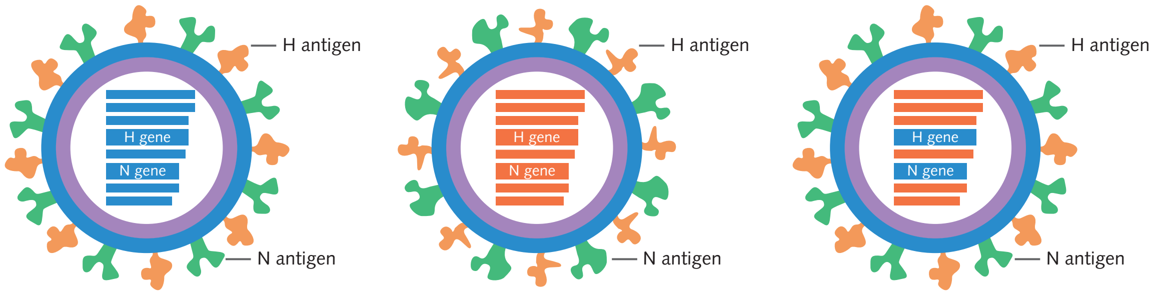


Adapted from http://www.eurosurveillance.org/images/dynamic/EE/V14N42/Australia_Fig1.jpg

Figure 8.27 Confirmed cases of influenza A/H1N1 in Victoria each day during the 2009 pandemic



1 Virus we want protection against

2 Harmless flu virus that grows well in chicken eggs but cannot infect human cells

3 This virus cannot infect humans but the H and N antigens are the same as those in the virus we want protection against. It is used in vaccines.

Figure 8.28 To make a vaccine against a new strain of influenza virus (virus 1), the genes for haemagglutinin (H) and neuraminidase (N) are taken from that virus and put into a virus that cannot infect humans (virus 2). This results in a new virus strain (virus 3), that is harmless to humans but produces the H and N antigens to which an immune response is required. Virus 3 is infected into chicken eggs so it replicates. Proteins expressed by this virus are used in the vaccine.

and can cause serious illness before an immune response develops. Melbourne is generally the first city in the southern hemisphere to succumb to new strains of influenza. Figure 8.27 shows the peak outbreaks of influenza infection during the 2009 H1N1 pandemic.

The WHO tracks influenza outbreaks globally. The WHO identifies the emergence and locations of new outbreaks, determines the type of influenza circulating, and measures the impact it has by the number of people hospitalised or killed. The most severe strains can be controlled by producing a vaccine. Information about influenza in the northern hemisphere can be used to prepare vaccines against flu strains for when they arrive in the southern hemisphere. Figure 8.28 shows how flu vaccines are prepared.

When a vaccine is injected into your arm, your body mounts an immune response against the haemagglutinin and neuraminidase antigens of the viruses in that vaccine. As a result, you produce memory cells that will provide a faster and larger immune response if you encounter that same antigen again. You will rapidly produce antibodies that control the infection. You probably will not experience any flu symptoms.

Questions

1 Construct a flow chart to show the sequence of events that occur when you are infected with influenza. On your flow chart, show both vaccinated and unvaccinated responses.

2 There is increasing pressure to make annual vaccinations against influenza mandatory for healthcare workers. The Peter MacCallum Cancer Centre has had a mandatory influenza vaccination program since 2009. However, there are no punitive consequences for staff who refuse to participate.

Do you think that annual influenza vaccinations should be mandatory for everyone? What ethical issues do you see? Use the ethical concepts in Table 8.2 to assist you in deciding on this issue.

Table 8.2 Ethical concepts used as a framework for analysing issues in biology

Ethical concepts	Description	Considerations
Integrity	Honest reporting of all information and communication of results	Is the information honest? Is information required by the public to make a decision open to public scrutiny?
Justice	Fair consideration of competing claims	Can everyone have equal access? How can discrimination be avoided?
Beneficence	A duty to do more good than harm, to consider the welfare of the research participant	Who will benefit from this technology? How will they benefit? (Can include physical, psychological, economic or social benefits) How many will benefit?
Non-maleficence	The duty not to cause harm	Who might be harmed by this technology? How are they harmed? (Can include physical, psychological, economic or social harms) How many are harmed? Is it possible to minimise the harm?
Respect	All living things have intrinsic value and can make their own decisions	Are people able to make their own decision based on their own criteria? Are people with diminished capacity protected where necessary?

Online Key Concepts
Chapter 8: Summary of key concepts

8 Summary of key concepts

8.1 Emerging and re-emerging pathogens

KEY CONCEPTS

p. 285

» Diseases that are caused by pathogens can be sporadic, endemic, epidemic or pandemic.
» Emerging pathogens are previously unrecognised pathogens or pathogens that have spread to new locations.
» Re-emerging pathogens are pathogens that have caused disease in the past and are now reappearing.
» Rapid global movement of humans, animals and other biological material means it is inevitable that the spread of pathogens has increased.
» Pathogens brought into Australia by European settlers, such as the viruses that caused measles, smallpox and the common cold, were deadly for Indigenous peoples who had not been exposed to these pathogens before.

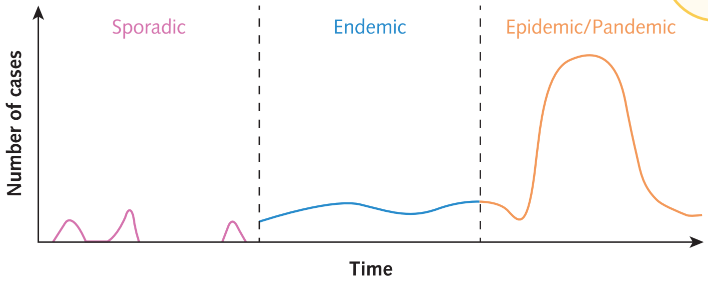


Figure 8.2 Within a population a disease may occur sporadically, at an endemic level, or at an epidemic/pandemic level.

8.2 Strategies for controlling pathogen transmission

KEY CONCEPTS

p. 290

» Factors relating to the host, pathogen, mode of transmission and environment all contribute to the measures taken to control the transmission of the pathogen.
» In an outbreak, it is important to identify the pathogen causing the disease.
» The transmission of disease-causing pathogens is complex and can be affected by many factors relating to pathogens, the environment and the host.
» Measures to control transmission include public health, education, good hygiene, social distancing and quarantine.

Figure 8.14 An increase in diarrhoeal illness in Kenya was observed after extensive flooding in 1998.

8.3 Vaccination programs

KEY CONCEPTS

p. 302

- Vaccination is a highly effective public health intervention that has substantially reduced worldwide morbidity and mortality from infectious diseases.
- Herd immunity occurs when a large enough proportion of the population is immune to a disease (usually due to a successful vaccination program), and there are too few susceptible individuals to sustain disease spread.

Figure 8.18 In 1901, one of these boys was vaccinated against smallpox and one was not vaccinated.

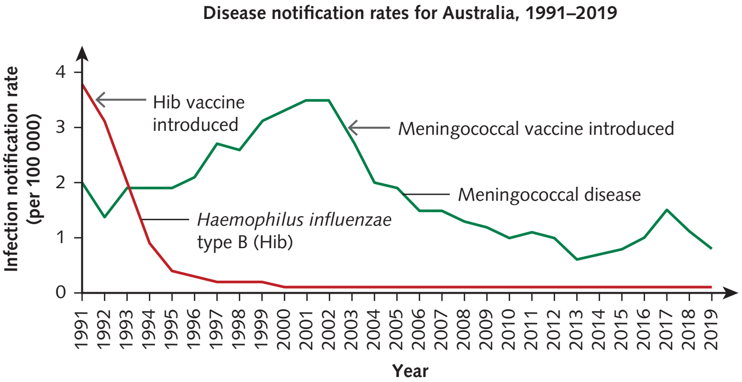


Figure 8.19 Rates of *Haemophilus influenzae* type B (Hib) and meningococcal infection since the introduction of vaccines against these organisms.

8.4 Immunotherapy strategies

KEY CONCEPTS

p. 306

- Cancer arises when a cell starts dividing uncontrollably and escapes detection by the immune system.
- An autoimmune condition is when the immune system starts attacking self-cells.
- Immunotherapy is a treatment that works to stimulate the immune system to either suppress or enhance its response in those suffering from either autoimmune diseases or cancer.
- Antibodies specific to an antigen can be produced through stimulation of B cells or they can be produced in a laboratory and are known as monoclonal antibodies.
- Monoclonal antibodies lock onto the antigen on the surface of the cancer cell, causing the immune system to attack it.
- Monoclonal antibodies block and neutralise cytokines, interrupting the transmission of signals between cells and tissues, limiting the actions of pro-inflammatory cytokines.

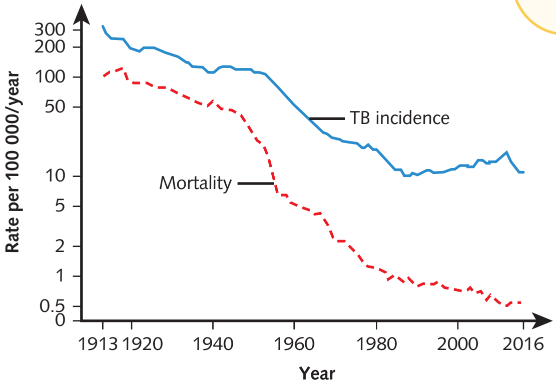


Figure 8.22 TB incidence and mortality rates per 100 000 people per year in England and Wales, 1913–2016

8

Chapter glossary

autoantibody an antibody produced by a person's immune system that is directed against the person's own proteins

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

cancer uncontrolled abnormal division of cells that are not kept in check by the immune system and invade other areas of the body

carrier a person who does not show symptoms of a disease but can transmit the infection to others

endemic restricted or native to a certain locality

epidemic the rapid spread of a disease across a number of countries

herd immunity when unvaccinated individuals are protected against a disease because a large number of people (between 60–95% depending on the disease) have been vaccinated, thereby making it unlikely that unvaccinated people will come in to contact with anyone suffering from the disease

hybridoma a cell involved in the production of large amounts of monoclonal antibodies

immunisation the process of making a person immune to a disease through vaccination

immunotherapy boosting the ability of a person's own immune system to fight cancer

infectivity the ability of a pathogen to spread from one host to another host

Koch's postulates a set of criteria to determine the causative agent of a disease

latent not active

monoclonal antibody a laboratory-produced molecule that serves as a substitute antibody to fight cancerous cells

pandemic the spread of a disease across the world

primary host an organism in which a pathogen reproduces

quarantine restricting the mobility of person or persons to a certain area so they reduce contact with other people in order to stop the spread of a pathogen

secondary host an organism in which the immature pathogen becomes mature

sporadic seen infrequently in a small number of people

T-cell transfer therapy a therapy that boosts the ability of T cells to fight cancer cells

tumour a mass of abnormally growing cells

vaccination the administration of a vaccine to protect someone from a disease

virulence the capacity of a pathogen to cause severe disease within its host

zoonotic describes a disease that can be transmitted from animals to humans

WB
8.5.1
EXAM PRACTICE
PAGE 179

8 Chapter review

Remembering

1 The following terms are associated with disease: virulence, infectivity, asymptomatic, symptomatic. Define each term and state why it is of concern.

2 Herd immunity is a useful concept in epidemiology. What does it mean? Use an example to discuss how it works.

3 European settlers introduced diseases that decimated Aboriginal populations in Australia, Maori populations in New Zealand and native American populations in North and South America. The Black Death was introduced into Europe through trade with the Orient in the 1300s and led to the death of more than 50 million people. The origins of the COVID-19 pandemic is currently under investigation.

a What aspects of diseases can make them very hard to control?
b Is it reasonable to ban travel and trade to prevent these horrific outcomes? Why?
c What do governments do today to arrest the movement of diseases around the world?

Understanding

4 The following steps show the WHO protocol for the preparation and use of blood products in the treatment of Ebola virus disease. This disease is characterised by significant blood loss.

1 A patient recovers from Ebola virus disease.
2 The same patient is disease-free for 28 days.
3 Blood is taken from the patient and screened for transmissible diseases.
4 Plasma is separated from the whole blood.
5 Plasma is transfused into another person with early signs of Ebola virus disease.

(Source: *Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease*, http://www.who.int)

a Explain why this protocol produces an effective treatment for Ebola virus disease.
b Does this protocol involve the use of a vaccine?
c What postulate does this relate to and how is it different?

5 Refer to Figure 8.15 on page 296. List the three main factors that affect the transmission of disease. Describe how these three factors interlink to affect disease transmission.

6 What are the main steps leading to the development of an autoimmune disease? How are immunotherapies being used in rheumatoid arthritis to limit symptoms in this debilitating condition?

Applying

7 Public health institutions regulate food handling to try to stop outbreaks and the passage of diseases such as hepatitis, typhoid, salmonellosis and shigellosis. All of these diseases can kill. Some countries have cultural practices around eating that are designed to prevent the spread of these diseases. However, cultural practices can also facilitate the progress of disease in a community. Research the cultural practice that leads to the spread of each of the following diseases. Describe public health measures that have been put in place to control outbreaks of:

a norovirus
b Creuzfeldt–Jakob disease
c kuru.

8 The Australian Government Department of Health website lists and defines Australian national notifiable diseases. The department states that incidences of the diseases on the list are to be notified nationally and provided to the Commonwealth's National Notifiable Diseases Surveillance System. The earliest date on this website is 2004.
   a What do you think happened before 2004?
   b Why are notifiable diseases a government concern?

9 Lack of fresh water in communities is a public health problem. Explain why.

10 What is the difference between 'social distancing' and 'quarantine'?

11 Malaria kills hundreds of thousands of people worldwide every year. Discuss historical and current measures used to control this disease.

Analysing

12 In the UK, at the beginning of the COVID-19 pandemic, the UK government considered herd immunity to be a possible pathway towards mass immunity in the UK population by allowing large numbers of people to contract the disease. The UK government then rejected the proposition. Why do you think this occurred?

13 Some herpes viruses are known to cause cancer. Gardasil immunisation is given to teenagers in Australia as part of the national vaccine program.
   a What cancer is Gardasil protecting against?
   b Initially the vaccine was only administered to females. Why do you think that was?
   c The vaccine is now administered to both males and females. Why do you think that is?

14 Figure 8.29 shows a model of disease transmission.

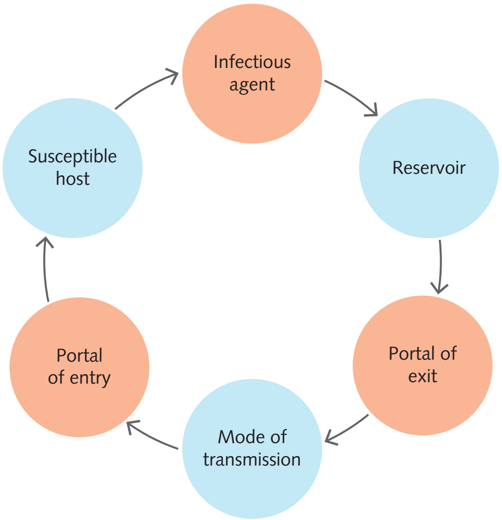


Figure 8.29 A model of disease transmission

   a What is:
      i a portal of entry?
      ii a susceptible host?
      iii an infectious agent?
      iv a reservoir?
      v a portal of exit?
   b A pathogen was identified as being non-adapted to dry conditions and as having the gastrointestinal tract as the portals of entry and exit. Is the mode of transmission most likely to be respiratory droplets, skin to skin or food?

15 Some pathogens mutate during the course of disease transmission in a community. Virulence can increase, decrease or stay the same. In the 1918 Spanish influenza pandemic, what was the pattern within communities?

16 In Australia, the operators of what modes of transport are compelled to report notifiable diseases under the *Biosecurity Act 2015?* Explain why this is.

17 Why isn't malaria found in Australia?

18 When a host is infected with a pathogen, why do they not show symptoms immediately?

19 Foodborne illness can kill (Figure 8.30). Some diseases tracked by health departments are caused by active pathogens in the host. Others may be caused by exotoxins, which are proteinaceous (made of or consisting of protein) particles. Why do we have strict rules around food preparation in Australia?

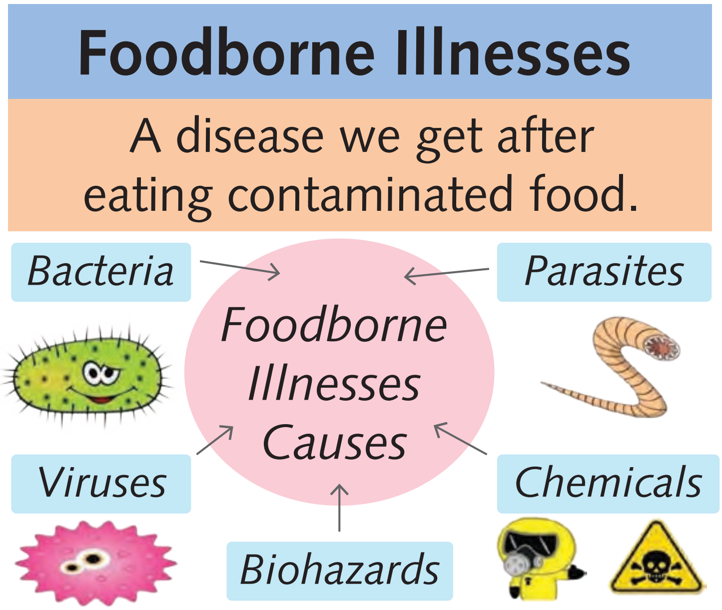


Figure 8.30 A poster explaining that foodborne illnesses are caused by consuming contaminated food or drink

20 Melanomas are characterised by uncontrolled cell division caused by mutations that continue to occur once the tumour has developed. Scientists have discovered that vaccines produced from antigens extracted from the patient's own melanoma cells can be useful in treating melanoma. When injected, the vaccines stimulate an immune response. What can be inferred from the scientists' discovery? Choose one alternative.

A Cancer cells carry unique antigens.
B Self-antigens are not present on cancer cells.
C The melanoma patient has a dysfunctional immune system.
D The body cannot mount an immune response against cancer cells.

21 Choose one alternative. The melanoma vaccine stimulates:

A T cells, which produce antibodies.
B cytotoxic T cells, which activate B cells.
C cell division to produce more lymphocytes.
D production of B cells, which destroy melanoma cells.

22 Even though Australia is an island and Melbourne is not near the northern coastline, each year Melbourne is the first place in Australia to register new patients with notifiable diseases. What might be some of the reasons for this?

23 Why does the influenza vaccine have to be redeveloped each year?

Evaluating

24 Influenza can be a deadly disease and has led to world pandemics. The US Centers for Disease Control and Prevention estimates that as many as 56 000 people die from the flu or a flu-like illness worldwide every year. In Australia, it is believed to cause 1500–3000 deaths, 18 000 hospitalisations and 300 000 visits to the GP each year. This is a huge load on health budgets. In 2019, more than 2.1 million vaccines were administered.

- **a** If there is a vaccine available, why isn't it mandatory to receive it?
- **b** Which parts of the community are most susceptible to the flu?
- **c** Consider your own experience in relation to influenza and other diseases. Do you think the Australian population has become complacent about influenza and did this affect community responses to COVID-19 in the initial stages of that pandemic?
- **d** List four factors that contribute to an individual's response to a disease in the community.

25 Several types of immunotherapies are used against cancer, including monoclonal antibodies, cancer vaccines, oncolytic virus therapy, T-cell therapy and non-specific immunotherapies. The therapies are still being developed, do not work for all types of cancers, vary in efficacy, and inhibit one cancer receptor pathway only. The pharmaceutical company Merck has been researching and developing the drug Pembrolizumab for more than 15 years. Pembrolizumab was accidentally discovered when researchers were looking for a way to use drugs to dampen down the immune response in patients with autoimmune disease. There were gaps in time in the research when the company went through two mergers and acquisitions and then in 2009 research was nearly cancelled. In 2013, research in lung cancer patients continued and in 2015 the drug went to market for a very limited use at about $100 000 per treatment and billions of dollars in research and development spent by the company. Since then, the market for use has increased as other cancers, through the use of drug trials in cancer patients, have been shown to respond to the drug.

- **a** Make a list of the cancers currently approved for treatment with this immunotherapy drug.
- **b** What does 'approved' mean?
- **c** Why aren't all cancer patients given this type of therapy?
- **d** Pembrolizumab (tradename Keytruda) is known as a humanised monoclonal antibody. What does this mean?
- **e** Pembrolizumab has a positive statistical effect in patients administered the drug in terms of efficacy, depending on the type of cancer with varying rates of destroying cancer cells with the treatment. Overall survival rates are still being researched. Why do you think that is?
- **f** The science that the drug is based on was recognised in 2018 when the Nobel Prize in Physiology or Medicine was awarded to James Allison and Tasuku Honjo. In the 1990s, they respectively made discoveries involving CTLA-4 and PD-1, which are two proteins that can act as a brake or check on tumour-fighting T cells. If you inhibit that process, you can reawaken the T cells to do their job. Keytruda is what is known as a checkpoint inhibitor.
  - **i** What type of material is a checkpoint inhibitor?
  - **ii** What is the function of a checkpoint inhibitor?
  - **iii** Where do checkpoint inhibitors operate in your body?
  - **iv** Why are checkpoint inhibitors important?
  - **v** Keytruda blocks the interaction between programmed cell death receptor-1 (PD-1) and the molecules to which it specifically binds. What is programmed death?
  - **vi** Does this process only affect tumour cells? If not, what else can it affect? Is that a problem?

Unit 4, Area of Study 1 review

Multiple choice

Question 1 ©VCAA 2013 Q39 ADAPTED HARD

Doctors are concerned about the overprescription of antibiotics. Many antibiotics have become ineffective against certain types of bacteria. This is due to

A antibiotics being less concentrated than they were 20 years ago.

B mutations occurring in the bacteria due to exposure to antibiotics.

C the antibiotic acting as a selection pressure selecting antibiotic-resistant phenotypes.

D selectively bred, antibiotic-resistant bacteria being introduced into the population.

Question 2 ©VCAA 2012 EXAM 1 Q9 ADAPTED HARD

Major histocompatibility complex (MHC) class 1 molecules

A are responsible for releasing antigens from the cell nucleus.

B display foreign antigens to cytotoxic T cells.

C produce antigen-specific antibodies.

D are found on all cells in the human body.

Question 3 ©VCAA 2014 Q14 EASY

An example of 'self' material in an adult human female is

A pollen inhaled from flowers in her garden.

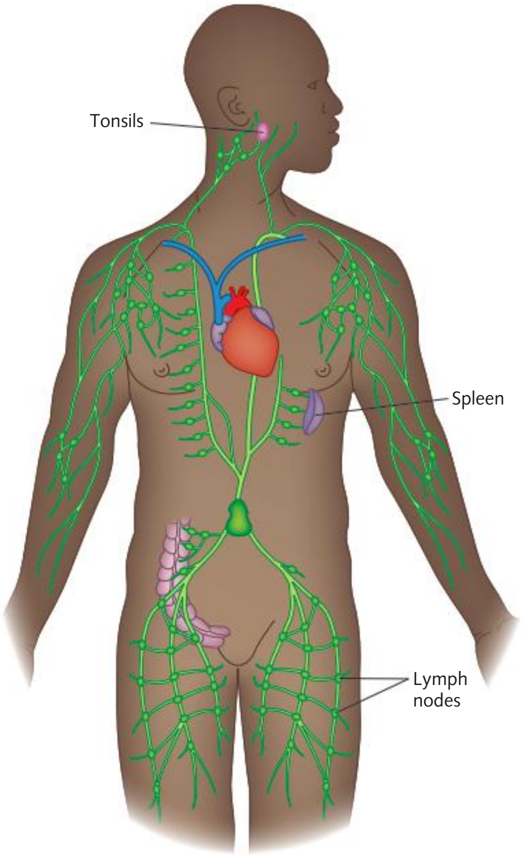
B sperm cells present in her reproductive tract.

C cells lining the inside of her arteries.

D bacteria inside the alveoli cells in her lungs.

Question 4 ©VCAA 2017 Q23 MEDIUM

The following diagram represents the lymphatic system, which includes the lymph nodes, spleen and tonsils.



The human lymphatic system

In these particular organs

A allergies trigger an initial response.

B clotting factors are inactivated to help seal a wound.

C red blood cells identify non-self antigens.

D B cells detect pathogens presented on dendritic cells.

Question 5 ©VCAA VCAA 2014 Q15 ADAPTED EASY

The first line of defence against pathogens includes the

A production of antibodies.

B release of tears from tear ducts in the eyes.

C release of histamine from mast cells.

D ingestion of viruses by phagocytes.

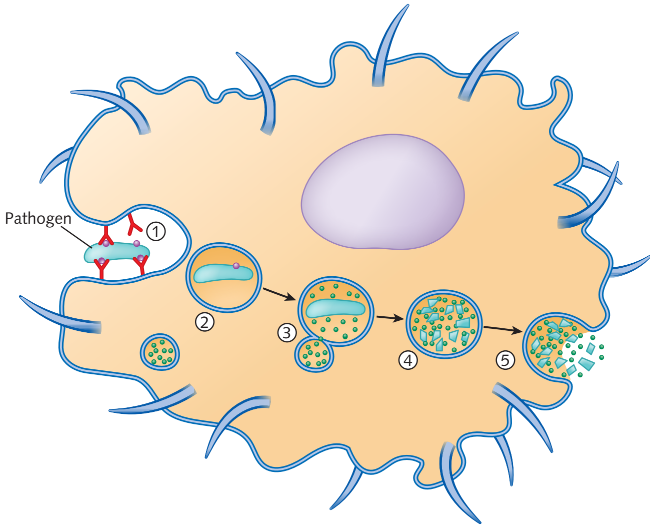
Question 6 ©VCAA 2018 Q23 ADAPTED **MEDIUM**

Chickenpox is caused by a virus. Once you have caught chickenpox and recovered, you will not develop symptoms of chickenpox again even if you are exposed to the virus. This is because you have

A natural passive immunity.
B natural active immunity.
C artificial passive immunity.
D artificial active immunity.

Question 7 ©VCAA 2019 Q20 ADAPTED **MEDIUM**

The following diagram shows the process of phagocytosis. This process is vital for immunity against extracellular infections.



What is happening at position 4?

A Enzymes that break down the micro-organism are released into the vesicle.
B Antibodies are added to the vesicle to kill the micro-organism.
C Enzymes are digesting the micro-organism.
D Intracellular microbes are attacking the micro-organism.

Question 8 ©VCAA 2016 Q20 ADAPTED **EASY**

Higher organisms have evolved the inflammatory response as a defence mechanism against infection and injury. If you step on a nail, you notice that the site around the wound goes red. This response

A is specific to the form of foreign body.
B is part of the adaptive immune system.
C involves activation of the complement system.
D involves lymphocyte production.

Question 9 ©VCAA 2014 Q16 ADAPTED **EASY**

An example of a specific response by the human immune system is

A production of cytotoxic C cells.
B epithelial cells producing defensins.
C cytokines being produced by macrophages.
D the engulfing of non-self material by a phagocyte.

Question 10 ©VCAA 2017 Q37 ADAPTED **MEDIUM**

Mosquitoes are responsible for the transmission of the viral disease yellow fever. An outbreak of yellow fever occurred in an area of Brazil in January 2017. The outbreak then spread to other areas within Brazil.

Which one of the following is a correct statement about this outbreak of yellow fever?

A This outbreak of yellow fever is defined as an epidemic.
B Bathing in the large bodies of still waters in the areas with yellow fever helped reduce the number of individuals affected.
C This outbreak of yellow fever occurred in populations with high vaccination rates for yellow fever.
D Infected individuals who travelled to other areas of Brazil did not increase the spread of the disease.

Question 11 ©VCAA 2016 Q24 ADAPTED **MEDIUM**

A protein called circumsporozoite protein (CSP) is being studied by scientists in their search for a malaria vaccine. CSP is secreted by the malaria parasite and is present on its surface. For the vaccination to work, the scientists want CSP to act as an

A antibody.
B allergen.
C antigen.
D antibiotic.

Question 12 ©VCAA 2016 Q23 ADAPTED **MEDIUM**

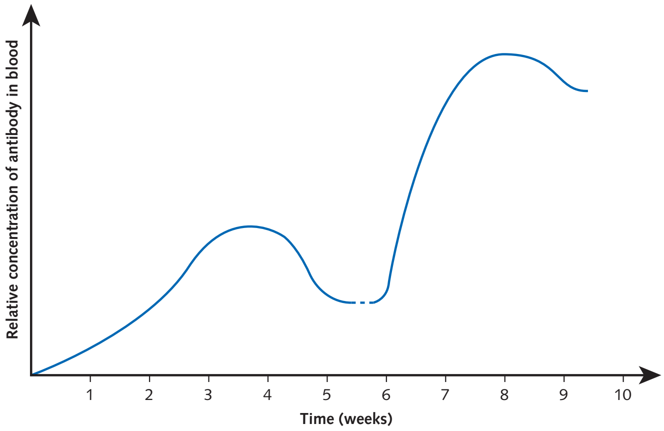
When bitten by a redback spider, a builder was treated with the injection of an antivenom serum. The treating doctor explained to him that the injection would not protect the builder against any future redback spider bites. This is because antivenom serum is used to achieve

A active and natural immunity.
B passive and artificial immunity.
C active and artificial immunity.
D passive and natural immunity.

Question 13

©VCAA **EASY**

The following graph shows the relative concentration of antibody in the blood after exposure to an antigen.



From this graph you can conclude that

A the highest level of antibody in the blood occurred in week 4.

B at week 6, the person was exposed to the same antigen for a second time.

C at week 1, the person had not been exposed to the antigen.

D antibody production was more rapid at week 2 than at week 6.

Question 14

©VCAA 2018 Q24 ADAPTED **MEDIUM**

Monoclonal antibodies can be produced and used to treat different types of cancers. Which one of the following statements about monoclonal antibodies is correct?

A Monoclonal antibodies are lipid molecules.

B Monoclonal antibodies pass through the plasma membrane of a cancer cell and attach to an antigen within the cell.

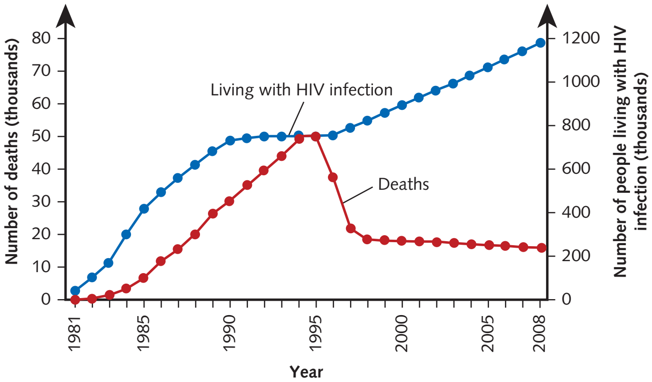
C Monoclonal antibodies are produced by fusing a B cell clone with cells extracted from a plasma cell tumour.

D Monoclonal antibodies produced to treat bowel cancer will be identical to monoclonal antibodies produced to treat throat cancer.

Question 15

©VCAA 2018 Q29 ADAPTED **MEDIUM**

The following graph shows the death rates from AIDS (acquired immune deficiency syndrome) and the number of people infected with the human immunodeficiency virus (HIV) in the period 1981–2008.



Centers for Disease Control and Prevention

Based on the information in the graph, you can conclude that

A a vaccination program for HIV was introduced in 1995 across many countries within a targeted population.

B more people were living with HIV infection in 1986 than were dying from the infection in 1995.

C HIV infection killed more people in 2005 than in 1985.

D before 1995, many people who were infected by HIV went on to develop AIDs, which led to their deaths.

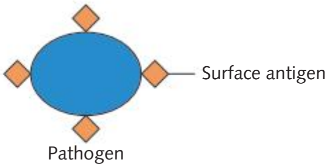
Short answer

Question 1 ©VCAA 2014 Q4 ADAPTED

a The term 'pathogen' is not a classification of any organism group. How is pathogen defined?

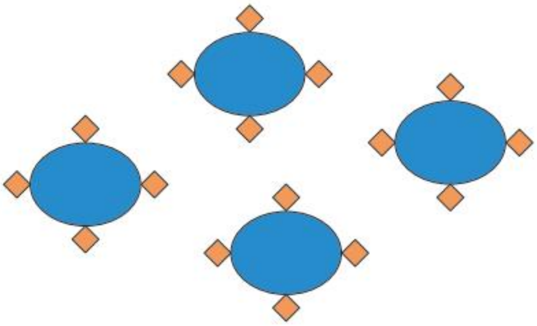
1 mark

The following diagram shows a generalised pathogen with antigens on its surface. The immune system responds to antigens by making antibodies.



b Draw an antibody that would be effective against the pathogen shown in the diagram above. Label the different parts of the antibody. 2 marks

Antibodies can work by forming antigen–antibody complexes. The following diagram shows four pathogens.



c **i** Illustrate on the diagram above how the antigen–antibody complex forms. Use at least four antibodies in your drawing.

2 marks

ii What is the purpose of the antigen–antibody complex?

2 marks

Question 2 ©VCAA 2018 Q3 ADAPTED

Many organisms, such as bacteria, fungi and viruses, can infect plant species. Plants do not have an immune system comparable to that evolved by animals. However, they have evolved physical barriers to stop invading pathogens from causing significant damage.

a Describe two physical barriers that a plant may have to protect itself from an invading pathogen.

2 marks

b Humans have a complex immune response to invading pathogens. State two physical barriers that the human body has to prevent pathogens from entering.

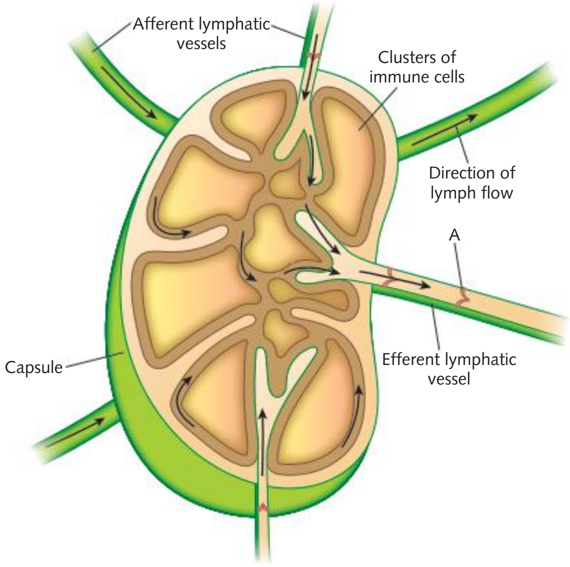
2 marks

c Once a pathogen has gained entry to the internal environment of a human, it can encounter macrophages and dendritic cells. Describe how these help defend the human body.

2 marks

Question 3 ©VCAA 2015 PART B Q5 ADAPTED

The following diagram represents a human lymph node.



a A substance enters the afferent lymphatic vessels and exits at the efferent lymphatic vessel. Name this substance.

1 mark

b Describe two roles of lymph nodes in the immune response.

2 marks

c One cell found within these clusters of immune cells in the lymph node has a large nucleus and extensive rough endoplasmic reticulum. Name this cell and explain its role in the adaptive immune system.

2 marks

Genetic changes in a population over time

9

By the end of this chapter you will have covered the following material.

Key knowledge

Genetic changes in a population over time

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles pp. 329–340; 343–346
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity pp. 341–343
- manipulation of gene pools through selective breeding programs pp. 346–349
- consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens pp. 349–357

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation pp. 339–340; 352–354
- identify independent, dependent and controlled variables in controlled experiments pp. 339–340; 352–354
- formulate hypotheses to focus investigation pp. 339–340
- predict possible outcomes pp. 339–340

Plan and conduct investigations

- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 339–340; 352–354
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 339–340; 352–354

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 339–340; 352–354
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 339–340; 352–354
- demonstrate ethical conduct when undertaking and reporting investigations pp. 339–340; 352–354

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 339–340
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 339–340; 352–354
- plot graphs involving two variables that show linear and non-linear relationships pp. 339–340

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 339–340
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 339–340; 352–354
- identify outliers, and contradictory or provisional data pp. 339–340; 352–354
- repeat experiments to ensure findings are robust pp. 339–340

Construct evidence-based arguments and draw conclusions

- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 339–340
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 339–340
- identify, describe and explain the limitations of conclusions, including identification of further evidence required pp. 339–340

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 339–340; 352–354
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 339–340; 352–354

Online Chapter Map
Chapter 9 map

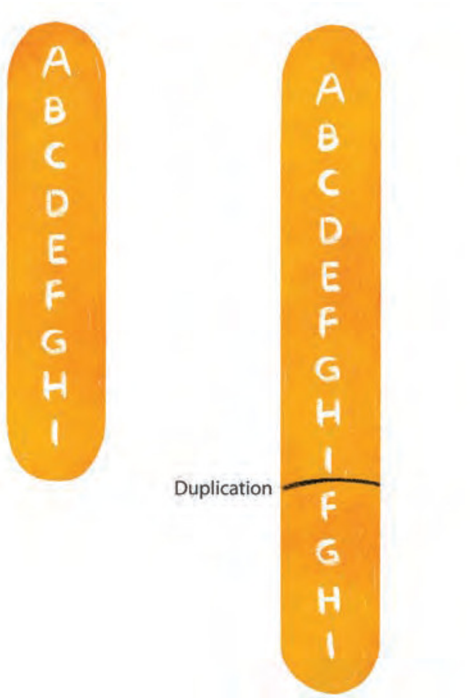
9 Genetic changes in a population over time

The gene pool of the Australian population today is very different from that of 100 years ago. New alleles have been introduced into the population by gene flow and genetic drift. Mutations have occured which would have introduced new alleles into the gene pool.

p. 329

9.1 Mutations are the source of new alleles

Mutations are permanent changes in DNA due to mistakes during replication. They are the source of new alleles and their effect can be neutral, harmful or advantageous for a species. They are the raw material for natural selection.



p. 333

9.2 Chromosomal mutations

Sometimes, whole chromosomes are rearranged. Bits of chromosomes break off and flip around, join to another chromosome, or become duplicated so there are many copies of the same gene. Sometimes due to incorrect separation of chromosomes in cell division, organisms may result with extra or fewer chromosomes, or whole sets.

p. 336

9.3 Changing allele frequencies in populations

The gene pool is made up of all the alleles in a population. Allele frequencies can change because of mutations, both gene and chromosomal; gene flow; migration into or out of a population; environmental selective pressures resulting in survival and reproduction of the better adapted individuals; and genetic drift in which factors cause random changes in the gene pool allele frequencies. because of genetic drift, when some alleles are not passed on and are lost from the gene pool; or when the population size changes dramatically.

p. 343

9.4 Natural selection

The allele frequencies of gene pools are altered by the process of natural selection. In a population with variation, the individuals with favourable traits (and alleles) that overcome selection pressures will survive and reproduce, passing those traits onto their offspring. This leads to a change in the gene pool of a population over time.

p. 346

9.5 Human manipulation of gene pools

Throughout history, humans have selected plants and animals with the most desirable traits for breeding. In this way, humans have selectively bred modern high-yielding crop varieties from wild species, hundreds of dog breeds with attractive features and horses for strength and speed. Humans are also reducing the capacity of wild species to adapt to changing conditions.

p. 349

9.6 Natural selection and consequences for disease

A consequence of natural selection is the emergence of antibiotic resistance in bacteria and 'superbugs', which are no longer controlled by antibiotics. New virus strains arise as a result of mutations and genetic rearrangements; new and updated vaccines are as important as ever.

Populations do not stay stable. Mutations introduce new alleles into populations. Natural selection acts on this variability to select the most well-adapted individuals to reproduce and pass on their alleles to a new generation. This has been going on for a very long time.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:

- Chapter 9 map (p. 326)

Online Key Terms:

- Flashcards (p. 328)

Weblinks:

- Natural selection (p. 344)
- Human impacts on Brazilian parrots (p. 348)
- Learn how influenza spreads (p. 355)

Online Worksheets:

- Natural selection (p. 344)
- Parrot gene pool (p. 348)

Online Key Concepts:

- Chapter 9: Summary of key concepts (p. 360)

Online Key Terms
Chapter 9 Flashcards

Know your key terms

alleles
amino acid sequence
antibiotic resistance
antibiotic resistance gene
antigenic drift
antigenic shift
artificial selection
beneficial mutation
block mutation
bottleneck effect
broad spectrum
chromosomal mutations
conserved
deleterious mutation
deletion mutation
double-strand break
duplication
extinct
fitness
fixed
founder effect
gene duplication
gene flow
gene pool
gene sequence
genetic drift
genotype
germline
heritable
horizontal gene transfer
insertion mutation
inversion mutation
missense mutation
monoculture
multidrug resistance
mutagen
mutation
natural selection
neutral mutation
nonsense mutation
phenotype
point mutation
population
population genetics
selection pressure
selective breeding
silent mutation
somatic
species
subspecies
substitution mutation
synonymous mutation
translocation

Remember

WB
REMEMBER
PAGE 181

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 A gene is a unit of inheritance. At the molecular level, a gene is a sequence of DNA that codes for a protein. Alternative forms of the same gene are called alleles.
2 Homologous chromosomes are matching chromosomes that have the same genes located at the same positions.
3 Genotype is an organism's genetic composition. Phenotype is how those genes are expressed in that organism.
4 Complementary base pairing in DNA is the pairing of the nitrogenous base adenine with thymine (or uracil in RNA) and cytosine with guanine.
5 Dominant alleles are represented by a capital letter whereas recessive alleles are represented by a lower-case letter.
6 Gene expression refers to the transfer of the DNA code in a gene, via transcription and translation, to ribosomes in the cytosol to produce a functional gene product, protein.
7 Natural active immunity develops when an organism comes into contact with a pathogen and develops memory B and memory T lymphocytes to the antigens of that pathogen or its products.
8 Antigens (weakened or dead microbes or their fragments) are introduced in vaccines. The body produces specialised lymphocytes (B plasma cells) and antibodies.
9 Herd immunity is developed when a large enough number of people in a population are vaccinated so that it halts the transmission of the disease to non-vaccinated people.

The peppered moth, *Biston betularia*, is widespread in the UK. Historically, the standard moth form, *typica*, was white, liberally speckled with black spots (Figure 9.1a). The dark *carbonaria* form (Figure 9.1b) was much rarer. During the 1800s, British cities and the countryside were transformed by the Industrial Revolution. Hundreds of coal-powered factories produced large quantities of airborne soot and other pollutants. By 1895, 95% of moths in industrial regions, such as Manchester, were the black *carbonaria* form. Lepidopterist J.W. Tutt proposed a link between the Industrial Revolution and changes in the moth population. The typical light-coloured tree trunks had become blackened by soot and now presented a new environment for the moth population. The black moths were better camouflaged against the black tree trunks than the common white speckled form. Consequently, the white speckled moths were easier for birds to see and were preyed on more than the black moths. Over time, black moths increased to become the main colour type in the populations.

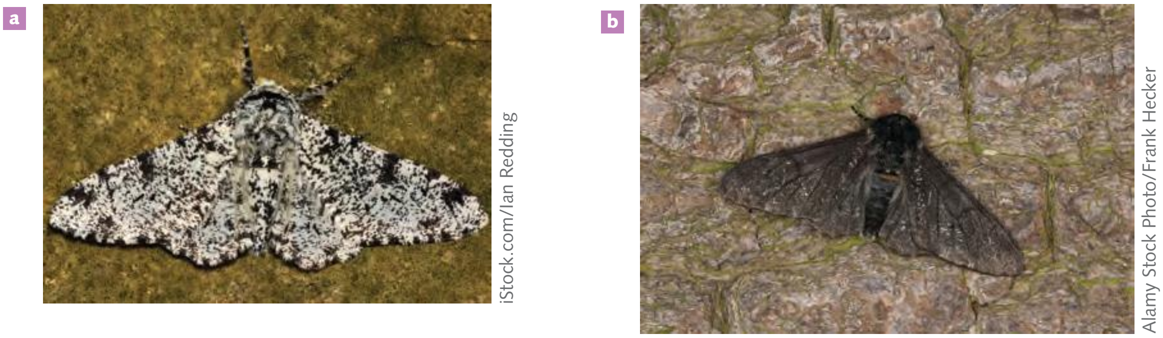


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Figure 9.1 The peppered moth, *Biston betularia*, has a **a** white speckled *typica* form and **b** dark *carbonaria* form.

In 1950, clean air legislation was passed, and tree trunks were no longer blackened with soot. Dark-coloured moths were again facing greater predation on the naturally white tree trunks and so they became less common. Both dark and white forms continue to exist in the population.

Individuals in any population, such as the peppered moths, have a range of different **phenotypes**, or characteristics expressed. This is because members of a population have variation in **genotypes**, or genetic make-up, that, together with environmental factors, results in variation in their phenotypes. This genetic variation is **heritable**; it can be passed to the next generation and under certain circumstances may give an individual an advantage in survival and reproduction compared to the rest of the population. In the peppered moth example, a **mutation** (genetic change) in genotype produced a dark-coloured form in this population. This dark phenotype conferred a survival advantage in the changed environment. The genotypic variation may also be a disadvantage or have no effect at all. Either way, genetic mutation introduces new alleles (alternative forms of a gene) and, therefore, new variation into populations.

9.1 Mutations – the source of new alleles

Phenotypic variation is crucial for a population to survive when changes occur in the environment. Consequently, genetic variation is essential for the survival of populations. New alleles generally come from existing alleles through mutation. Mutations are rare and barely noticeable in a large population but they are essential because they are the ultimate source of variation within a population.

Mutations are changes to DNA. A spontaneous mutation may arise from a mistake when DNA is copied during cell division, induced by physical or chemical agents called **mutagens**. Mutations may also arise through the action of biological agents, such as viruses that insert their genetic sequences into the host's DNA. Mutations that occur in genes sometimes result in changes to the translated proteins they code for. These changes may be subtle, or they may be severe with potentially catastrophic effects for the survival of the organism. Occasionally, the mutation enhances the function of the protein or makes the organism better suited to the environment.

CONNECT

Translation of proteins is discussed in Chapter 3.

The effect of a mutation also depends on whether it has occurred in non-reproductive (body, or **somatic**) cells or in reproductive (gametes, or **germline**) cells (Figure 9.2).

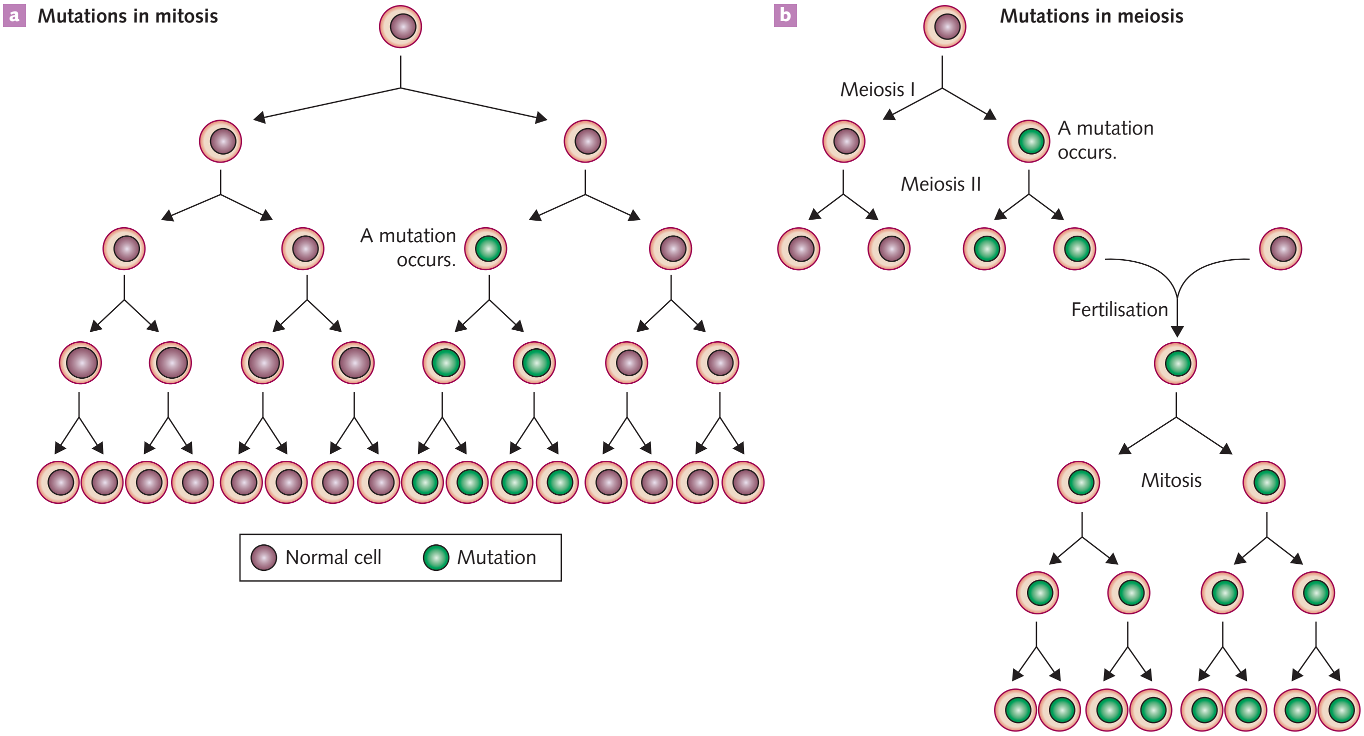

Figure 9.2 a Mutations in somatic cells affect only the cell in which it occurred and all its daughter cells. **b** Mutations in germline cells affect all body cells of the individual who inherits them.

A mutation in a somatic cell occurs only in the affected body cell and the daughter cells produced from it by mitosis. All other cells of that organism lack the mutation. Cancer is one possible outcome of mutations in somatic cells. The mutations accumulate in particular genes or regions of the DNA that accelerate the rate of cell division, abolish the cell's ability to undergo apoptosis or increase the rate of mutations within the cell.

Mutations that occur in germline cells affect gametes and can be inherited or passed on to the next generation so that they are incorporated into every cell of the offspring. Often, the germline mutation results in developmental abnormalities that cause the affected embryo or foetus to spontaneously abort. If carried through to birth, the germline mutation may result in congenital disorders in the offspring with varying severity. Occasionally, a gene mutation changes or enhances the function of the encoded protein, which, if circumstances suit, enhances the survival of the organism. If the mutation is consistently passed on from one generation to the next, a new allele has entered the population.

Point mutations

WB
9.1.1
POINT MUTATIONS
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The simplest form of mutation is a **point mutation** in which just a single nucleotide within the original DNA sequence is affected. If the point mutation occurs in a gene, the mutated **gene sequence** can be transcribed and translated into a protein that may be the same as that encoded by the original form of the gene, or it may be altered. When the protein is altered, the mutation may have a subtle or a dramatic effect on its structure and function.

Substitution

A substitution occurs when one nucleotide is replaced by another (e.g. adenine substituted by guanine). **Substitution mutations** have a number of possible effects on the translated protein.

A **silent mutation**, also referred to as a **synonymous mutation**, occurs when the substituted base results in a nucleotide triplet, or codon, that codes for the same amino acid as the original. For example, AGA and AGG both specify for the addition of an arginine amino acid in the polypeptide chain (Figure 9.3). Therefore, the protein encoded by the mutated gene is identical to that encoded by the original gene. Silent mutations are possible because there is a level of redundancy in the genetic code. Recall that the genetic code consists of 64 codons that code for 20 amino acids plus the instructions to start and stop translation. Therefore, any individual amino acid can be encoded by more than one codon.

A **missense mutation** arises when a single nucleotide substitution changes the amino acid. For example, substitution in an AGA codon to generate an AGC codon results in a serine amino acid being added to the polypeptide instead of the original arginine (Figure 9.4).

A **nonsense mutation** occurs when a single nucleotide substitution creates a new stop codon within the original gene sequence; for example, substitution in a GAG codon to generate a TAG codon (Figure 9.5). This leads to early termination of translation of the transcribed gene sequence because the remaining sequence downstream of the new stop codon is not translated. This results in an incomplete polypeptide.

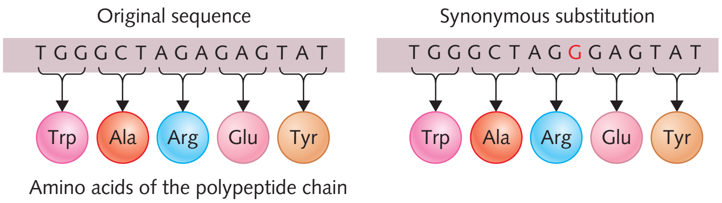

Figure 9.3 A silent mutation

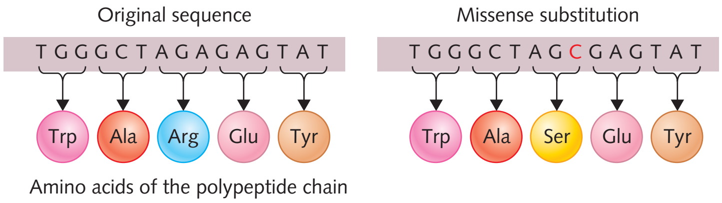

Figure 9.4 A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.

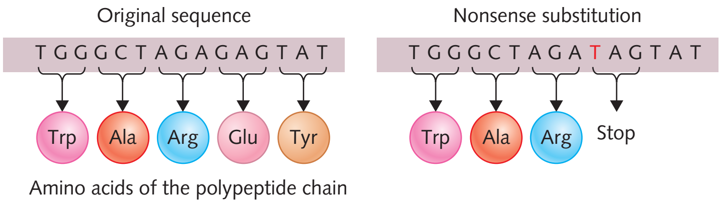

Figure 9.5 A nonsense mutation in the gene sequence results in premature termination of translation.

Insertions and deletions

An **insertion mutation** occurs when one or more nucleotides are added at a site within the original gene sequence. A **deletion mutation** occurs when nucleotides are lost from a site within the original gene sequence. The effect of the insertion or deletion is frequently a frameshift mutation, in which the reading frame for the corresponding amino acids has been shifted away from the original and all the codons downstream of the mutation are affected. The consequence for the translated protein is that the amino acids downstream of the mutation bear no resemblance to those of the original polypeptide (Figure 9.6). It is also probable that a new stop codon will be introduced in a different position from that in the original gene sequence. Under such circumstances, even a single nucleotide insertion or deletion can have a profound effect on the corresponding protein.

WB
9.1.2
CYSTIC FIBROSIS MUTATION
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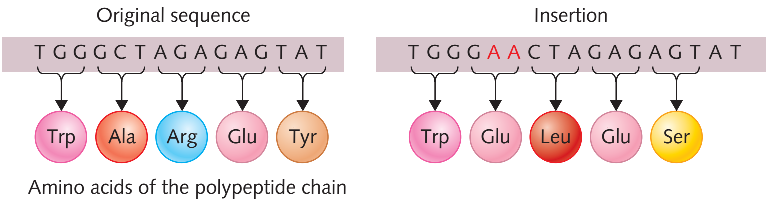
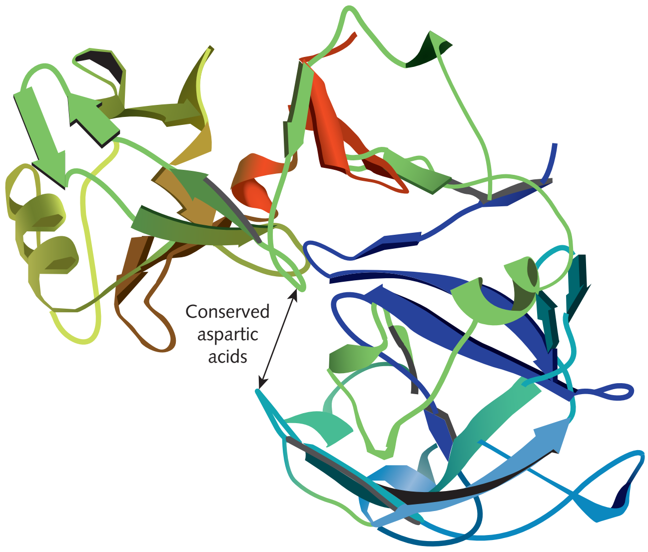


Figure 9.6 An insertion in the gene sequence results in a frameshift mutation. Here, two adenines have been inserted.

Mutations affect protein structure and function

A protein's function ultimately depends on its shape, which is determined by the protein's primary structure. Although mutations affect protein primary structure directly, it is how they influence protein folding that ultimately impacts on an organism's survival.

CONNECT
Revise protein structure on page 57



Science Photo Library/Laguna Design

Figure 9.7 **Human pepsin, an example of an aspartyl protease. The aspartic acid residues in the active site are conserved in the aspartyl proteases of fungi and animals.**

Some regions of a protein's three-dimensional structure are much more sensitive to alteration than others. Changes to the amino acids lining the active site of an enzyme can profoundly influence its function. Even a single missense substitution that leads to the loss of an enzyme's function is likely to be harmful. The critical amino acids involved in binding substrates or carrying out reactions in the active site of enzymes are often **conserved**. These amino acids are retained in the protein over time and across different species, whereas other amino acids have become substituted. For example, the **amino acid sequences** and three-dimensional structures in the active site of a family of enzymes called the aspartyl proteases are consistent across diverse eukaryotic organisms from fungi to mammals, even though the details of the rest of the proteins vary considerably (Figure 9.7).

Effects of mutations on survival

A protein's function depends on its structure. Mutations that change a protein's structure can affect protein function, impacting on the organism's survival. Therefore, mutations can also be classified according to how they affect the protein's function and expression and whether the organism's survival is unchanged, changed for the worse, or changed for the better.

Neutral mutations

Silent mutations do not change the protein product, so the organism's survival is unaffected. This is a **neutral mutation**. Missense substitutions are sometimes also neutral mutations, provided that the original amino acid is swapped with another that has similar properties. For example, in the ABCA1 gene, which codes for a protein involved in cholesterol transport, a missense substitution in a single GAA codon generates a GAC codon. This causes the amino acid glutamic acid to be swapped for an aspartic acid. Both amino acids are negatively charged and reside on the surface of the protein where they interact with surrounding water, so the properties and function of the protein remain essentially the same.

Deleterious mutations

Living organisms are very complex and random mutations that disrupt the function of an encoded protein can affect the whole organism, undermining the organism's overall ability to carry out its basic processes and survive. Such mutations are referred to as **deleterious mutations**. Most mutations are deleterious.

Nonsense mutations are typically deleterious because they result in the production of an incomplete protein that is non-functional. However, these deleterious mutations may persist if the individual who carries them also has a copy of the normal allele that encodes for the functional version of the protein. The deleterious mutation is thus masked within the phenotype of the organism. If the organism only has two non-functional alleles for a particular gene, the condition usually results in the death of the organism before they have the opportunity to reproduce and pass the alleles onto any offspring.

Beneficial mutations

Occasionally, gene mutations generate a new allele that benefits the survival of the organism. The type of **beneficial mutation** can vary; it could be a missense mutation that changes the function of the original protein, or it could be a nonsense mutation that eliminates a protein that may have been harmful to the organism in some circumstances.

Many mutations produce recessive alleles that can be masked by the effects of the original allele, which becomes the allele for the dominant phenotype. Each human may carry several hundred mutations, most of which will never be noticed, particularly if they have children with partners who are not closely related.

Conversely, recessive alleles are an important source of variation within populations. This was the case with the peppered moth in the United Kingdom. Before the Industrial Revolution, the dominant *carbonaria* forms were extremely rare. During the Industrial Revolution, alleles coding for the recessive white (*typica*) trait survived mainly in heterozygous members of the population at a low level. Only the extremely rare homozygous individuals experienced the selective pressure of increased predation.

Sexual reproduction has been key to producing populations with variation, through the random mixing and assortment of traits from one generation to the next through meiosis and chance fusion of gametes.

KEY CONCEPTS

- » Mutations are changes in DNA. Mutations occur spontaneously or are caused by mutagens. The potential effect of a mutation depends on whether the mutation occurs in somatic or germline cells.
- » Point mutations can cause changes in a DNA sequence by substitution, insertion or deletion of a nucleotide.
- » Substitutions can be silent mutations, which cause no change in the encoded protein, or they can be missense or nonsense mutations, which alter the structure and function of the encoded protein.
- » Insertions or deletions can cause frameshift mutations that affect the amino acid sequence downstream, severely affecting the encoded protein.

Concept questions 9.1

1 Mutations can be beneficial, harmful or neutral. What does this mean?

2 What is the difference between somatic cells and germline cells?

3 Describe three possible effects a mutation could have on an organism's survival.

4 A frequent effect of a frameshift mutation is to produce a stop codon earlier than normal. What effect would this have on the structure and function of the encoded protein?

5 List the types of point mutations in the order of potential severity of their effects from least to most severe.

HOT Challenge

6 Variation in a species is a function of mutations and can be vital for a species' survival. Greater variation occurs within species that engage in sexual reproduction. How could sexual reproduction produce greater variation?

9.2 Chromosomal mutations

In addition to gene mutations, genetic variation can be driven by wholesale changes to the chromosomes. Alterations to chromosomes contrast with single point mutations because they can affect many genes simultaneously. Rearrangements of whole segments of chromosomes are described as **block mutations**. Some of the variations that occur with chromosomes, such as chromosome number, are natural in certain situations and are therefore integral to the functioning and continuity of the species. Others arise because of anomalies that occur during cell division or the formation of the gametes.

WB
9.2
CHROMOSOMAL
REARRANGEMENTS
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Block mutations

Block mutations lead to changes in chromosome structure. A block mutation may occur within a single chromosome or between different chromosomes. In the process, a segment of a chromosome containing multiple genes may be lost, duplicated, switched in orientation, or swapped between chromosomes.

Deletions

A chromosome may undergo **double-strand breaks** at two positions and the section in between may drop out, removing all its genes with it. If the two ends then re-join, a shorter chromosome results with a segment missing. This is called a chromosome deletion (Figure 9.8a). It can have a profound effect on the development of an organism because it leads to an absence of certain genes. All but the shortest deletions are usually fatal and the few that are not fatal are associated with adverse effects.

Inversions

Another kind of chromosomal rearrangement occurs if a chromosome breaks in two places and the middle segment rotates 180° before being re-joined within the chromosome. This reverses the normal sequence of genes (Figure 9.8b) and is called **inversion**. The effects of inversions are usually less dramatic than other types of chromosomal changes because genes have been neither gained nor lost and the genes within the inverted segment can still function normally. However, the inversion may disrupt a gene through which it occurs or cause two different genes to be fused together. Also, if the chromosomes do not align properly for meiosis, the affected individual may have reduced fertility.

Translocations

Sometimes a section of one chromosome breaks off and attaches to another chromosome. This is known as **translocation** (Figure 9.8c). In humans, translocation can occur between chromosomes 8 and 14. Normal control over the genes in that segment is lost, often resulting in a form of cancer.

Duplications

A **duplication** occurs when an extra copy is made of a section of chromosome and inserted into the same chromosome or another chromosome (Figure 9.8d). Gene sequences can be replicated many, sometimes thousands, of times. Like other chromosomal abnormalities that change the number of copies of particular genes, duplications are frequently harmful. However, on occasions, they can be advantageous. The various genes that control the different haemoglobins produced in human red blood cells are thought to have arisen by duplications.

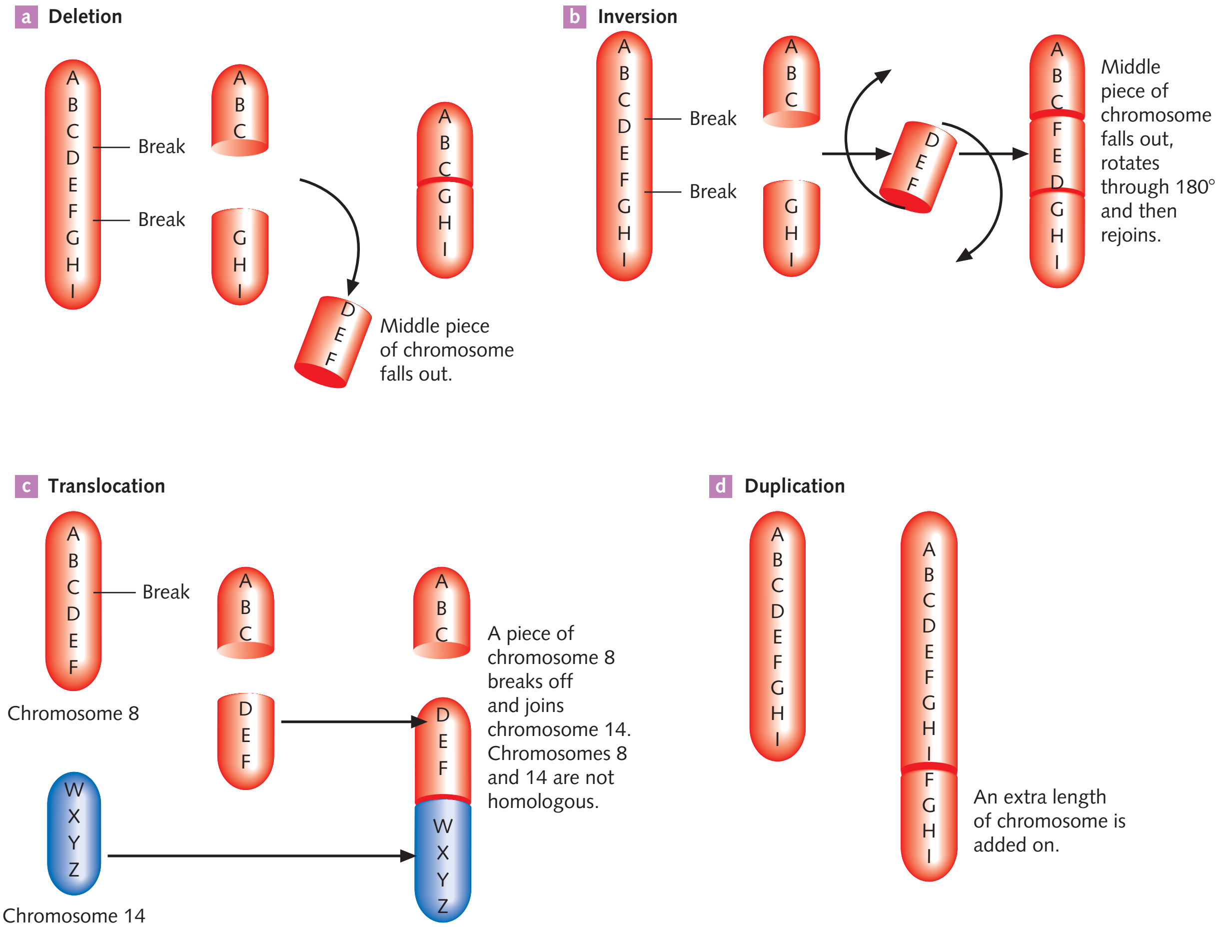


Figure 9.8 Abnormalities caused by chromosomal or 'block' mutations may arise by **a** deletion, **b** inversion, **c** translocation or **d** duplication.

Changes in chromosome numbers

As well as changes in structure due to block mutations, the genetic make-up of an organism can change due to gain or loss of whole chromosomes or sets of chromosomes. These mutations are called chromosomal abnormalities or **chromosomal mutations**. They usually occur due to failure of the chromosomes to separate correctly during meiosis (called non-disjunction) resulting in gametes with more or fewer chromosomes than normal and therefore more or fewer genes for particular traits. Such mutations would alter the frequency of the alleles for those genes in the gene pool.

There are two types of chromosomal mutations: aneuploidy and polyploidy.

Aneuploidy is a condition where there is one extra or one less chromosome in the genome. Compared to the normal human chromosome number of 46 per body cell (2n=23), Down syndrome individuals have 47 with an extra chromosome 21; Turners syndrome females have 45 with one less X chromosome; and Klinefelters syndrome males have 47 with an extra X chromosome.

Polyploidy is a condition in which individuals have more than the normal 2 sets of chromosomes. This would alter the allele frequencies in the gene pool of a population. There are few surviving cases of polyploidy in animals and it is a fatal condition in humans so the few that are born die very early. However, it is common in plants, in which the plants often thrive with such a condition. Examples include macaroni wheat which has 4 sets of chromosomes and bread wheat has 6 sets of chromosomes.

Acquiring new genes

A combination of block and point mutations generate entirely new genes coding for novel proteins. New genes arise primarily through **gene duplication** and subsequent point mutation. This contrasts with the appearance of new alleles, which arise by point mutation in an existing gene. The gene duplication event is normally the result of chromosomal duplication. Following gene duplication, one of the gene copies usually retains its original function. The fate of the other copy can vary (Figure 9.9). In some cases, the copy

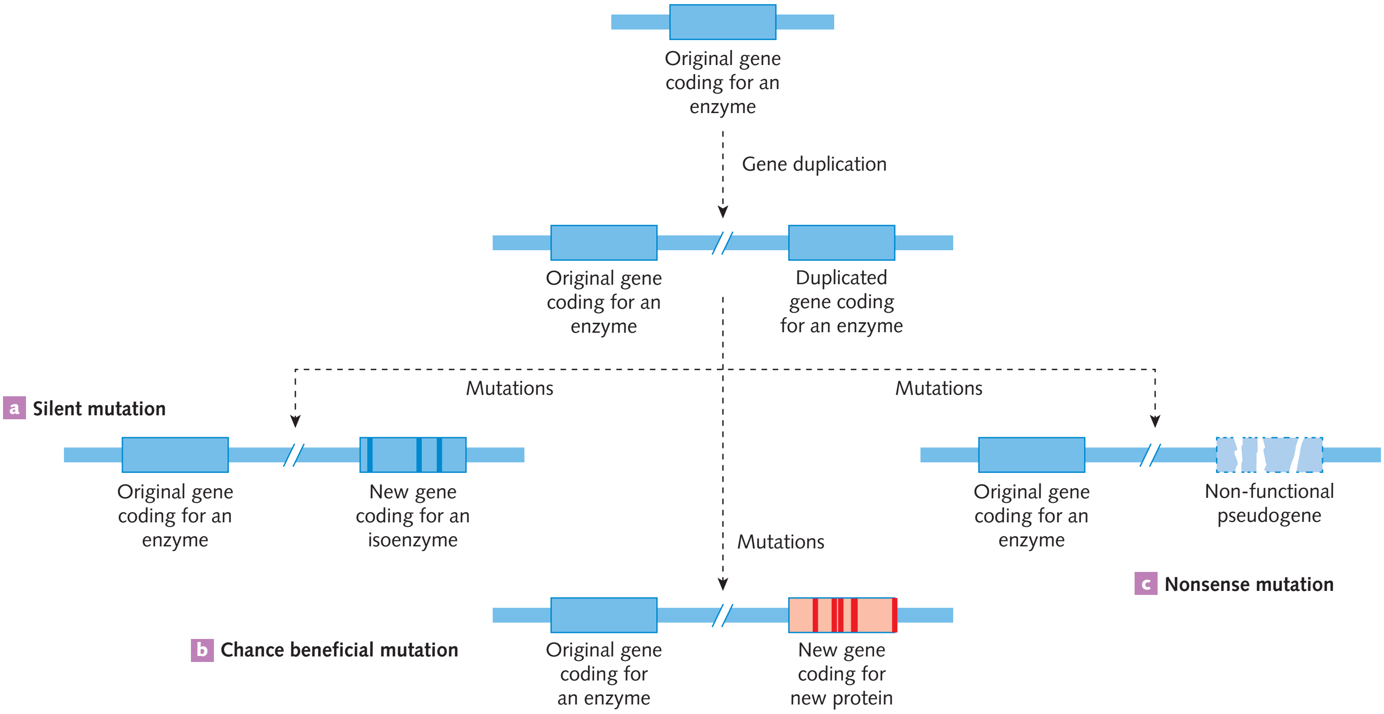


Figure 9.9 An example of duplication and subsequent mutation of a gene coding for an enzyme. **a** Silent mutations result in two genes for enzymes with the same function, so-called 'isoenzymes'. **b** Chance beneficial mutations may result in a new gene for a protein with a novel function. **c** Nonsense mutations in a duplicated gene may result in a non-functional pseudogene.

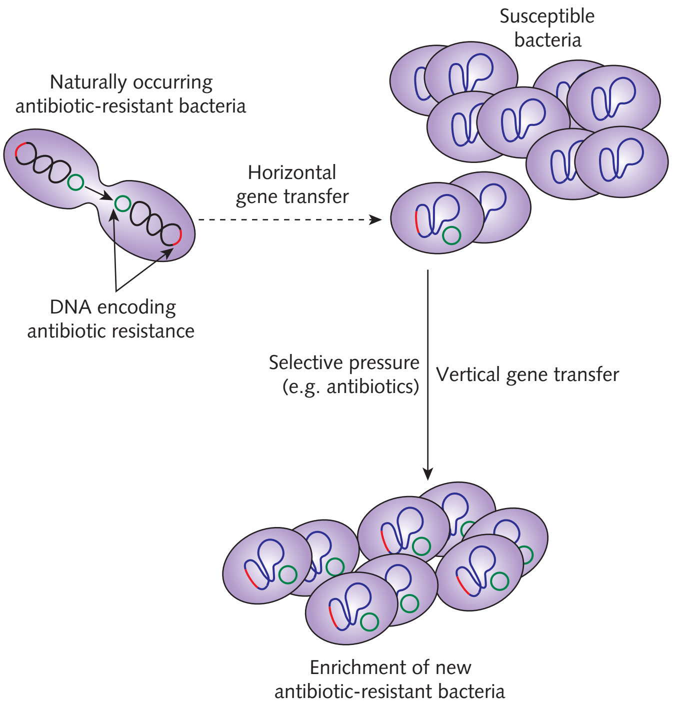


Figure 9.10 New species and strains of antibiotic-resistant bacteria are sometimes generated by horizontal gene transfer.

fortuitously mutates into a new gene, and the consequent new protein has an innovative property or function that benefits the organism.

Gaining new genes by horizontal gene transfer

Genes for proteins with novel properties can also arise by **horizontal gene transfer**. This is the process by which an organism obtains a new gene directly from another organism, even another species, rather than by mutation or inheritance from a parent.

Horizontal gene transfer is common in prokaryotes. Horizontal transfer mechanisms include acquiring naked pieces of DNA from their environment, new genes through infection by a virus (bacteriophage), or new genes directly from another bacterium through cell-to-cell transfer (conjugation). Conjugation enables genes that enhance survival to spread through a population of bacteria in a relatively short time. It is believed to be one reason for the emergence of new strains of multi-drug resistant bacteria (Figure 9.10).

In contrast, vertical gene transfer is when genetic material is transferred from parent to offspring.

KEY CONCEPTS

- Mutations can cause changes in chromosome structure and chromosome number.
- Deletions, inversions, translocations and duplications may involve particular segments of a chromosome and may change the number of alleles in affected cells.
- Mutations can be silent, nonsense, beneficial or disadvantageous.
- New genes may be acquired by gene duplication and subsequent point mutation or by horizontal gene transfer.

Concept questions 9.2

1 What are homologous chromosomes?
2 When would chromosomal rearrangements occur in the production of a new organism?
3 Draw a diagram to show the four main types of mutations that affect whole segments of chromosomes. Which would have the most severe effect on an organism?
4 Contrast how a new allele and a new gene may be introduced into a population.
5 Contrast the acquisition of a new gene by horizontal gene transfer with that by gene duplication.

HOT Challenge

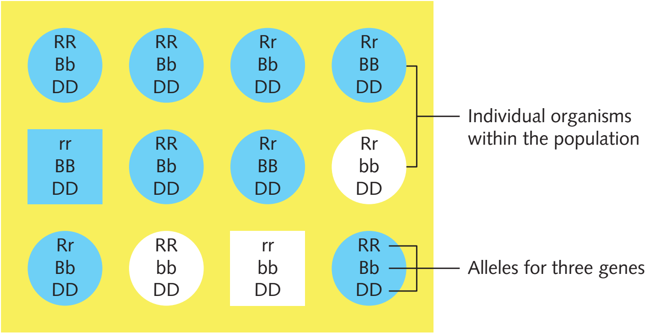
6 Most eukaryotic organisms show diploidy. Polyploidy is relatively common in flowering plants but is lethal in humans. How can diploidy mask mutations in the genome?

9.3 Changing allele frequencies in populations

Mutations introduce new variations into a population, and so can many external influences. The collection of alleles within a population is shaped by the movement of individuals and by environmental events that can sometimes rapidly and considerably change the composition of populations. The study of allele frequencies in populations and how they change over time in response to various environmental circumstances is called **population genetics**.

Gene pools

Genes are the means of transmitting the genotype from one generation to the next. Many genes exist in different forms as alleles, and the characteristics of individuals are largely determined by the alleles they inherit. Sometimes there may only be one version of a gene (one possible allele), or there may be many versions or alleles of a gene (many possible alleles). The variation in alleles carried by different individuals leads to most of the variation in a population. The total collection of all alleles for all genes within a population is referred to as a **gene pool** (Figure 9.11). In biological terms, a **population** is a group of individuals of the same **species** that live in the same geographical area and readily interbreed to produce fertile offspring, so they share the same gene pool.



Alleles: R = round, r = square; B = blue, b = white; D = broad

Figure 9.11 **A simple example of a gene pool depicting three genes. For the third gene, the allele D is fixed in the population.**

The range of variation possible in a population is restricted by the genes and the alleles available in its gene pool. For example, frill-necked lizards do not carry genes for characteristics related to wings or hard-shelled eggs or for the enzymes required to synthesise chlorophyll or to digest cellulose. However, all frill-necked lizards carry genes for a tail, rudimentary teeth, scales and four legs.

Many genes may have only one possible allele in a gene pool. Such genes do not contribute to any variation and the incumbent allele is said to be fixed in the population (Figure 9.11). Scientists believe that 80–85% of human genes are fixed in this way. For variation to occur in phenotypes, more than one allele of a gene must exist. These alleles occur because of mutations that have generated different versions of the gene.

Allele frequencies refer to the proportion of alleles of each type at a particular gene position (locus) in a gene pool. If there are 2 alleles for a gene that codes for a particular characteristic (eg. R and r), their frequencies could be calculated by totalling the number of a particular allele (eg. R) and dividing it by the total number of alleles (r+R) in the population. The allele frequencies are usually expressed as percentages or fractions. If you examine figure 9.11, the number of alleles of R=15/24=62.5% and the allele frequency of r=9/24=37.5%

The frequency of different alleles is not usually constant and can be affected by further mutation of an allele, immigration of individuals into the population, emigration of individuals out of the population and environmental selective pressures. This results in survival and reproduction of the better adapted organisms and random changes in allele frequencies due to chance events and genetic drift.

Migration and gene flow

In a biological sense, populations are defined by their reproductive and genetic isolation. Few populations are completely isolated from each other, and generally some migration takes place both into and out of the population. **Gene flow** is the transfer of alleles that results from emigration and immigration of individuals between different populations. The flow of genes occurs if the migrants to a new population breed. Immigrants may add new alleles to the gene pool, and emigrants may completely remove some alleles or significantly change the frequency of others (Figure 9.12).

Humans are polymorphic for the ABO blood types; that is, there are several different blood types. Some alleles are present in Indigenous Australians at different frequencies from other populations in the world. Indigenous Australians have largely been isolated for at least the last 50 000 years, except for some gene flow from Asia and New Guinea in the northern regions of Australia. Most Indigenous Australians do not have the B allele of the ABO blood group that results in either the B or the AB blood type. The B allele occurs at a frequency of up to 10% in European populations and up to 20% in Asian populations. The overall frequency of the B allele is increasing within the Indigenous Australian population as a result of migration from Asia and Europe into Australia and the gene flow between these populations. The most common allele

WB
9.3.1
FOWLER'S TOAD AND THE AMERICAN TOAD
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EXAM TIP
Remember that 'gene flow' refers to the movement of alleles between populations. Gene flow is not simply another term for the migration of individuals.

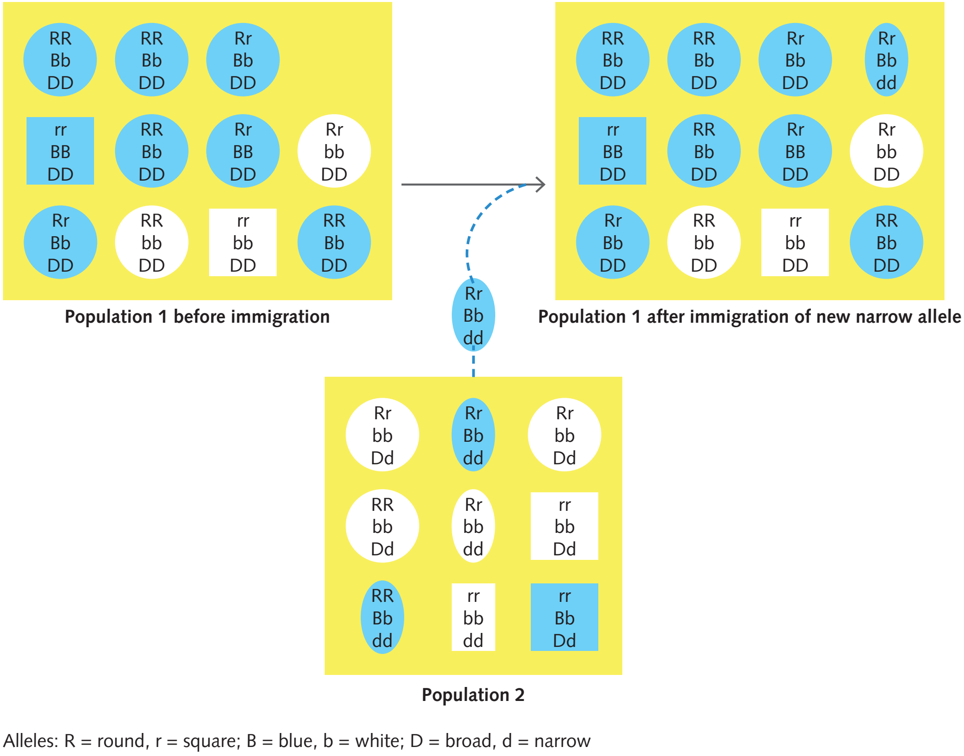

Figure 9.12 A new allele d enters Population 1.

in the Australian population and the world today is the O allele. The A allele is the most ancient allele and evolved before the human species diverged from its hominin ancestors.

Genetic drift

The term **genetic drift** applies generally to random changes in allele frequencies in a gene pool due to a chance event, especially affecting that of a small population. Every reproductive event involves chance. Each of us inherited half our alleles from our mother and half from our father. Which half of their alleles our parents passed on to us was a matter of chance. It depended on the random assortment of chromosomes and recombination during meiosis and whichever gametes met at fertilisation. In large populations, this randomness in inheritance of alleles is not noticeable overall and the allele frequency of the gene pool tends to remain fairly stable. But if a population is small, there is a chance that some alleles present in a parental group will not be passed on at all. For example, if a family group of koalas in a small area of bushland died in a bushfire. These alleles may be permanently lost from the gene pool (Figure 9.13). Alleles may be easy to lose, but they are virtually impossible to replace.

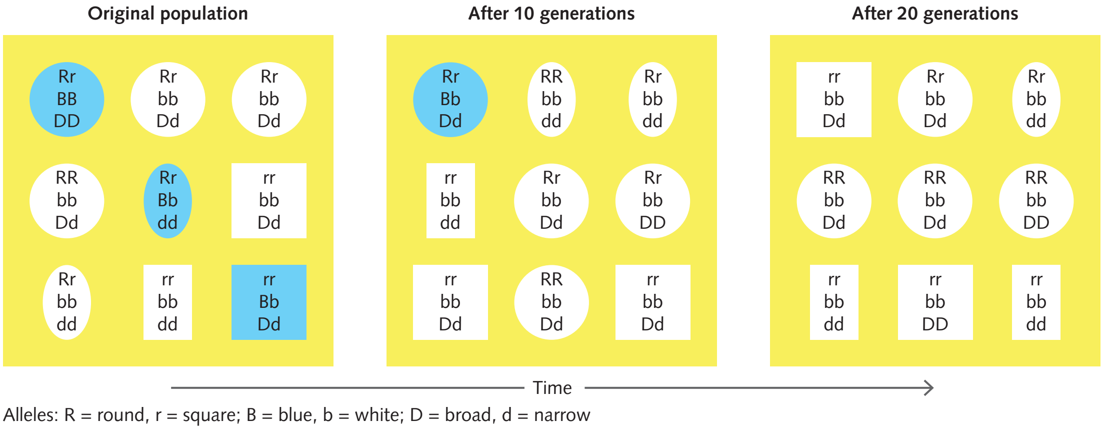

Figure 9.13 Allele b for colour has become fixed in the population as a result of genetic drift.

Genetic drift can occur in a small population or when a large population is suddenly reduced by a catastrophic event. This can give rise to a **bottleneck effect**. When a small group of individuals migrates and establishes a population in a new location, the **founder effect** may occur.

Developed by Southern Biological

INVESTIGATION 9.1

Natural selection

Aim

To explore how hatching viability of brine shrimp (*Artemia* species) is affected by different saline level environments

Time requirement

45 minutes

Materials

- Brine shrimp eggs (cysts)
- 0.5%, 1.0% and 2.0% solutions of salt water
- Distilled water
- 4 Petri dishes
- Fine brush
- 4 Microscope slides
- 4 Strips of double-sided tape
- Stereo microscope
- Permanent marker
- Graduated cylinder

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Brine shrimp may cause an allergic reaction.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at the end of the activity. Inform your teacher if you have an allergy.

Method

Preparing eggs for hatching (Day 1)

1. Using a permanent marker, label four Petri dishes: 0%, 0.5%, 1.0%, 2.0%.
2. Form your hypothesis; for example, ‘If the salinity of the hatching solution is 2%, then more cysts will hatch and thrive.’
3. Using the graduated cylinder, measure 30 mL of each saline solution and pour it into the appropriately labelled Petri dish.
4. Collect four microscope slides. Measure and cut four 1.5 cm strips of double-sided tape and gently adhere one of them to each of the microscope slides.
5. Lightly touch the fine brush to the side of the dish containing the brine shrimp eggs. Collect 20–30 eggs on the brush. Do not collect too many eggs because you will be required to count them.
6. To adhere the eggs to the double-sided tape, lightly press the brush onto the tape on the first microscope slide. Repeat this step for the remaining three microscope slides.
7. Using a microscope, count the number of eggs on the first slide. Record this information in the results table.
8. Once the eggs have been counted, place this slide into the 0% salt solution Petri dish. Place the slide with the tape side facing up.
9. Count the eggs on each slide and place them in the respective salt solutions. Copy Results table 1 into your logbook and record the egg count information (at 0 hours).
10. Place the Petri dishes under a light bank for 24 hours at room temperature.

Data collection (days 2 and 3)

1. After 24 hours, examine the contents of each Petri dish under the stereomicroscope. You should see that some brine shrimp have hatched and are swimming in the salt solution. Record the number of eggs, the number of dead or partially hatched eggs and the number of swimming brine shrimp information in your results table.
2. After 48 hours, examine the contents of the Petri dishes again and record your observations in the results table. Calculate the hatching viability of each dish at 48 hours by dividing the number of shrimp swimming by the initial number of eggs in the Petri dish. Round up your calculations to the nearest hundredth and add this information to the class results table.
3. Draw a bar graph that shows the sample means from the class results.

Results

Results table 1 **Hatching viability of brine shrimp at varying levels of salinity**

NaCl (%)	0 h	24 h			48 h			
	No. eggs	No. eggs	No. dead or partially hatched	No. swimming	No. eggs	No. dead or partially hatched	No. swimming	Hatching viability (%)
0								
0.5								
1								
2								

Results table 2 **Class results of hatching viability of brine shrimp in varying levels of salinity**

		Salinity (%)			
		2	1.5	0.5	0
Hatching viability	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
Calculation	Mean hatching viability				

Draw a graph of your results, ensuring you include labels.

Discussion

1 Describe two conditions that were controlled in this experiment.
2 Which Petri dish had the highest hatching viability? Which had the lowest? Suggest possible reasons for these results.
3 Was your hypothesis supported? Explain why.
4 Based on your individual data and the class data, is there enough evidence to conclude that environments of different salinities affect the hatching viability of brine shrimp?

Taking it further

What other conditions may affect the hatching viability of brine shrimp? Design an experiment to investigate another environmental factor that may impact hatching viability.

Bottleneck effect

WB
9.3.2
BOTTLENECK
EFFECT
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Sometimes a catastrophic event or a period of adverse conditions drastically reduces the size of a population. In this scenario, certain alleles may be lost through chance (Figure 9.14). If a portion of the population survives the catastrophe, the original population's gene pool cannot be recovered. The expanded population can only carry the alleles that existed in the population that survived the event. Therefore, the gene pool will now carry an indication of the bottleneck that occurred long after the population has recovered.

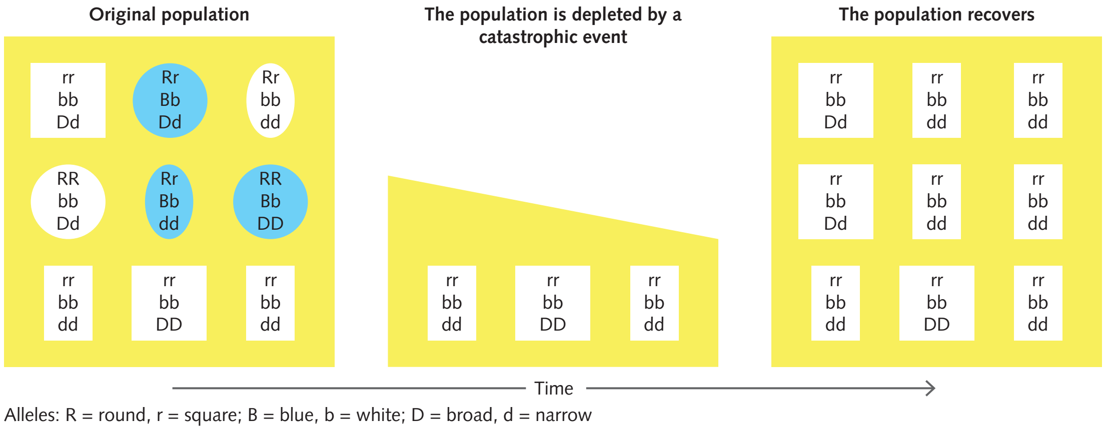

Figure 9.14 When the population recovers from a bottleneck effect, the gene pool is less diverse than that of the original population.

Cheetahs are an endangered species that have survived a genetic bottleneck (Figure 9.15). In a declining population, parents mated with their own offspring, and the resulting generations were left with strikingly similar alleles. One of these is a mutated allele with negative effects on fertility. Typically, a male cheetah's sperm count is low and 70% of the sperm are abnormal. Other shared alleles result in lowered resistance to disease. Infections that are seldom life-threatening to other cat species can be lethal in cheetahs. Today, there are only about 7000 cheetahs left in the world.


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Figure 9.15 Cheetahs survived a severe bottleneck that increased the frequency of some mutated alleles.

Founder effect

The founder effect is a particular example of gene flow. A few individuals who move to a new area and become isolated from a larger population might not carry all the alleles that were present in the original population (Figure 9.16). This means that the isolated population has less genetic diversity than the original population and recessive alleles that may be deleterious have a higher chance of meeting during fertilisation than they did in the original population.

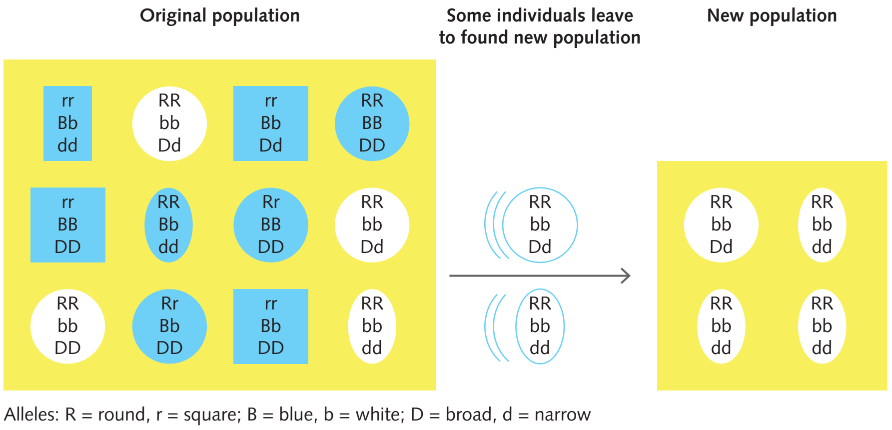

Figure 9.16 The founder effect occurs when individuals migrate to an isolated area and form a new population with allele frequencies different from the original population.

This effect has been observed in human populations when small groups of people with particular religious or ethnic backgrounds have settled somewhere new and mixed very little with other populations. About 200 people originally settled the Amish community of the USA, and at least one of the settlers had a recessive allele for Ellis–van Creveld syndrome. This syndrome has been relatively common among Amish people of this region ever since. Ellis–van Creveld syndrome includes symptoms of dwarfism, polydactyly (extra toes or fingers, Figure 9.17) and sometimes a hole in the heart.

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Figure 9.17 Examples of polydactyly, one of the symptoms of Ellis–van Creveld syndrome

KEY CONCEPTS

- Genetic drift is the change in allele frequency in a population due to the random assortment and selection of certain genes during meiosis and fertilisation.
- The bottleneck effect occurs when an event causes a large reduction in the gene pool of a population, decreasing genetic diversity in subsequent generations.
- Gene flow results from the transfer of alleles into or out of a gene pool because of the migration of individuals between populations.
- The founder effect occurs when a small number of individuals, carrying a restricted number of alleles, form a new population with reduced genetic diversity compared with the original population.

Concept questions 9.3

1 Recall the relationship between genotype and phenotype.
2 Distinguish between a gene and an allele.
3 Outline why variations have to be inheritable for them to be relevant to evolutionary change.
4 Define the following terms and describe examples of where these processes may have occurred.
  a Founder effect
  b Genetic drift

5 Define 'population' and 'gene pool'. Describe the mechanisms that can lead to changes in the gene pool of a population.

HOT Challenge

6 a Outline how gene flow can affect allele frequency.
  b How can small populations have low allele frequency?
  c For a population that is in genetic equilibrium, the sum of the frequencies of all the alleles is 100%. The conditions to maintain this equilibrium are no mutations, no gene flow, large population, random mating and no natural selection.
    i Is this a realistic situation in a population?
    ii Could a population in genetic equilibrium be achieved through the founder effect?
    iii What are some possible effects of achieving a genetic equilibrium in a population if this was possible?
    iv How might a bottleneck effect change the genetic equilibrium of a population?
    v Alleles that are removed from a population are not easy to replace. What might be some of the processes that could restore more genetic diversity to a population?

9.4 Natural selection

The allele frequencies in gene pools can change as a result of mutations, migration and chance events. Environmental factors also play a role in altering gene pools. **Natural selection** is the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals, leading to changes in the gene pool of a population. Natural selection is the mechanism that drives evolution. Through natural selection, favourable traits are selected for, inherited and become more common in subsequent generations. The capacity of an individual to survive and reproduce is sometimes referred to as its **fitness**.

CONNECT
Evolution is discussed in Chapter 10.

Selection pressures

Selection pressures are environmental or ecological factors that promote the survival of some individuals in a population over others. When conditions are favourable, most members of a population prosper. However, if environmental conditions become adverse, individuals of the population compete to survive and reproduce. Those individuals unable to endure the environmental challenge (the selection pressure) tend to die young, leaving no or very few offspring. In effect, the selection pressure weeds out the inadequate members of the population and their traits. Those individuals that endure the environmental challenge have a selective advantage; they prevail in the 'struggle for survival' and are prone to reproduce and leave relatively more offspring. The next generation predominantly inherits the characteristics of the survivors. The characteristics of the subsequent generation are better attuned to survive the selection pressure. If the selection pressure persists for many generations, the population becomes adjusted, or 'adapted', to cope with it.

Selection pressures include predator–prey interactions, such as the case of the peppered moth outlined at the beginning of this chapter. Other examples are competition between species for food or territory; competition within species for food, water, territory, mates or breeding sites; and the differing susceptibility of members of a population to an environmental stressor, such as heat, poisons or disease.

WB 9.4.1 SELECTION PRESSURES PAGE 193

Principles of natural selection

Selection pressures act on phenotypes but they lead to changes in the gene pool. The processes of natural selection and genetics allow us to make some key propositions.

1 Individuals differ from one another; that is, individuals within populations show variation.
2 Many of these variations are caused by mutations that create new alleles. All the alleles are heritable.
3 In general, more offspring are born than can survive to maturity so only some organisms survive to reproduce.
4 Some individuals have traits that make them more suited than others to their environment; those individuals with a selective advantage are better able to reproduce and pass on their alleles to the next generation.

Natural selection is thus represented in Figure 9.18.

WB 9.4.2 PRINCIPLES OF NATURAL SELECTION PAGE 194

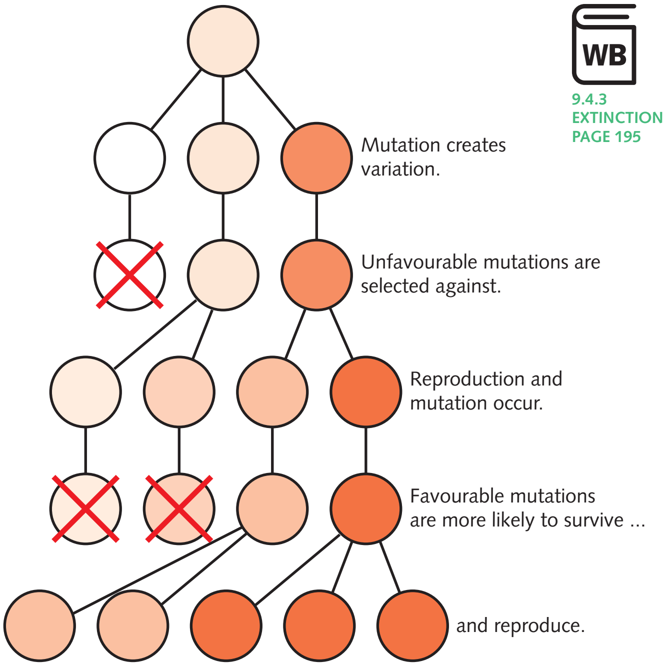

Figure 9.18 A diagrammatic representation of natural selection over successive generations. In this example, the darker traits confer an advantage over lighter traits.

WB
9.4.3
EXTINCTION
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Vulnerability and extinction

A large population with a diverse gene pool is relatively well equipped to respond to selection pressures. However, this is not always the case. Populations that have arisen through founder or bottleneck effects, even if they grow large in number, tend to have a gene pool with limited variation. This may have a couple of detrimental consequences for the population.

First, certain recessive alleles with potentially harmful phenotypes become concentrated in the gene pool. The result is that the potentially harmful condition occurs more frequently in the population; for example, reduced fertility among the cheetah population.

Second, a restricted gene pool means the population has less genetic and phenotypic reserves to respond to selection pressures. When environmental conditions become unfavourable, the population may suffer a substantial reduction in numbers as many of the individuals are incapable of surviving. This results in a bottleneck effect. If the population lacks the phenotypes required to survive an adverse environmental change, the whole population could die out. If all the members of a population have died out, the population is said to have become **extinct**.

Weblink
Natural selection

Online Worksheet
Natural selection

Experimental evolution

One of the approaches biologists use to study the impacts of selection pressures is to experimentally replicate the effects on populations and their gene pools. Biologists can manipulate and measure the distribution of phenotypes and alleles in the populations. Such experiments are typically conducted on populations of small, rapidly reproducing organisms whose life cycles are well understood and are easy to maintain in the laboratory. Organisms suited to studies of experimental evolution include bacteria, microscopic algae and shrimp species.

Experiments using the shrimp species *Americamysis bahia* (Figure 9.19) show the consequences when populations become small or have a limited genetic diversity. Initial small populations with high diversity grew to much larger sizes than comparable initial populations with low diversity (Figure 9.20a). An added effect was that the diversity of the larger population continued to increase while that of the smaller, low-diversity population stagnated or even decreased. In the process of applying a bottleneck effect, about 20% of the low-diversity populations died out before the biologists had the chance to trial them in experiments, possibly because the population accumulated alleles unfavourable for long-term survival through inbreeding. When a selection pressure was applied, in this case fresh water that is normally harsh to a species that prefers saline conditions, all of the high-diversity populations survived. By contrast, more than two-thirds of the low-diversity populations went extinct, with all the members of those populations dying out (Figure 9.20b). Therefore, it can be demonstrated experimentally that populations with low genetic diversity are at greater risk of extinction when conditions become unfavourable.


Alamy Stock Photo/Science Photo Library

Figure 9.19 Scientists use the mysid shrimp *Americamysis bahia* to study the effects of natural selection.

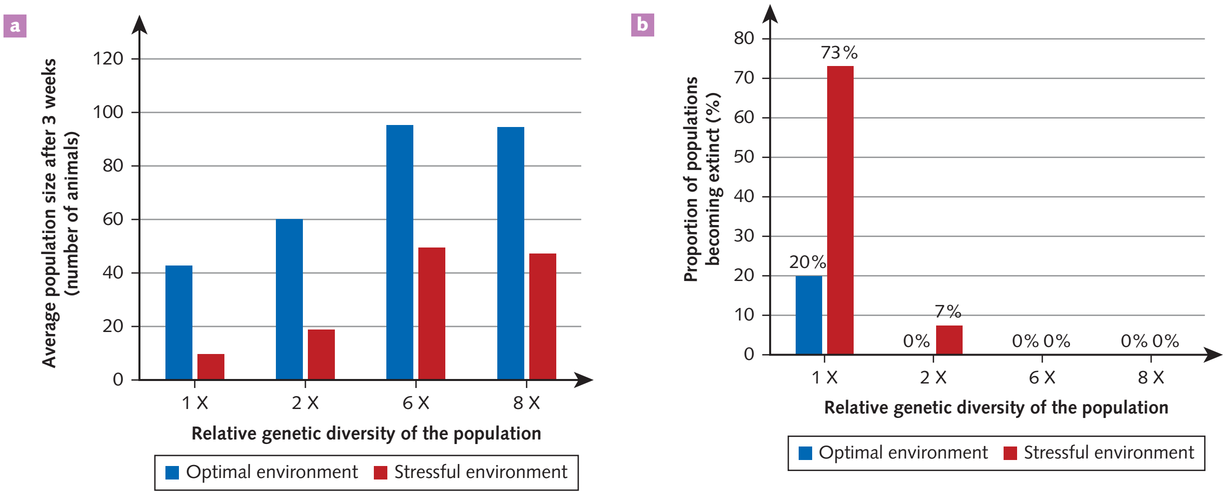

Figure 9.20 a The average population of mysid shrimps after 3 weeks of growth in saline (optimal) or freshwater (stressful) environments. Each average represents 15 replicate populations that each started with 12 animals. The 'relative genetic diversity' was determined using genetic markers. The 8X population is 8 times more diverse than the 1X population. **b** Results from the same experiment showing the proportion of the 15 starting populations of each condition that became extinct.

Multiple selection pressures

Another aspect of natural selection is that, if conditions change dramatically, two or more selection pressures may be acting simultaneously. For example, a population may be coping with drought stress. Individuals would be suffering from the heat and competing for the little available water and food. The population may become further stressed if a disease is introduced to it. As the selection pressures increase, there are fewer individuals in the population with the required phenotypes to cope with all the stressors at the same time. Multiple and extreme selection pressures can drive even large, diverse populations to extinction.

KEY CONCEPTS

- Selection pressures favour the survival of some members of a population over others; those individuals with a selective advantage are better able to survive and reproduce.
- Natural selection is the driving force that causes populations to adapt to changing environmental circumstances.
- Populations with low genetic diversity are more vulnerable to extinction.
- Experimental evolution enables the effects of selection pressures on populations to be tested and measured.
- There may be multiple selection pressures acting on a population at the same time.

Concept questions 9.4

1 List as many examples of selection pressures as you can.
2 Identify the role of variation in natural selection.
3 Summarise the key principles of natural selection.
4 Define 'extinction'. Can an allele be 'extinct'? What is the leading cause of extinction in species?

HOT Challenge

5 'Fitness' is a term applied to species in terms of natural selection. What does 'survival of the fittest' mean?

9.5 Human manipulation of gene pools

Humans have a long history of manipulating gene pools, either intentionally or unintentionally. The classic case is that of **selective breeding**, in which humans are the agents of selection, choosing parents with the most desirable traits (to humans) to breed the next generation. The result is the domestication of species suited to human subsistence. Humans have also had a substantial effect on populations of wild species.

Artificial selection: animal and plant selective breeding

WB 9.5.1 ARTIFICIAL SELECTION: ANIMAL AND PLANT BREEDING PAGE 196

Principles of natural selection can be applied to breeding programs for domesticated animals and plants. The processes for breeding have been understood and practised for centuries. Such breeding programs built on the observation that there was variation in the population. Parental stock with certain desirable traits were selected and mated, and it was understood that these traits were often passed on to the offspring. Over time, the new traits could be established in later populations. This process is called **artificial selection**, also called **selective breeding**, and relies upon human intervention to determine which traits are selected for.

Many of the familiar forms of domesticated plants and animals have arisen as a result of selective breeding. This process relies on human intervention to determine which animals are allowed to breed, removing alleles that produce undesirable traits from the gene pool and increasing the frequency of alleles that produce desirable traits.

The choice of traits selected and considered desirable in an organism depends on the use of the organism and the preference of the breeder. The Belgian blue breed of cattle contains an allele that promotes abnormal muscle growth (Figure 9.21). Farmers have only allowed the cows and bulls with the highest muscle mass to breed, producing more profitable offspring.

Dog breeds have been subjected to intensive selective breeding programs, producing forms that are aesthetically pleasing to breeders but which sometimes come with a number of considerable health problems for the dogs. This is because their acquired form may not be the best for optimal functioning, and because selective breeding processes may cause deleterious recessive alleles to become homozygous.


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Figure 9.21 Belgian blue cattle have been selectively bred to fix an allele for abnormal muscle growth that appeared in the 19th century.

Biological consequences of selective breeding

Once alleles have been lost from a population through natural selection or selective breeding, the traits they confer are permanently lost with them. Figure 9.22 shows the dramatic changes to English bulldog skulls that are a result of selective breeding.

age-fotostock/Alamy/Natural History Museum, London
age-fotostock/Alamy/Natural History Museum, London
Alamy Stock Photo/The Natural History Museum

Figure 9.22 Skulls of English bulldogs showing the effects of selective breeding: **a** the original English bulldog, with a functional skull in 1860, **b** 1867 and **c** 1906, showing a very exaggerated skull. The skull has changed dramatically in a short span of time.

Many of the food crops that are now commercially and domestically grown have been selectively bred to favour traits that make them better foods or better products for the producers; for example, bananas (Figure 9.23). Traits that have been selected for include yield, fruit size and longevity, the timing of grain or fruit maturation, and resistance to diseases.

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Figure 9.23a Wild bananas with large seeds and **b** selectively bred commercial bananas

While artificial selection generates species that are valuable to humans, it also risks decreasing the 'fitness' of domesticated breeds and makes them more vulnerable to natural selection pressures. The risk is exacerbated by combining artificial selection with large-scale propagation of relatively uniform populations, described as a **monoculture**. Populations with low diversity are more susceptible to being eliminated when exposed to natural selection pressures.

A historical example took place during the Great Famine of Ireland commencing in 1845. The Irish population of about 4 million people was heavily dependent on potatoes as a staple food crop. However, the potato crop had low genetic diversity across the country due to selective breeding of desirable varieties. The summer of 1845 was relatively cool and wet, ideal conditions for the infestation of the water mould *Phytophthora infestans* in the country's potato crops (Figure 9.24). About half the potato crops across Ireland were lost that year, and severe losses continued for several years after. During those years, approximately a million people starved to death and another million were forced to emigrate.

Alamy Stock Photo/Nigel Cattlin

Figure 9.24 A potato infected with *Phytophthora infestans*

Human effects on wild populations

Human actions have also affected gene pools in wild populations of animals and plants. Hunting for food or animal parts or culling to protect domesticated species has reduced the sizes of many animal populations.

Centuries of commercial hunting severely depleted populations of marine mammals, such as seals and whales. For example, the northern elephant seal (*Mirounga angustirostris*, Figure 9.25), which lived in the eastern Pacific Ocean along the west coast of North America, was declared extinct in the late 19th century. However, a handful survived on a remote island off Mexico and the population recovered during the 20th century, spreading, and repopulating much of its original home range. Numbers now exceed 250 000. The rebound is encouraging,

age-fotostock/imageBROKER/Winfried Wisniewski

Figure 9.25 The northern elephant seal has experienced a human-induced bottleneck effect.

but the species has been subjected to a human-induced bottleneck effect that severely reduced its level of genetic variation. The pattern has been repeated many times where populations have been depleted by human hunting.

Humans impose many selection pressures on wild species. Land clearing diminishes the habitat and resources available to resident wild populations. Introducing non-native invasive species, such as rats, foxes and feral cats, increases competition and predation of wild populations. Chemical and plastics pollution and the geographical spread of parasites and pathogens add further pressures on survival.

Many human-induced stressors are augmenting existing selection pressures in the natural environment. At the same time, humans are decreasing the size and variability of many wild populations, reducing their capacity to adapt. The risks of wild species extinctions are consequently amplified. For example, it has been estimated that amphibian species are now becoming extinct more than 200 times faster than background rates of extinction. Many small populations of animals with low diversity have already been driven extinct, including the Caspian, Balinese and Javan **subspecies** of tiger (*Panthera tigris*), the West African black rhinoceros (*Diceros bicornis longipes*), the Pinta Island tortoise (*Chelonoidis nigra abingdonii*), the Iberian ibex (*Capra pyrenaica pyrenaica*), and the Tasmanian tiger (*Thylacinus cynocephalus*).

Weblink
Human impacts on Brazilian parrots

Online Worksheet
Parrot gene pool

KEY CONCEPTS

- Selective breeding, or artificial selection, occurs when humans selectively breed organisms for desired traits.
- In selective breeding, the frequency of alleles encoding for desired traits increases and the frequency of other alleles at the locus decreases, and this reduces genetic diversity.
- Human activities have resulted in decreased genetic diversity in many wild species, making them vulnerable to extinction.

Concept questions 9.5

1 Explain the effects of selective breeding on the gene pool of a population.

2 Give an example of selective breeding other than those described here.

3 Describe the relationships between the genetic diversity of a population, the number and intensity of the selection pressures acting on it, and the likelihood of the population becoming extinct.

4 The formation of a monoculture through large-scale breeding of domestic animals can be a problem. The artificial selection of characteristics based on culture and taste has led to physical and functional issues with some dog breeds. Labradors are prone to hip dysplasia, dachshunds are prone to both hip and elbow dysplasia, and pugs can suffer from breathing difficulties and conjunctivitis because of the contraction of their nasopharynx area. Deleterious alleles can become fixed within a population and thus pose serious health concerns and mortalities.

   a What does ‘fixed’ mean in this sense?

   b If the fixed deleterious allele becomes homozygous recessive in the population, what does that mean for any offspring?

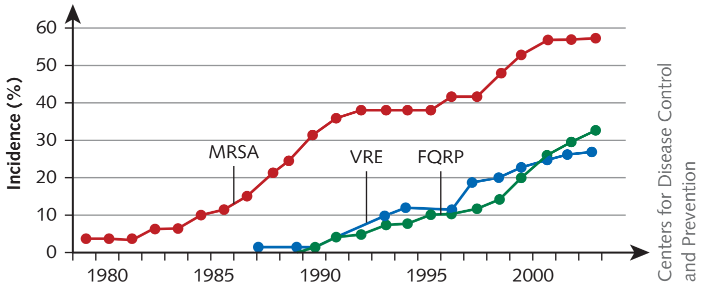
5 a What are the selection pressures acting on the northern elephant seal?

   b Explain how human-induced stressors are augmenting existing selection pressures in the natural environment. Provide one example.

HOT challenge

6 Malaria kills hundreds of thousands of people every year. It is endemic to equatorial regions of Africa. Usually, if a disease kills all the hosts, it dies off. Malaria is caused by a number of the *Plasmodium* species and the mode of transmission is the infective *Anopheles* mosquito. Malarial plasmodia undergo a life cycle that includes spending some time in human blood. In African nations where malaria is endemic, the genetic disorder sickle cell anaemia is also prevalent. The sickle cell (red blood cell) is marked by defective haemoglobin. The sickle cell blocks blood flow to vital organs. People who inherit two copies of the allele for sickle cell anaemia usually die quite young. But people who are heterozygous for the allele (carriers) survive and have children. They seem to have an advantage in malaria-prone areas because of the lower concentration of oxygen in their blood caused by the sickle cell trait. Based on your understanding of fitness, selective advantage, selective pressures and natural selection, write a hypothesis that seeks to answer the question: ‘Why does malaria remain endemic in some areas of the world where sickle cell anaemia occurs, when malaria is a killer?’.

9.6 Natural selection and consequences for disease



Centers for Disease Control and Prevention

Figure 9.26 The increasing prevalence of some antibiotic resistant bacteria over time. MRSA = methicillin-resistant *Staphylococcus aureus* ('golden staph'); VRE = vancomycin-resistant *Enterococcus*; FQRP = fluoroquinolone-resistant *Pseudomonas aeruginosa*. Based on USA data sourced from Centers for Disease Control and Prevention (2013).

Antibiotics are chemicals that are toxic to bacteria. Almost as soon as antibiotic production was industrialised in the 1940s, it was clear that their therapeutic use could be compromised by pathogenic bacteria developing resistance to them. Throughout the 20th century, each time a new antibiotic was introduced, **antibiotic resistance** eventually followed. The repeated pattern has resulted in a global increase in antibiotic resistance (Figure 9.26).

When a bacterium becomes resistant to two or more antibiotics it is described as **multidrug-resistant** or is called a 'superbug'. One of the most pervasive multidrug-resistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA), sometimes referred to as 'golden staph'. Some strains of MRSA are resistant to at least six different types of antibiotics. Such MRSA strains are also insensitive to disinfectants and they are a major source of hospital-acquired infections. The rise of multidrug-resistant pathogenic bacteria is a serious concern because fewer antibiotics are available to treat the infections the pathogens cause.

WB
9.6.1 NATURAL SELECTION EXPLAINS ANTIBIOTIC RESISTANCE
PAGE 198

Natural selection explains antibiotic resistance

Bacteria have many different mechanisms for rendering antibiotics ineffective. These include:

- modifying the chemical structure of the antibiotic
- producing cellular transporters that remove the antibiotic from the cell
- adjusting the bacterium's own physiology to evade the antibiotic.

In all cases, the effects are carried out by proteins. These proteins are not essential to the normal survival of the bacterium but they may be expressed under certain circumstances, such as when the bacterium encounters a particular toxin in its environment. Proteins that mediate antibiotic resistance are encoded by genes collectively referred to as **antibiotic resistance genes**. The antibiotic resistance genes are typically found on bacterial plasmids. Multidrug-resistant bacteria have two or more antibiotic resistance genes.

A key observation for understanding how bacteria develop drug resistance is that the capacity for resistance is already present in the population, even when the bacteria have not been exposed to a therapeutic dose of the antibiotic. New genes for such antibiotic properties arise by a combination of gene duplication and mutation. Once the gene has entered the gene pool, a few members of the population will have the antibiotic-resistant phenotype.

If only a low proportion of bacteria in the population are antibiotic resistant, and the dose of antibiotic is sufficient, then the whole population will die off. In such a case, the therapeutic dose of antibiotic successfully clears the infection. However, antibiotic resistance may arise if the dose of antibiotic is not enough to kill all of the bacteria. The dose may temporarily inhibit bacterial growth but it does not exterminate the population. This level of antibiotic acts as a selection pressure that favours the survival of the bacteria that have the resistant phenotype (Figure 9.27).

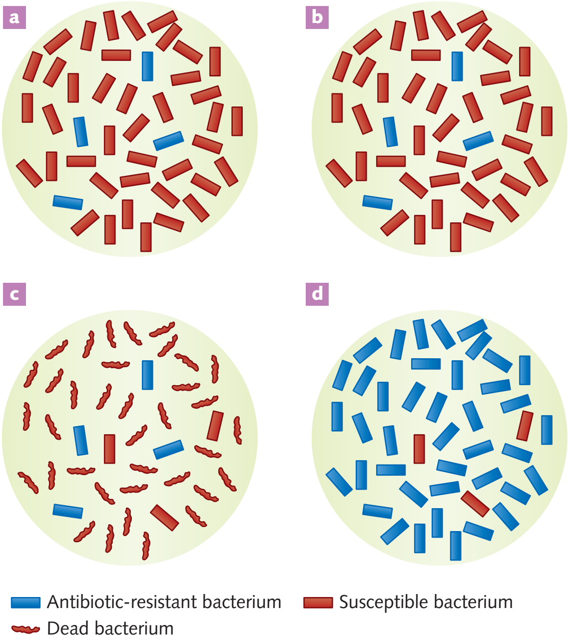


Figure 9.27 a An initial population of bacteria includes a few antibiotic-resistant individuals. **b** On exposure to the antibiotic, a new selection pressure is introduced. **c** Many of the susceptible bacteria die off; the survivors tend to be those with antibiotic resistance. **d** Under continuing selection pressure, these bacteria continue to reproduce until, eventually, the population thrives again.

The surviving resistant bacteria reproduce passing on the genes for antibiotic resistance to their offspring. In time, under continuing exposure to the antibiotic, the population recovers and almost all are equipped for antibiotic resistance. Successive exposures to the antibiotic have no detrimental effects on the bacterial population.

Once a large proportion of the population of bacteria has an antibiotic-resistance gene, members of the population can acquire a second antibiotic-resistance gene from an unrelated bacterium through horizontal gene transfer (p. 336). Combinations of subsequent exposures to different antibiotics, natural selection and horizontal gene transfer result in multidrug-resistant bacteria.

iStock.com/jetstream4wd

Figure 9.28 In some countries, antibiotics are widely used as growth promoters in agriculture.

Emergence of a global health threat

The growing scale of multidrug-resistant bacteria poses a major threat to global health, officially recognised by the World Health Organization (WHO). Multidrug-resistant pathogenic bacteria increase the risk of medical complications, extended hospitalisations, and fatalities caused by infections. In Australia, approximately 1600 deaths per year are the result of antibiotic-resistant bacteria.

There are many selection pressures acting to increase the prevalence of multidrug resistance. One of these is the over-prescription of antibiotics, especially when patients have a viral rather than a bacterial infection. This is exacerbated when patients who are appropriately prescribed antibiotics do not finish their course. In many countries, antibiotics are available without prescription. This situation allows indiscriminate use of antibiotics by the public. Many of the over-the-counter antibiotics are **broad-spectrum** antibiotics, which are effective against several different bacterial species. They are frequently purchased to treat short-term and self-resolving illnesses, such as upper respiratory tract or gastrointestinal infections.

Another selection pressure occurs when antibiotics are used in agriculture, farming and aquaculture. Approximately three-quarters of the antibiotics consumed in the USA are used in these sectors. The most common use is for cattle, pigs and poultry as a feed supplement, where they act as growth promoters. The antibiotics inhibit gut microbes, stimulate the animals' immune systems and provide some protection against disease for animals kept in close quarters (Figure 9.28). The occurrence of antibiotic-resistant bacteria in farm animals continues to rise, with the highest rates in low- to middle-income countries in Asia, Africa and South America where livestock farming has increased rapidly in the last two decades.

Excess antibiotics escape into the environment where they continue to exert selection pressure on environmental microbes. Antibiotic-resistant populations of bacteria have been detected in soil, freshwater and marine samples far from the likely sources of commercial antibiotics. For example, in a population of dolphins in southeastern USA (Figure 9.29), the prevalence of erythromycin-resistant bacteria growing around the blowholes and in the gastrointestinal tracts of the dolphins has increased during the last decade. It is thought that the source of the erythromycin is sewage in Florida.

Multiple strategies are required to counter multidrug-resistant bacteria. These include:

- prohibiting the sale of over-the-counter antibiotics
- delaying the prescription of therapeutic antibiotics
- tighter management and use of prescribed antibiotics
- enforcing stricter regulation of antibiotics in agriculture
- more rigorous surveillance and reporting of multidrug-resistant bacteria
- educational programs for both the public and practitioners.

It is also important that new antibiotics (naturally occurring or chemically synthesised) are discovered and developed. However, pharmaceutical companies have become reluctant to develop new antibiotics because they are less profitable than other drugs. The situation may be alleviated by governmental policies and incentives.

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Figure 9.29 **Antibiotics are leaking into the environment, resulting in antibiotic-resistant bacteria growing in wild animal populations.**

Lessons from nature

The environmental context for antibiotic resistance is the perpetual competition for resources between different species of fungi and microbes. One species can eliminate its competitors by producing an antibiotic that is toxic to the other species. Many antibiotics have been isolated from naturally occurring sources. For example, penicillin was isolated from the *Penicillium* fungus. The target species may improve its chances of survival by developing resistance to the antibiotic. The first species may in time produce another antibiotic to which the second species eventually develops resistance, and so on it goes. Human activity is now replicating this natural 'arms race'.

Bacteria are ideally suited to develop antibiotic resistance for several reasons.

- They are vastly numerous. A single teaspoon could hold literally trillions of bacteria.
- Their small size makes them highly mobile and prone to encountering new and different environments with corresponding new and different selection pressures.
- Bacteria have relatively compact genomes and reproduce rapidly with consequently increased levels of mutation.
- Short generational times and the capacity for horizontal gene transfer enable bacteria to rapidly transmit beneficial genes from generation to generation and between members of the same generation.

Viruses

Viruses share many attributes with bacteria. They are extremely small and highly mobile, have compact genomes, reproduce quickly in large numbers and have relatively high mutation rates. However, viruses must infect hosts to reproduce. Transmission between susceptible hosts is important for the survival and replication of viruses.

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INVESTIGATION 9.2

Antibiotic resistance

Antibiotics are molecules that are produced by bacteria and fungi as a defence against other microbes. The discovery of penicillin and other antibiotics in the 20th century provided us with a way to treat disease and save lives. Antibiotics have been developed for use against a broad range of pathogenic microbes; each has its strengths and weaknesses. Unfortunately, overuse has led to antibiotic resistance. No antibiotic works against all bacteria and it is important to know which antibiotic to use to treat different bacterial diseases.

Aim

To investigate antibiotic effectiveness against common bacteria

Time requirement

45 minutes

Materials

- *Escherichia coli* broth culture
- *Staphylococcus epidermidis* broth culture
- 4 nutrient agar plates
- Bleach or 70% alcohol
- 2 sterile plastic pipettes
- 2 disposable spreaders
- 2 Mastring antibiotic discs
- Forceps
- Measuring ruler or callipers
- Sticky tape
- Permanent marker
- Bunsen burner
- Contaminated waste bag
- Disposable gloves

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Although lab strains are usually harmless, bacteria may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at the end of the activity. Decontaminate benches before and after the activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

Note: to use aseptic technique, wipe your bench down with 70% alcohol or bleach and keep your work near the Bunsen burner to take advantage of the updraught the flame will create to waft potential contaminants away from your materials.

1. Label the bottom of your four agar plates with your name and the date. Label two plates *E.coli* and two plates *S. epidermidis*. Label one plate of each type of bacteria with ‘E’ for experiment and label the other ‘C’ for control (Figure 9.30).
2. Using a sterile plastic pipette, transfer a drop of the *E. coli* bacterial broth onto the surface of the agar on your two *E. coli* plates.
3. Working in close proximity of the Bunsen burner, use a spreader to spread the bacterial broth evenly over the plates. If you are using a glass spreader, pass it through the flame of the Bunsen burner before each use.

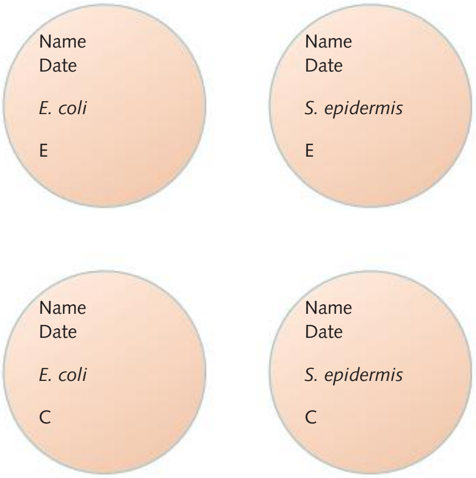


Figure 9.30 The four agar plates

4 Replace the lids on the plates immediately to avoid contamination.
5 Repeat steps 2–4 for *S. epidermidis*, using a new pipette and spreader.
6 Wait 10–15 minutes before applying the Mastring to ensure the bacteria has a chance to grow.
7 Flame your forceps and let them cool before picking up the Mastring. Place the Mastring in the middle of one of your experiment plates and push (very gently) with the forceps to help it stay in place. Each lobe of the Mastring is impregnated with a different antibiotic; use the code on the packet to differentiate them.
8 Repeat step 7 for your other experiment plate. Ensure you flame the forceps between each application.
9 Seal all four plates with sticky tape and incubate them for 24 hours at 37°C, upside down so that the agar is at the top.
10 Wipe your bench down with 70% alcohol or bleach and clean your hands thoroughly.
11 Dispose of all materials safely in a contaminated-waste bag.
12 The next day, observe the plates for the presence or absence of growth near each disc and measure the diameter of any zones of inhibition.
13 Copy the results tables into your logbook and record your results.

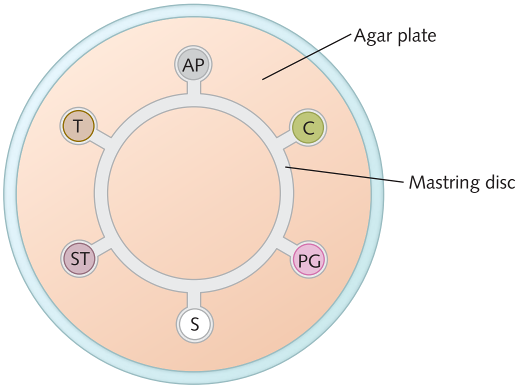


Figure 9.31 The Mastring antibiotic disc; AP, ampicillin; C, chloramphenicol; PG, penicillin; S, streptomycin; ST, sulphatriad; T, tetracycline

Results

1 Draw a labelled diagram of what you see on each plate.
2 Complete Results table 1 with the results of your experiment.

Results table 1 Experimental results

Antibiotic	Diameter of zone of inhibition (mm)	
	Escherichia coli	*Staphylococcus epidermidis*
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

3 Calculate the mean class diameter of zone of inhibition for each antibiotic and copy and complete Results table 2.

Results table 2 **Class means**

Antibiotic	**Mean diameter of zone of inhibition (mm)**	
	Escherichia coli	***Staphylococcus epidermidis***
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

Discussion

1 Explain the function of the control plate. How could a control plate be helpful if there is no growth on the experiment plate?
2 What were four variables that you kept constant in this experiment? How did you control them?
3 What is a zone of inhibition? How were they created in your experiment?
4 Why is it important to pool data from the class results and find the mean zone of inhibition for each antibiotic?
5 Which antibiotic had the greatest zone of inhibition? Explain why this might be.
6 Did your individual results differ from the class results? If so, suggest possible reasons.
7 Which antibiotic would be most suitable to treat an infection by *Staphylococcus epidermidis*?
8 Which antibiotic would you use if you were unsure of the pathogen in an infection? Explain your answer.
9 Did your results show any signs of antibiotic resistance?
10 Discuss the impacts that antibiotic resistance has on medical treatment.
11 Why have antibiotics become a less effective treatment for infection in recent years?

EXAM TIP

In a written exam question, do not simply describe the control as a 'control'. It helps to describe how the result for the control is compared to that of the treatment and how the difference explains the effect of the treatment.

Conclusion

Write a conclusion for this investigation.

Taking it further

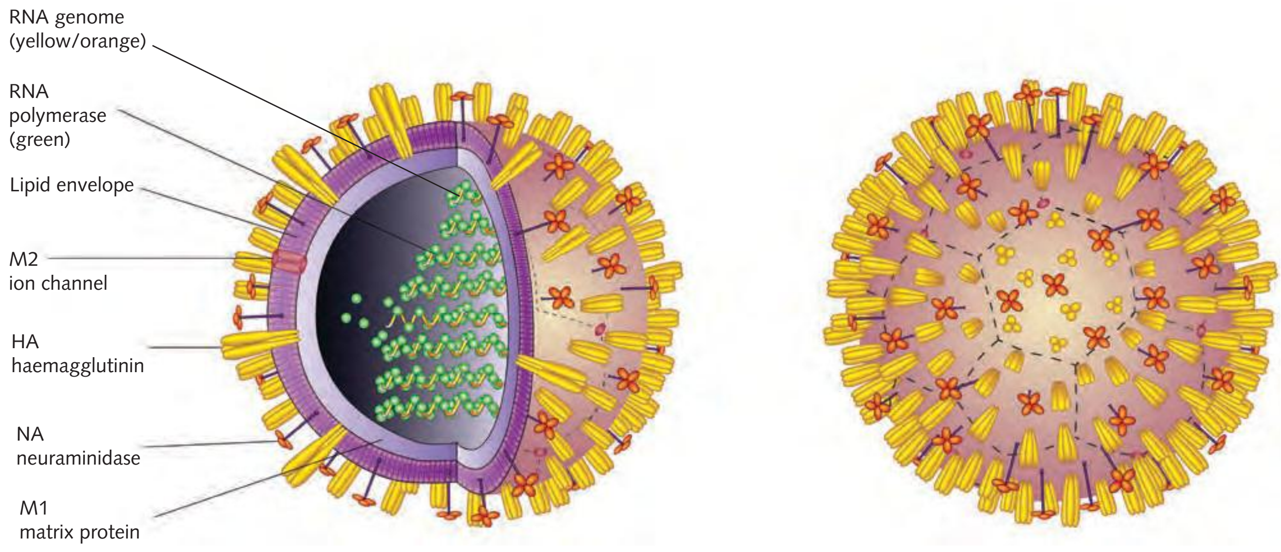
Test the efficacy of natural antibiotics on similar bacteria.

Once your body is exposed to a viral infection, the adaptive immune response helps clear the infection and also prepares it for future infection from the same virus. Memory B and T cells and circulating antibodies recognise the antigens of previous viral pathogens. Viruses bearing those antigens are doomed by the immune system if they re-infect the host. The virus's capacity to replicate stalls when the population of susceptible hosts is exhausted, and transmission is restricted.

Antigenic drift

During a pandemic, a viral infection migrates across geographically dispersed hosts. Substitution mutations accumulate in the population of viruses as they replicate, some of them altering the antigens that would otherwise be recognisable by the adaptive immune system. If the antigen is altered enough, the result is a new strain of the virus capable of evading the adaptive immune system and re-infecting the host, even if the host was previously infected or vaccinated against the original strain of the virus. This is described as **antigenic drift**.

Antigenic drift helps to explain seasonal influenza (flu) epidemics. The influenza virus is an RNA virus that bears the antigenic surface glycoproteins haemagglutinin and neuraminidase (Figure 9.32). Haemagglutinin helps the virus attach to a susceptible host cell on infection. Neuraminidase helps the newly replicated viruses detach from an infected host cell.



Science Photo Library/Research Illustration, Francis Crick Institute/Joe Brock

Figure 9.32 A cutaway diagram of an influenza virus

There are four types of influenza virus: A, B, C and D. The four types infect different groups of host animals. Humans are susceptible to types A, B and C. Only type A is known to have caused global pandemics in human populations. Type A influenza viruses are subdivided into different subtypes identified by their particular haemagglutinin (H) and neuraminidase (N) proteins. For example, the subtypes H1N1 and H1N2 have the same haemagglutinin but different neuraminidase proteins. The different proteins reflect the different genetic variants of each subtype. More than 130 subtypes of influenza type A have been detected in nature.

As influenza spreads across global human populations, substitution mutations occur in the replicating virus, creating new strains. A particular subtype (e.g. H3N2) may be prevalent in Australia during a given year. By the time the epidemic has run its course, a new strain of the H3N2 subtype will have emerged elsewhere in the world and migrated to Australia, causing a new epidemic the following year. For this reason, specific strains of flu virus are distinguished according to when and where the strain was first isolated (Table 9.1). Each new strain emerges because of antigenic drift.

Weblink
Learn how influenza spreads

Table 9.1 Examples of influenza virus strains

Strain name	Influenza virus type	Influenza virus subtype	Location first identified	Year first identified
A/Nanchang/933/1995 (H3N2)	A	H3N2	Nanchung, China	1995
A/Sydney/5/1997 (H3N2)	A	H3N2	Sydney, Australia	1997
A/Panama/2007/1999 (H3N2)	A	H3N2	Republic of Panama	1999

WB
9.6.2
VIRAL ANTIGENIC DRIFT AND SHIFT
PAGE 200

Antigenic shift

Normally a single subtype of influenza infects an individual host. However, on rare occasions a single host may be infected by two different subtypes of influenza. When this happens, the genetic material of the two subtypes is shuffled into a new combination in the replicating viruses. The result is the sudden appearance of a new subtype of the virus. Its antigens are substantially different from those of the original viruses. The adaptive immune system of hosts that were previously infected by or vaccinated against the original viruses will not recognise the new antigens. This is referred to as **antigenic shift**.

Antigenic shift typically occurs when different host animals are living close together. Each reservoir of host animals is infected by different subtypes of the virus, increasing the chances that the two different subtypes will meet in a common host animal. New influenza subtypes have appeared in rural environments where humans live closely with aquatic birds and pigs. Sometimes, the subtype infecting birds jumps directly to a human host bringing the two subtypes together. On other occasions, a susceptible third species may act as a 'mixing pot' that brings the two subtypes together.

If the new subtype of virus is highly contagious in humans, it can lead to a global pandemic. A 2009 pandemic of influenza originated in pig farms was thus informally referred to as 'swine flu'. The deadliest global influenza pandemic in history was the 1918 Spanish influenza outbreak, during which at least 50 million people died of both the flu and opportunistic secondary infections of flu victims. Figure 9.33 summarises the difference between antigenic shift and antigenic drift.

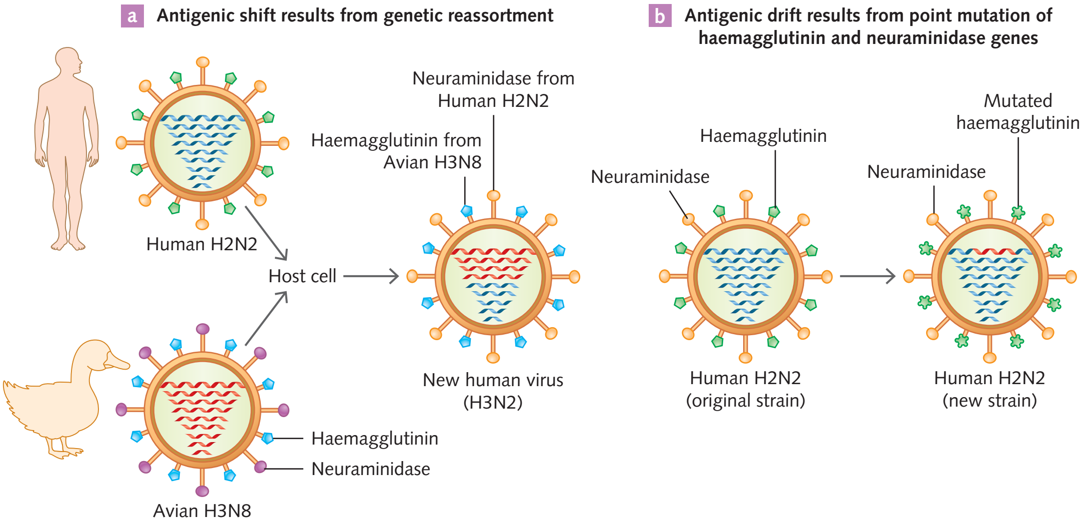


Figure 9.33 a This antigenic shift example models the emergence of the 1968 H3N2 subtype of influenza virus. **b** Antigenic drift occurs when an existing virus steadily mutates into a new strain.

Scientific and social challenges for treatment strategies and vaccination programs

If an influenza outbreak of a new virus subtype occurs that appears to have a high mortality rate, policy makers may act to limit the spread by restricting contact between people. This may involve shutting down public transport within the affected area as well as travel into and out of the affected area. It may also require closing down communal hubs, such as schools and sports venues where large numbers of people normally congregate. Antiviral drugs (e.g. Relenza) may be prescribed to treat infected individuals. However, if drug stocks are limited, the supply may be reserved for personnel involved in health or emergency services. Once an outbreak has begun, it is difficult to contain. The preferred option is preventing the outbreak in the first place; for example, through vaccinations.

Annual vaccination is the chief preventative measure against influenza. Current guidelines recommend vaccinations for people over the age of six months, including pregnant women. Building herd immunity in the population reduces the spread of the virus to vulnerable people, such as children under the age of six months.

The vaccination normally protects against three or four subtypes of influenza; however, the formulation may change from year to year. Influenza surveillance is conducted at a network of centres in more than 100 countries, including at the Victorian Infectious Diseases Reference Laboratory in Melbourne. The network monitors the emergence and spread of influenza subtypes, how contagious they are, and the severity of their disease symptoms. The WHO convenes a meeting of the network twice a year, one each for the northern and southern hemisphere flu seasons, to assess the data and determine which subtypes should be the focus of the upcoming season's vaccine. Recommendations must be made six months in advance of the flu season to allow time for production of the updated vaccine.

KEY CONCEPTS

- The global rise in antibiotic-resistant bacteria is explained by the principles of natural selection. Natural selection and horizontal gene transfer contribute to the emergence of multidrug-resistant bacteria.
- Managing the rise of multidrug-resistant bacteria requires measures to regulate antibiotic use and develop a pipeline of new antibiotics.
- Antigenic drift and antigenic shift account for the emergence of new strains and subtypes of viruses.
- Preventing outbreaks of new virus strains or subtypes is best achieved by vaccination; managing an outbreak requires measures that limit contact within the population and regulating access to antiviral drugs.

Concept questions 9.6

1. Describe the characteristics of bacteria and viruses that enable them to adapt readily to changing environments.
2. Outline how the process of natural selection most likely led to the emergence of fluoroquinolone-resistant *Pseudomonas aeruginosa*.
3. List the ways that human activity is causing the worldwide increase in antibiotic-resistant bacteria.
4. Explain the difference between antigenic drift and antigenic shift. What is the difference in the relative amount of antigenic change between the two?
5. How does multidrug antibiotic resistance arise?

HOT Challenge

6. About 90% of an antibiotic dose may be excreted from the body and end up in the sewers of towns and cities. *Escherichia coli* is a natural inhabitant of the human gut and is excreted in large numbers into the sewer system when people use the toilet. Run-off from farms can include large amounts of antibiotics and *E. coli*. Multidrug resistance has been found in *E. coli* and this has led to the use of the term 'superbug'.
   - a List the steps that could lead to drug-resistant *E. coli* in the human population through natural selection.
   - b What is a superbug?
   - c Antibiotics do not discriminate between beneficial and harmful bacteria. Why could this be a problem for us?

BRANCHING OUT

Wildlife reservoirs of antibiotic-resistant bacteria

Pigs are not native to Australia. They were introduced as livestock by early European settlers in the late 19th century but soon escaped into the wild. Owing to their hardy disposition and capacity to roam long distances, pigs flourished in Australian conditions (Figure 9.34). Feral pigs are a pest, damaging native ecosystems through habitat destruction, predation, resource competition and disease transmission. Feral pigs are difficult to control and a burden to Australian agriculture, costing the sector about $100 million per year.

Dreamstime.com/John Carnemolla

Figure 9.34 **It is estimated that today there are up to 24 million feral pigs in Australia covering more than one-third of the continent.**

One population of feral pigs inhabits the semi-arid Kimberley region of Western Australia. This population is isolated from other feral pig populations to the east and south. The land is relatively arid, sparsely populated by humans and used chiefly for beef cattle grazing. The cattle experience minimal contact with humans so veterinarian care is limited, but the cattle and the feral pigs compete for habitat and resources.

Concerned by antibiotic resistance appearing in the environment, Australian scientists investigated the potential for antibiotic resistance in *Escherichia coli* (*E.coli*) resident in the feral pig population in the Kimberley region. The scientists reasoned that, because these pigs have little to no contact with antibiotics, antibiotic-resistance data for their *E.coli* would provide an understanding of the natural occurrence of antibiotic resistance in the wild. The scientists collected samples from the colon and rectum of recently culled feral pigs, isolated individual colonies of *E.coli* bacteria from the samples, and then tested each of the *E.coli* isolates against antibiotics. The results of these experiments are shown in Table 9.2.

The study showed the presence of incomplete antibiotic resistance in *E. coli* from the feral pigs. Given the pig population's isolation and the characteristics of local farming practices, the scientists concluded that transmission of the resistance from livestock was unlikely. The scientists noted that freshwater sources normally contain the most diverse range of antibiotic resistance, although the antibiotic sulfadimethoxine was not detected in soil or groundwater. Other antibiotics such as chlortetracycline absorb strongly to clay, soil and sediments. Pigs normally push their snouts into soil and sediments in search of food.

»

Aim

To investigate antibiotic resistance in bacteria from a remote population of feral pigs and relate it to the risks associated with therapeutic antibiotic use

Questions

1 Suggest how it is possible that *E. coli* from such a remote and isolated population of feral pigs could show resistance to:
  - a sulfadimethoxine
  - b chlortetracycline.

2 Assuming that the antibiotics are equally effective treatments for an infection:
  - a which would you recommend administering to grazing beef cattle in the area and why?
  - b what other advice could you provide a farmer putting cattle on a course of antibiotics?

3 What risks for transmission might there be for the domestic cattle if they and the feral pigs congregate around common water sources?

4 The scientists conducting the investigation proposed that the data could be used as a 'baseline' for surveillance of antibiotic resistance in domestic pigs.
  - a Suggest how the surveillance could be conducted.
  - b Propose what sort of data you would be seeking.

Table 9.2 **Susceptibility to veterinary antimicrobials of 115 *E. coli* isolates from feral pigs in north-western Australia**

Veterinary antibiotic	Number of resistant isolates
Ampicillin	0
Ceftiofur	0
Chlortetracycline	6
Danofloxacin	0
Enrofloxacin	0
Florfenicol	31
Gentamicin	0
Neomycin	0
Oxytetracycline	0
Spectinomycin	0
Sulfadimethoxine	58
Trimethoprim-sulfamethoxazole	0
Tulathromycin	0
Tylosin	0

Online Key Concepts
Chapter 9 Summary

9 Summary of key concepts

9.1 Mutations are the source of new alleles

KEY CONCEPTS

p. 329

- Mutations are changes in DNA. Mutations occur spontaneously or are caused by mutagens. The potential effect of a mutation depends on whether the mutation occurs in somatic or germline cells.
- Point mutations can cause changes in a DNA sequence by substitution, insertion or deletion of a nucleotide.
- Substitutions can be silent mutations, which cause no change in the encoded protein, or they can be missense or nonsense mutations, which alter the structure and function of the encoded protein.
- Insertions or deletions can cause frameshift mutations that affect the amino acid sequence downstream, severely affecting the encoded protein.

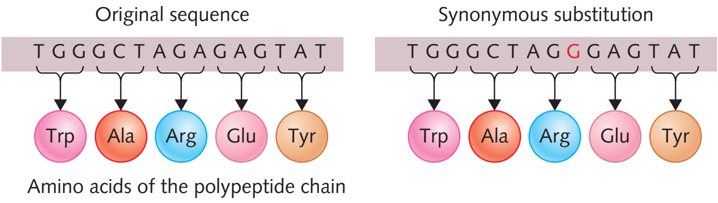


Figure 9.3 A silent mutation

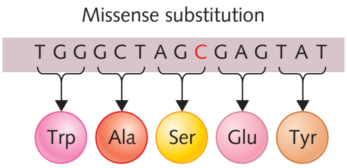


Figure 9.4 A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.

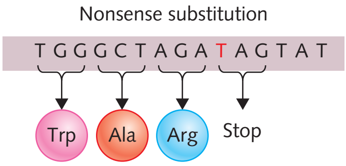


Figure 9.5 A nonsense mutation in the gene sequence results in premature termination of translation.

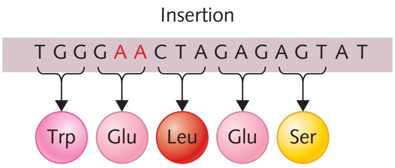


Figure 9.6 An insertion in the gene sequence results in a frameshift mutation. Here, two adenines have been inserted.

9.2 Chromosomal rearrangements

KEY CONCEPTS

p. 333

- » Mutations can cause changes in chromosome structure and chromosome number.
- » Deletions, inversions, translocations and duplications may involve particular segments of a chromosome and may change the number of alleles in affected areas.
- » Mutations can be silent, nonsense, beneficial or disadvantageous.
- » New genes may be acquired by gene duplication or by horizontal gene transfer.

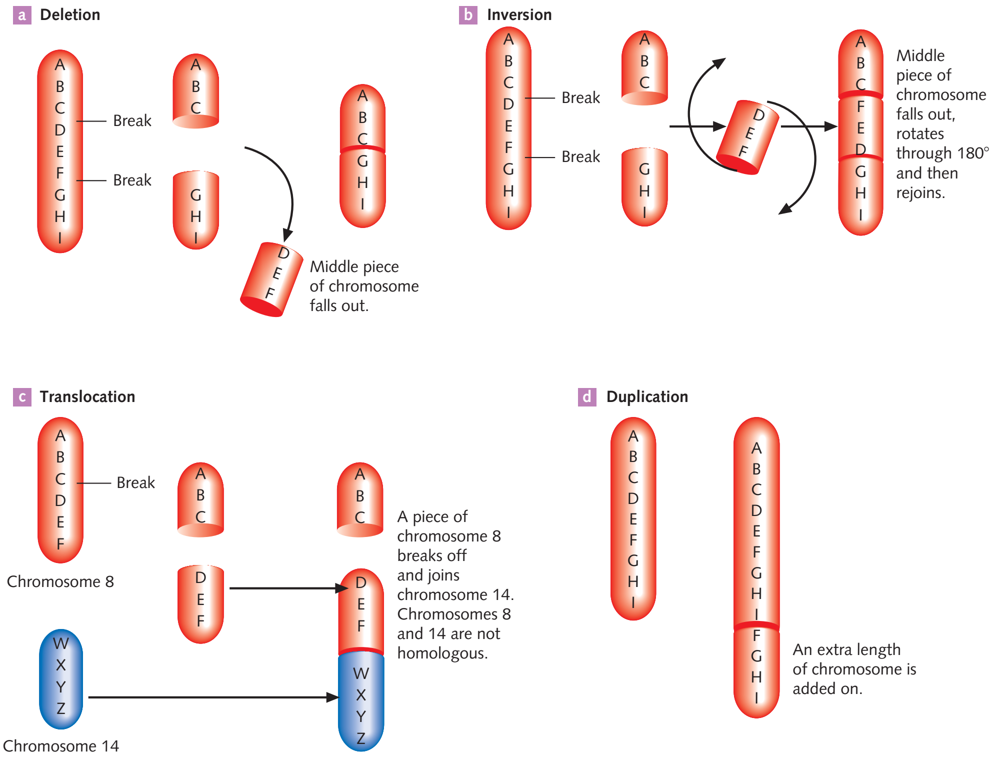


Figure 9.8 **Abnormalities caused by chromosomal or 'block' mutations may arise by a deletion, b inversion, c translocation or d duplication.**

9.3 Challenging allele frequencies in populations

KEY CONCEPTS

p. 336

- » Genetic drift is the change in allele frequency in a population due to the random assortment and selection of certain genes during meiosis and fertilisation.
- » The bottleneck effect occurs when an event causes a large reduction in the gene pool of a population, decreasing genetic diversity in subsequent generations.
- » Gene flow results from the transfer of alleles into or out of a gene pool because of the migration of individuals between populations.
- » The founder effect occurs when a small number of individuals, carrying a restricted number of alleles, form a new population with reduced genetic diversity compared with the original population.

9.4 Natural selection

KEY CONCEPTS

p. 343

- Selection pressures favour the survival of some members of a population over others; those individuals with a selective advantage are better able to survive and reproduce.
- Natural selection is the driving force that causes populations to adapt to changing environmental circumstances.
- Populations with low genetic diversity are more vulnerable to extinction.
- Experimental evolution enables the effects of selection pressures on populations to be tested and measured.
- There may be multiple selection pressures acting on a population at the same time.

9.5 Human manipulation of gene pools

KEY CONCEPTS

p. 346

- Selective breeding, or artificial selection, occurs when humans selectively breed organisms for desired traits.
- In selective breeding, the frequency of alleles encoding for desired traits increases and the frequency of other alleles at the locus decreases, and this reduces genetic diversity.
- Human activities have resulted in decreased genetic diversity in many wild species, making them vulnerable to extinction.

9.6 Natural selection and consequences for disease

KEY CONCEPTS

p. 349

- The global rise in antibiotic-resistant bacteria is explained by the principles of natural selection. Natural selection and horizontal gene transfer contribute to the emergence of multidrug-resistant bacteria.
- Managing the rise of multidrug-resistant bacteria requires measures to regulate antibiotic use and develop a pipeline of new antibiotics.
- Antigenic drift and antigenic shift account for the emergence of new strains and subtypes of viruses.
- Preventing outbreaks of new virus strains or subtypes is best achieved by vaccination; managing an outbreak requires measures that limit contact within the population and regulating access to antiviral drugs.

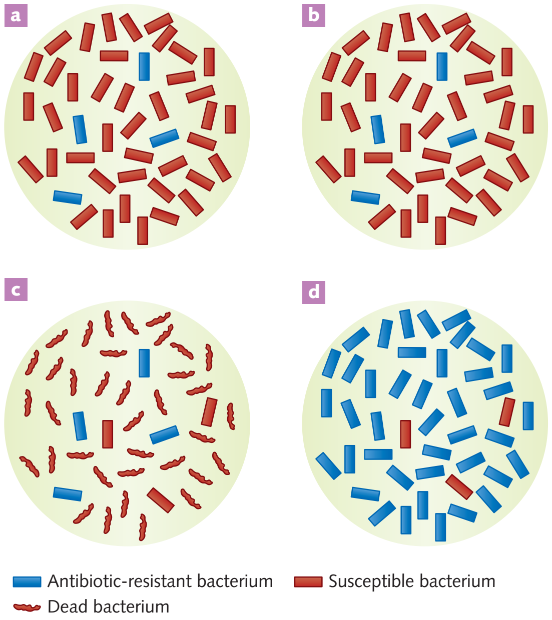


Figure 9.27 a An initial population of bacteria include a few antibiotic-resistant individuals. **b** On exposure to the antibiotic, a new selection pressure is introduced. **c** Many of the susceptible bacteria die off; the survivors tend to be those with antibiotic resistance. **d** Under continuing selection pressure, these bacteria continue to reproduce until, eventually, the population thrives again.

WB
9.7.1 KEY TERMS
PAGE 201

9 Chapter glossary

amino acid sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide, sometimes referred to as 'polypeptide sequence'

antibiotic resistance the capacity for a microbe to withstand the lethal effects of an antibiotic to which it was once susceptible

antibiotic resistance gene a gene that codes for an antibiotic resistant phenotype

antigenic drift a change in the antigen of a virus that would otherwise be recognisable by the adaptive immune system, resulting from the gradual accumulation of mutations in the virus

antigenic shift a sudden change in the antigen of a virus resulting from the rearrangement of genetic material from two or more strains or subtypes of the virus

artificial selection breeding of plants and animals over successive generations to produce traits that are desirable to humans; also known as 'selective breeding'

beneficial mutation a mutation that increases the organism's chances of survival and reproduction

block mutation a mutation involving rearrangements of chromosomal segments

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population and its genetic diversity

broad spectrum describes an antibiotic (or insecticide) that is effective against a variety of organisms

conserved amino acids of polypeptide sequences or nucleotides of DNA sequences that remain consistent across species

deleterious mutation a mutation that decreases the organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

double-strand break a mutation involving breaks in the sugar-phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

duplication a mutation that occurs when one or more extra copies are made of a section of chromosome

extinct when all the members of a population or species have died out

fitness the capacity of an individual to survive and produce viable offspring

fixed describes an allele when it is the only variant available for a particular gene in the gene pool of a population

founder effect the type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

gene duplication generating an extra copy of a gene within a genome as a result of duplication of a chromosomal segment

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

gene sequence the sequence of nucleotides in a gene

genetic drift the change in the gene pool of a population as a result of chance; usually occurs in small populations

genotype a specific combination of alleles for a particular gene locus belonging to an individual

germline a cell line in eukaryotic organisms from which the gametes are derived

heritable capable of being passed on to the next generation

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

inversion mutation a mutation resulting in the normal sequence of genes being reversed in a chromosome

missense mutation a mutation that results in one amino acid being replaced by another amino acid in the encoded protein

monoculture the practice of cultivating a single genetically uniform breed of plant or livestock

multidrug resistance when a bacterium becomes resistant to two or more antibiotics

mutagen an agent capable of inducing mutations

mutation when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

natural selection the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals

neutral mutation a mutation that has no effect on the organism's chances of survival and reproduction

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

point mutation a mutation that affects a single base-pair position within a gene

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time

selection pressure a factor that favours the survival of some individuals over others within a population

selective breeding *see* artificial selection

silent mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a synonymous mutation

somatic describes a body cell that will not pass its genes on to the next generation

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same; also describes the lowest formal taxonomic rank and forms the second part of an organism's scientific name

subspecies the level of classification below species, referring to races of a species that are geographically isolated from each other

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

synonymous mutation *see* silent mutation

translocation a mutation occurring when a section of one chromosome breaks off and reattaches to another chromosome

WB
9.7.2 EXAM PRACTICE 202

9 Chapter review

Remembering

1 Define:
  - a gene pool
  - b allele frequency
  - c genetic drift.

2 Draw diagrams depicting a:
  - a bottleneck effect
  - b founder effect.

Understanding

3 Classify the following mutations as neutral, deleterious or beneficial to an organism's chances of survival.
  - a An insertion mutation in the human hexosaminidase A gene results in improper neural development.
  - b A mutation in the beta-lactamase gene of the bacterium *Escherichia coli* generates a new version of the enzyme that detoxifies the antibiotic ampicillin.
  - c A nonsense mutation in the human SURF1 gene encodes a protein crucial for formation of a key metabolic enzyme.
  - d A silent mutation in the codon for an amino acid occurs at the active site of bovine salivary amylase.
  - e Various mutations in a gene for the enzyme alcohol dehydrogenase result in different versions of the functional enzyme.
  - f A mutation that extends expression of a human lactase gene enables lactose digestion into adulthood.

4 Draw an annotated diagram of two chromosomes showing that one of them has experienced two double-strand breaks. Draw the possible chromosomal rearrangements that might occur when the fragments of the broken chromosome are re-joined.

5 Figure 9.35 shows segments of chromosomes with genes numbered along their lengths. Identify the mutation that has occurred to produce each of these structural rearrangements from the original segment.

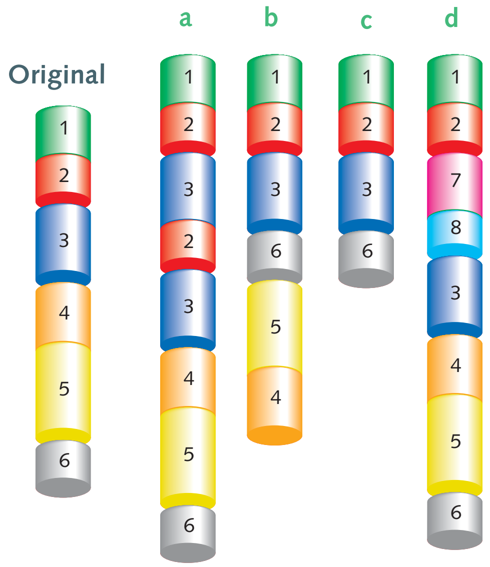


Figure 9.35 Chromosomal mutations

6 Discuss the relationship between substitution, silent, missense and nonsense mutations.

7 Mimicry is a common phenomenon in natural systems. The mimic seeks to take on the appearance of another organism. The organism being mimicked (the model) is harmful, distasteful or unpalatable to predators. Predators learn to avoid the model and therefore the mimic. It is assumed that the origins of mimicry lie in random, spontaneous gene mutations, recombinations and chromosome alterations that result in colour, structure or pattern change.
   - a Explain the possible advantages of mimicry.
   - b Explain what you would expect the ratio of models to mimics to be in natural systems.
   - c Describe how the disappearance of the model might affect the mimic.

Applying

8 Amylase is an enzyme that digests starch. Two forms of amylase are found in humans: one that is secreted in saliva, the other secreted from the pancreas. The amylases are coded by two separate but closely located genes on chromosome 1.
   - a Explain how this situation has arisen.
   - b In populations with a history of farming starchy grains, most individuals have two or more copies of the salivary amylase gene. Every gene is expressed. How has this situation come about?
   - c Draw an annotated diagram of the genes on chromosome 1, depicting your answers to parts **a** and **b**.

9 The FTO gene, a gene associated with obesity in humans, is widespread in vertebrate animals (fish, birds, reptiles and mammals). FTO is absent in insects, worms and fungi but is found in a few genera of single-celled algae. What might explain the peculiar distribution of the FTO gene across these organisms?

10 Construct a diagram to summarise the natural selection that occurred among the peppered moths of the UK as described in this chapter.

11 Copy and complete Table 9.3 using information provided in Table 9.4. Note that more than one type of mutation may describe the effect on the protein.

Table 9.3

Genetic mutation	Amino acid	Type of genetic mutation	Effect on protein
GTCCCA ↓ GTCCCT	Valine–Proline ↓ Valine–Proline	Substitution	Silent
TCAATA ↓ TAATA	Serine–Isoleucine ↓		
AGAGGT ↓ AGATGT	Arginine–Glycine ↓		
GCAAGA ↓ GAAAGA	Alanine–Arginine ↓		
CAGTAC ↓ CACGTAC	Glutamine–Tyrosine ↓		

12 A new subtype of influenza virus, H2N8 emerges from pre-existing human H2N1 and avian (bird) H1N8 subtype.
  a Use annotated diagrams to show how this new subtype may have arisen.
  b Explain why the new subtype has the potential to cause a global pandemic.
  c Describe what the WHO does to prepare for such a scenario.

13 Calculate the allele frequency of the D allele and the d allele in Figure 9.12 Population 1 before immigration. Compare this to the allele frequency in Population 1 after immigration.

Analysing

14 Using Table 9.4, list all the codons that could result from a silent mutation of GGG. What observation can you make about which of the three nucleotides in the codon is most prone to being mutated?

Table 9.4 **Properties, names and DNA codons for each of the 20 amino acids**

Characteristics	Name	DNA codons
Small, hydrophobic	Glycine	GGT, GGC, GGA, GGG
	Alanine	GCT, GCC, GCA, GCG
	Valine	GTT, GTC, GTA, GTG
	Leucine	TTA, TTG, CTT, CTC, CTA, CTG
	Isoleucine	ATT, ATC, ATA
Cyclic	Proline	CCT, CCC, CCA, CCG
Bulky, hydrophobic	Phenylalanine	TTT, TTC
	Tyrosine	TAT, TAC
	Tryptophan	TGG
Sulfur-containing, hydrophobic	Methionine (START)	ATG
	Cysteine	TGT, TGC
Hydrophilic	Serine	TCT, TCC, TCA, TCG, AGT, AGC
	Threonine	ACT, ACC, ACA, ACG
	Asparagine	AAT, AAC
	Glutamine	CAA, CAG
Negatively charged, hydrophilic	Aspartic acid	GAT, GAC
	Glutamic acid	GAA, GAG
Positively charged, hydrophilic	Histidine	CAT, CAC
	Lysine	AAA, AAG
	Arginine	CGT, CGC, CGA, CGG, AGA, AGG
	STOP	TAA, TAG, TGA

15 Construct a diagram that illustrates how recessive traits that are deleterious can survive in a population.

16 Explain why processes such as genetic drift and the founder effect are not regarded as examples of natural selection.

17 When a mutation occurs in a large population, it has very little effect on the population as a whole. Explain why mutations are still vital to the process of natural selection despite this small effect.

18 Artificial breeding of horses and cattle is not an example of natural selection but does lead to change in populations. Explain how artificial breeding is relevant to understanding natural selection.

19 The world's stock of Cavendish bananas is sterile and has been derived by cloning from a single original stock. Cavendish bananas in Australia, Africa and Asia are threatened by infection from the fungus *Fusarium oxysporum*. Why is the Cavendish banana so vulnerable to *Fusarium oxysporum* infection and what are the prospects for the population?

20 The red-legged earth mite (*Halotydeus destructor*) is considered a pest of canola and pea crops. In Western Australia, repeated applications of synthetic pyrethroid insecticides over several seasons became ineffective against the mite.

- a Explain this observation.
- b What might be the long-term consequences for red-legged earth mites in other states if the insecticide-resistant mites breach quarantine borders?

21 Explain how herd immunity reduces the antigenic drift of viruses.

Evaluating

22 Trypsin and chymotrypsin are proteases (enzymes that digest proteins) with very similar structures, but they preferentially split proteins at the site of different amino acids. The enzymes are coded by different genes; however, scientists propose that the two genes arose from a common ancestral gene. Discuss, with annotated diagrams where appropriate, what mutations may have occurred to generate the two different genes from the one original gene.

23 A measles outbreak occurs among an unvaccinated segment of the population. In time, vaccinated members of the population start developing measles. Explain using annotated diagrams how this situation could have emerged. Discuss what strategy health authorities may take to restrict the outbreak.

24 Imagine a situation in which the offspring of dark-skinned parents has inherited a mutated form of a gene that confers light skin pigmentation. Predict whether this mutation would be neutral, beneficial or deleterious if the individual is located in the Arctic Circle as compared with equatorial Africa. Explain your reasoning. Discuss how, if at all, your interpretation of 'neutral', 'beneficial' and 'deleterious' is influenced by the individual's environment.

Creating

25 Imagine you were initiating a captive breeding program for the Tasmanian devil (*Sarcophilus harrisii*), a species threatened by the spread of the transmissible devil facial tumour disease. While most of the population is susceptible, some individual devils show resistance to the disease.

- a Explain why a captive breeding program might be valuable even if resistance is showing up naturally in the wild.
- b Outline what your objective would be for the gene pool of the captive population.
- c Explain how you would seek to achieve your objective.
- d Explain which aspects of your scenario are an example of natural selection and which are an example of artificial selection.

Changes in species over time

10

By the end of this chapter you will have covered the following material.

Key knowledge

Changes in species over time

- changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils pp. 373–386
- evidence of speciation as a consequence of isolation and genetic divergence, including Galapagos finches as an example of allopatric speciation and *Howea* palms on Lord Howe Island as an example of sympatric speciation pp. 386–393

Determining the relatedness of species

- evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences pp. 393–404
- the use and interpretation of phylogenetic trees as evidence for the relatedness between species pp. 404–406

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- formulate hypotheses to focus investigation pp. 406–409

Generate, collate and record data

- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 406–409
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 406–409

Construct evidence-based arguments and draw conclusions

- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 406–409
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 406–409

Online Chapter Map
Chapter 10 Map

10 Changes in species over time

There is plenty of evidence to tell us that species have not always been as they are today. Species have come and gone, changed and also stayed the same. How do we know? It is written in the rocks!

10.2 Patterns in evolution

p. 381

Fossils reveal evidence for past mass extinctions followed by evolution of new groups of organisms. Transitional fossils with characteristics of both earlier and later species show how organisms are related. Divergent evolution occurs when new species emerge from one population under different selection pressures. Convergent evolution occurs when unrelated species evolve similar features under the same selection pressures.

10.1 Studying fossils

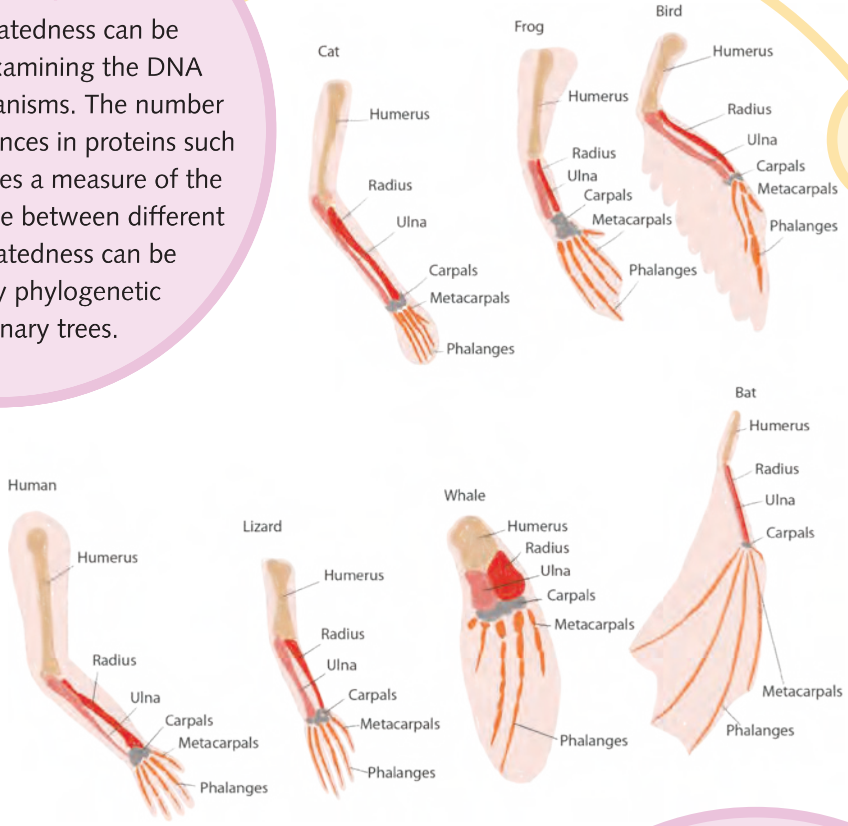
p. 374

Changes in species over millions of years can be seen in fossil evidence. Fossils are evidence of past life. They can be dated by either comparing the sequence of the rocks in which they are found (relative dating) or using the predictable natural decay of radioactive elements in rocks or fossils to determine a numerical age – (absolute dating).

p. 399

10.5 Molecular evidence for relatedness of species

Evolutionary relatedness can be investigated by examining the DNA and proteins of organisms. The number of amino acid differences in proteins such as cytochrome c gives a measure of the evolutionary distance between different species. This relatedness can be represented by phylogenetic or evolutionary trees.



p. 386

10.3 Emergence of new species

If a population becomes divided, then the members of each isolated population are unable to interbreed and exchange alleles. If each new population is exposed to different selection pressures, they will evolve independently so that eventually they cannot interbreed even if they are brought together again – they are new and separate species.

p. 393

10.4 Determining the relatedness of species

Even though species may look completely different from each other, close study of their structural morphology, embryonic stages and homologous structures give clues to their relationships. For example, the human arm has similarities with the whale flipper, bat wing and cat foreleg, showing that all these species had a common ancestor.

Evidence for relatedness of species can be found in the fossil record, how species grow and are built and even in their molecular structures. This knowledge can be applied to the human species to trace their evolution over time.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 10 map (p. 370)

Online Key Terms:
- Chapter 10 flashcards (p. 372)

Weblinks:
- Video: how fossils are formed (p. 374)
- Evidence for evolution (p. 393)

Online Worksheets:
- Fossils (p. 374)
- Evidence for evolution (p. 393)

Online Key Concepts:
- Chapter 10: Summary of key concepts (p. 412)

Chapter 10 flashcards

Know your key terms

absolute dating
adaptation
adaptive radiation
allopatric speciation
analogous structure
bioinformatics
biological species concept
clade
cladogram
comparative dating
convergent evolution
correlation
divergent evolution
electron spin resonance
evolutionary distance
fossil
fossil record
fossil succession
homologous
homologous structure
index fossil
isotope
lineage
luminescence
mass extinction
maternally inherited
mineralisation
molecular clock
molecular homology
monophyletic
morphological species concept
mya
niche
node
optically stimulated luminescence
pairwise comparison
phylogenetic tree
phylogeny
phylogram
radioactive decay
radiometric dating
relative dating
reproductively isolated
sequence alignment
speciation
strata
structural morphology
superposition
sympatric speciation
taxonomy
tetrapod
thermoluminescence
trace fossil
transitional fossil
vestigial structure

Remember

REMEMBER
PAGE 204

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Mutations are the source of new alleles in a gene pool.
2 A point mutation is when a single nucleotide is changed within a DNA sequence. If the mutation occurs within a gene, it could alter the translated protein.
3 A selection pressure is an environmental or ecological factor that promotes the survival of some individuals in a population over others.
4 Natural selection is when selection pressures cause a change in the gene pool of a population.
5 Extinction is when all the members of a population or species have died out.

In 1858, two publications were released simultaneously through the Royal Society in London. These were papers by Charles Darwin and Alfred Russel Wallace. The papers outlined Darwin's and Wallace's ideas on the evolution of species, which they referred to as 'descent with modification'. This term highlights the important idea that all life that exists today has descended from shared ancestors. Darwin and Wallace proposed that this happened by the process of natural selection, which has shaped nearly every feature of living things in the world today. Through natural selection, favourable traits are selected for and inherited and become more common in subsequent generations.

The idea of adaptive evolution through natural selection is one of the most important ideas in Biology. Although Darwin and Wallace did not have a good understanding of the underlying causes of inheritance, they did realise that variable traits must be heritable. Subsequent discoveries of the genetic basis of inheritance, initially through the work of Mendel, fitted perfectly with their theories to produce a coherent theory of evolution referred to as the 'modern synthesis'.

The evidence for evolution has accumulated in Earth's geology, in the form and function of living organisms, and in the structure of their cells and molecules. Moreover, we can use the evidence to hypothesise and construct the relationships between organisms over time. We can reconstruct the actual and hypothetical lines of descent that connect many present-day groups of species through a shared ancestry.

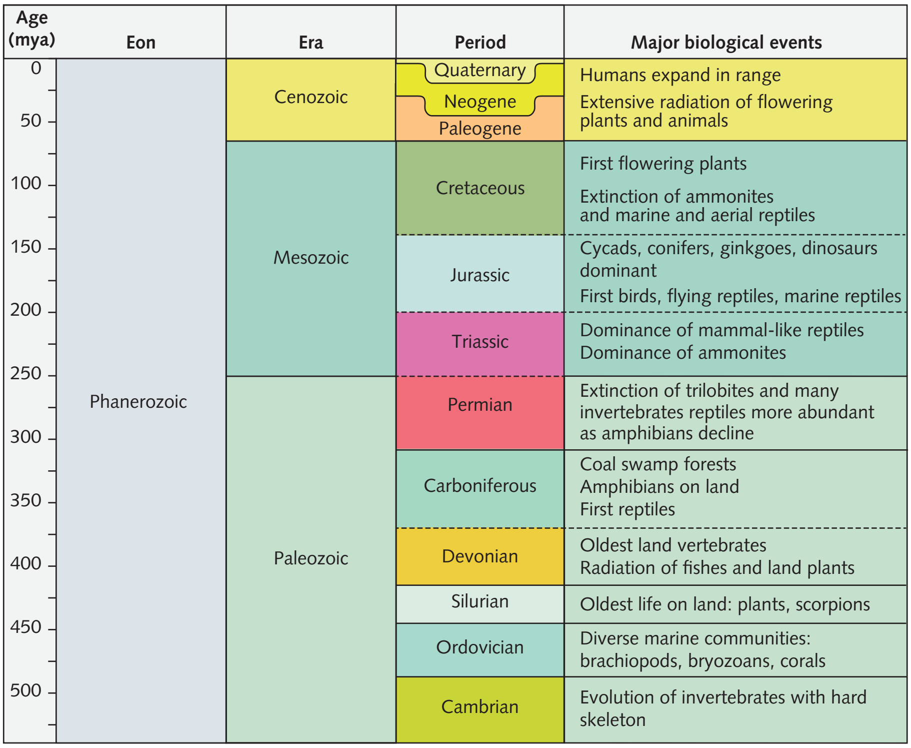
The worldwide collection of fossils as they occur in the surface layers of Earth constitutes the **fossil record**. The species appear in an order of 'fossil succession', from single-celled forms through to more structurally complex multicellular forms.

Before studying fossils, their types, methods of dating them, and interpretations that can be made from the successive layers over millions of years, it is necessary to understand that the Earth's history can be represented on a geological time scale as a 'calendar' showing a series of chronological events divided into distinct periods and eras.

Geological timescales

Dating techniques credibly demonstrate the colossal time scales it takes for species to diversify. Instead of understanding Earth's history in years, decades or even centuries, we have devised other ways of measuring 'deep time' in segments covering millions, sometimes billions, of years, such as periods, eras, epochs and eons. These measurements are known as geological time and are expressed in millions of years ago (**mya**). Fossils that are large enough to see with the naked eye become apparent from around 600 mya. This fossil record provides another means of refining Earth's geological time scales. A summary of the geological time scales is presented in Figure 10.1.

Examine Figure 10.1 to gain an understanding of some of the key events that have occured in our geological time, including prokaryote appearance, photosynthesis, first eukaryotes, first multicellular organisms, appearance of land animals, mammals, and flowering plants. However you are not expected to learn these dates or the order of events, rather just gain an understanding before studying the fossil record.



Eon	Era	Period	Major biological events
Phanerozoic	Cenozoic	Quaternary	Humans expand in range
		Neogene	Extensive radiation of flowering plants and animals
		Paleogene	
	Mesozoic	Cretaceous	First flowering plants Extinction of ammonites and marine and aerial reptiles
		Jurassic	Cycads, conifers, ginkgoes, dinosaurs dominant First birds, flying reptiles, marine reptiles
		Triassic	Dominance of mammal-like reptiles Dominance of ammonites
	Paleozoic	Permian	Extinction of trilobites and many invertebrates reptiles more abundant as amphibians decline
		Carboniferous	Coal swamp forests Amphibians on land First reptiles
		Devonian	Oldest land vertebrates Radiation of fishes and land plants
		Silurian	Oldest life on land: plants, scorpions
		Ordovician	Diverse marine communities: brachiopods, bryozoans, corals
		Cambrian	Evolution of invertebrates with hard skeleton

Figure 10.1 The geological time scales constructed from relative and absolute dating methods

10.1 Studying fossils

Fossils are the preserved remains and traces of organisms. They provide evidence of past life. These remains can be hard parts, such as teeth, bones and shells, or impressions in the rock where the organism's tissue decayed. Most fossilised 'hard parts' of animals or plants are found in rocks that have been derived from sediment; that is, sand, silt or clay. Along with animal bones, such as the skeleton of *Ceratoichthys pinnatiformis* (Figure 10.2), fossils can also include trace fossils: footprints, burrows or other evidence for the organism's existence.

To make sense of the fossil record and examine it for evidence of evolution, you need to understand some basic information about the fossils and their geological setting, including how fossils are formed and why some organisms are well represented in the fossil record while others are not. We need to be able to estimate how old the fossils are, which organisms arose first, and which organisms lived together.

iStock.com/MarcelC

Figure 10.2 An immaculately preserved fossil of the extinct fish *Ceratoichthys pinnatiformis*: a rare example of a complete fossilised skeleton.

WB
10.1.1 FOSSILISATION PROCESS
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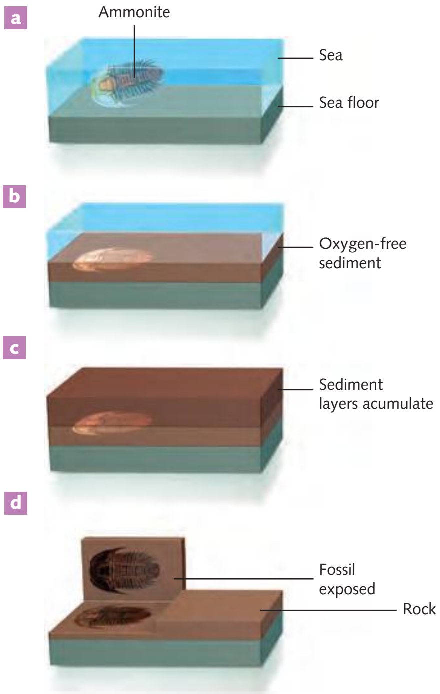
Fossilisation

The process of fossilisation requires very specific and rare conditions. The remains of the vast majority of long-extinct animals may never be found, and consequently the fossil record is incomplete and biased towards organisms that fossilise more easily. To become a fossil, organic matter needs to be deposited and covered in sediments in an environment that lacks oxygen. The lack of oxygen reduces the rate of decomposition by bacteria. The overlying sedimentary material also protects the organic matter from scavengers. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay, depriving the remains of oxygen, as can happen in the beds of lakes and rivers or in calcium-rich seabeds (Figure 10.3). In many cases, minerals from the sediments have replaced the natural bone or shell material, making the remains harder and more likely to fossilise. This type of fossilisation is called **mineralisation**. The resulting fossils are generally only the 'hard parts' of organisms, such as bones and teeth that are slow to decay, and rarely more delicate tissue, such as feathers. These layers of mud or silts are consolidated under pressure and over prolonged stretches of time to form sedimentary rock.

Fossils are not ordinarily found in volcanic rocks because molten lava solidifies at about 1000°C, which is hot enough to burn any organic material. However, fossils can be found in sedimentary layers of eroded volcanic ash. Metamorphic rock does not usually bear fossils because the pressure and heat of metamorphism generally (although not always) destroys any remnant of the fossil.

Thin tissue, such as leaves and muscle, is sometimes preserved as films or impressions left in the sedimentary rock. Fossils are also formed when the impression left by a decomposed organism is later filled by soft material such as volcanic ash, or by



Science Photo Library/Mikkel Juul Jensen

Figure 10.3 Fossil formation. **a** An ammonite dies and sinks to the sea floor. **b** The soft parts of the animal decompose and leave the shell. **c** Left undisturbed, the shell becomes buried under sand and silt. **d** Over millions of years, the shell becomes mineralised and the sedimentary layer hardens into rock.

Weblink
Video: how fossils are formed

Online Worksheet
Fossils

a mineral solution, resulting in fossils such as those composed of opal. These processes also support formation of trace fossils. **Trace fossils** are not formed directly from the organism itself but from the organism's activites; for example, footprints (Figure 10.4), burrows and even preserved waste products such as 'coprolites' – fossilised faeces – are trace fossils.

There are several other ways fossils can form. They can form through freezing and subsequent dehydration. Plant material may be partly dissolved, and some tissue replaced with dissolved salts (a type of mineralisation) that petrify the material; that is, they turn it to rock. Entire tree trunks have been preserved by petrification in fossilised forests in Arizona and Antarctica.

Shutterstock.com/Danielle Beder

Figure 10.4 An example of a trace fossil: dinosaur footprints left in ancient mud flats on what is today called Dinosaur Ridge, Colorado, USA

Fossil strata

The fossil record is delineated by layers of successive rocks, or **strata**. Each stratum comprises unique collections of fossils and represents a unique age range. The sequence of strata can be interpreted as depicting the progress of biological evolution on Earth.

To understand the fossil record as a basis for evolution, you need to understand how the strata are formed.

Stratification

Sedimentary rock is composed of weathered material from Earth's surface, such as gravels, silts, sands and muds that have been transported by water and deposited on riverbeds, flood plains and sea floors. Sediment transport and deposition is an ongoing process; it has been occurring continuously on Earth for billions of years and can still be observed today. Over time, these deposited sediments form defined strata that consolidate into sequences of sedimentary rock. Older strata gradually become buried under progressively newer formations. These sequenced layers are the strata, and a section showing successive layers of sedimentary deposition is called stratification (Figure 10.5).

age-fotostock/Zoonar/Graham Mulroo

Figure 10.5 Sequential strata of marine sedimentary strata are visible in these weathered sandstone cliffs. The youngest strata are at the top and the oldest are at the bottom.

Strata formed at the same time and under similar circumstances are composed of the same sorts of minerals and have the same physical properties. These strata often also contain the same sorts of fossils. Strata become deformed and disrupted over long periods of time by the geological processes that destroy and rebuild Earth's crust. Over millions of years, these geological processes gradually push strata that have formed on the sea floor up above sea level, in many cases forming new mountain ranges. There they are subjected to the weathering effects of wind and rain. After a very long time, ancient fossils may become exposed.

Only a very small percentage of organisms ultimately leave fossilised remains. Many fossils are destroyed by weathering and erosion. Many more may lie buried or hidden. The fossil record is incomplete; however, it is continually augmented by new discoveries, and curated fossils may be re-examined as new experimental techniques are developed.

Relative dating techniques

Relative dating (also called **comparative dating**) is used to determine the age of a rock, or a fossil contained in the rock, relative to other rocks or fossils found nearby. This approach to dating relies on our understanding of how sedimentary rock is formed.

WB
10.1.2
RELATIVE
DATING
TECHNIQUES
PAGE 206

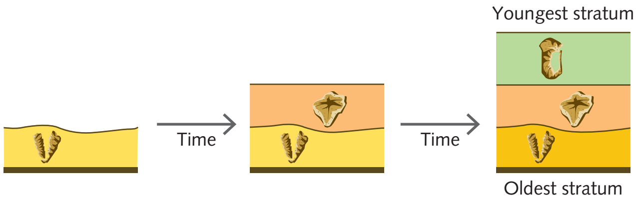


Figure 10.6 Over geological time, younger strata are deposited over older strata. The youngest fossils are found in the uppermost stratum and the oldest are found at the bottom.

Strata are deposited in a time sequence, with younger strata progressively formed on top of older strata (Figure 10.6). This is the basis of the principle of **superposition**. Assuming that the movement of Earth's crust has not twisted or inverted the strata, palaeontologists can assign relative ages to fossils according to the strata in which they are found. The fossils found in the uppermost strata must be younger than those found in the older strata underneath. While this technique cannot give an age in years, it can estimate the ages of the sequence of the strata relative to each other.

Index fossils

Strata that are located in distant sites but have identical mineral and fossil composition can be assumed to be the same age. This is the principle of **correlation**.

Correlating the ages of strata that are far apart is expedited by identifying index fossils. **Index fossils** are fossils that are representative of specific geological ages. Ideally, index fossils appear in only a limited segment of the fossil record so they are tightly linked with a particular geological age. They occur over relatively wide geographical ranges so they can be used to calibrate ages between far-flung sites (Figure 10.7). If rock beds hundreds of kilometres apart contain specimens of the same index fossil, it can be inferred they are of the same geological age.

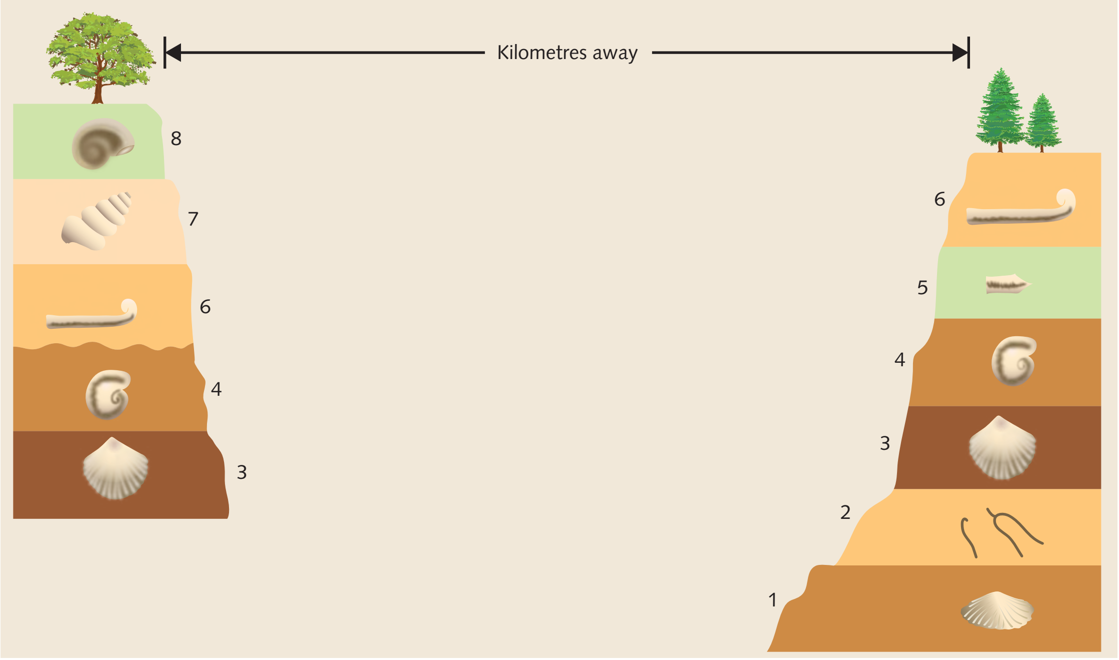


Photo courtesy of The Changing Earth - Introduction to Geology (2nd ed.), by Mears, Jr., D. Van Nostrand Co., 1977

Figure 10.7 Index fossils are unique to specific strata and enable the ages of strata from distant sites to be correlated.

Fossil succession

As early as the beginning of the 19th century, when fossils were becoming increasingly studied, it was recognised that each stratum bore a unique collection of fossil animals and plants. By advancing upwards through the sequence of strata (essentially travelling forward in time), one collection of organisms is replaced by another.

Also, many fossil organisms occur in more than one stratum. However, if you proceed down through the sequence (travelling back in time), you reach a stratum beyond which none of that fossil organism is found. In effect, initially the organism does not exist in the fossil record but, after it first appears, it may persist in the fossil record for some time, possibly even to the present.

These observations highlight the principle of **fossil succession**. They demonstrate that the history of life on Earth is described by a number of distinctive time spans, each dominated by a characteristic collection of animals and plants. Progressively, through the course of evolution, one collection abruptly gives way to another.

Absolute dating

Absolute dating is a technique that assigns a numerical age in years to a fossil or rock. Unlike comparative dating, absolute dating is based on the physical or chemical properties of materials in the rock, rather than the assumption-based sequences that relative dating provides. There are several different absolute dating techniques for absolute dating on vastly different time scales from thousands of years to hundreds of millions of years.

WB
10.1.3
ABSOLUTE DATING
PAGE 207

Radiometric dating

The most common method of absolute dating is **radiometric dating**, which is based on the predictable rates of decay of naturally occurring radioactive isotopes present in a rock or fossil. By testing for the proportion of different radioactive isotopes, an age in years can be estimated for the sample.

Many elements occur as **isotopes**: they have the same atomic number (the same number of protons) but a different atomic mass (different numbers of neutrons). For example, carbon has three isotopes: carbon-12 (^{12}C), carbon-13 (^{13}C) and carbon-14 (^{14}C). ^{12}C has six protons and six neutrons in each nucleus, ^{13}C has six protons and seven neutrons, and ^{14}C has six protons and eight neutrons. Some isotopes have an unstable nucleus that splits and emits energy in the form of radioactivity (alpha, beta or gamma rays) at a measurable rate. This process is referred to as **radioactive decay**. The half-life of an isotope is the time taken for half of the radioactive atoms in an initial sample to decay.

^{14}C is a radioactive isotope that forms when cosmic rays strike nitrogen atoms in the upper atmosphere. ^{14}C decays at a known rate to produce nitrogen-14 (^{14}N). Its measurable rate of decay is the basis of carbon dating. When an organism dies, the ^{14}C it contains decays at a steady rate. We can use the half-life of ^{14}C (5730 years) and the ratio of ^{14}C to ^{12}C in the sample to determine the age of the sample – in other words, the time taken for the original ratio to decay to the present ratio (Figure 10.8).

In its simplest form, carbon dating assumes that the proportion of ^{14}C in the atmosphere is constant, but data from tree rings shows that it can change with time. For this reason, the calculated age has to be corrected into calendar years. Ages are expressed with their degree of accuracy (usually as ± x years).

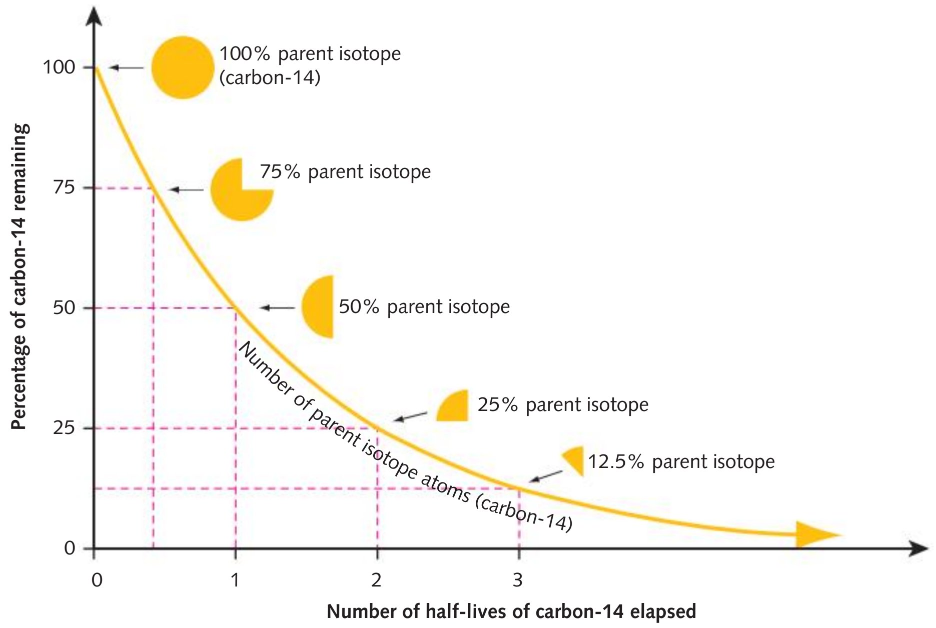


Figure 10.8 A graph of the decay of ^{14}C (half-life 5730 years)

The older the object, the greater the margin of error. Carbon dating – corrected for atmospheric variation – is thought to be accurate for samples up to about 60 000 years old. After this time it is difficult to measure the level of ^{14}C accurately and so other radioisotopes, such as potassium-40 (^{40}K), which decays into argon, are used instead (Table 10.1).

^{14}C dating is generally used for artefacts such as ash deposits but is not applied directly to fossils for two main reasons.

- In most cases, fossils have been mineralised, meaning that the organic (carbon-containing) tissue has been chemically altered or replaced.
- The process of fossilisation generally takes longer than the maximum age of accuracy for ^{14}C.

EXAM TIP

Many radiometric dating methods are suitable for dating sediments on the scale of mya, but ^{14}C is limited to determining ages less than 60 kya.

Table 10.1 The half-lives and products of decay of some elements used in radiometric dating

Element	Product	Half-life (years)
Carbon-14 (^{14}C)	Nitrogen-14 (^{14}N)	5730
Uranium-235 (^{235}U)	Protactinium-231 (^{231}Pa)	704 million (7.04×10^8)
Uranium-234 (^{234}U)	Thorium-230 (^{230}Th)	246 000
Potassium-40 (^{40}K)	Argon-40 (^{40}Ar)	1.25 billion (1.25×10^9)
Thorium-232 (^{232}Th)	Lead-208 (^{208}Pb)	14 billion (1.4×10^{10})
Rubidium-87 (^{87}Ru)	Strontium-87 (^{87}Sr)	48 billion (4.8×10^{10})

Certain types of rocks are more suited to radiometric dating than others. Igneous and metamorphic rocks are suitable because the radioactive atoms are generated and incorporated into the rock when the rock is first formed. For example, feldspar is a common type of igneous rock that contains potassium but not argon; radioactive decay of ^{40}K to ^{40}Ar (argon-40) in the feldspar thus provides an accurate reflection of the age of the rock. Sedimentary rocks are not suited to radiometric dating because they are formed when older rocks are weathered, and the particles are subsequently deposited in the sedimentary layer. Radioactive decay would have already occurred in the minerals before they formed the sedimentary rock so radiometric dating of the sedimentary layer is misleading. A more reliable estimate for the age of sedimentary rock is to date the igneous or metamorphic layers either side and define an age bracket for the sedimentary rock in the middle.

Luminescence techniques

Thermoluminescence and **optically stimulated luminescence** are absolute dating techniques that measure the properties of electrons in the crystals of minerals (Figure 10.9). The atoms in mineral crystals mostly pack uniformly into a regular lattice structure; however, there are imperfections or holes where atoms are missing from the structure. The energy from natural radioactivity within the mineral lattice and in the surrounding environment chips electrons off atoms in the mineral and they can become trapped as isolated solitary electrons in the holes of the lattice. The rate at which solitary electrons are produced depends on the amount of radioactivity experienced by the mineral. The total amount of solitary electrons trapped in the mineral depends on both the rate at which they are produced and the amount of time since the mineral was last exposed to radiation (i.e. light or heat).

The trapped solitary electrons are subsequently released when the mineral crystal is again exposed to light or heat (Figure 10.9). As the electrons escape, they emit a corresponding amount of energy in the form of light, which can be measured by a specialised instrument. **Luminescence** techniques differ depending on how the solitary electrons are released from the mineral. In optically stimulated luminescence, the electrons escape when the mineral is exposed to coloured light. In thermoluminescence, the electrons escape when the mineral is heated. Either way, the amount of light emitted is a measure of the quantity of solitary electrons trapped in the mineral grain.

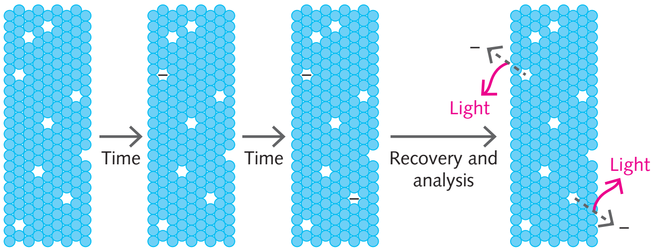


Figure 10.9 The basis of luminescence dating techniques. A long time after burial (e.g. thousands of years), natural radioactivity in the mineral and the environment drives the production of free electrons (–) that become trapped in imperfections in the mineral crystal. The amount of electrons that are trapped depends on the rate at which they are produced and the total amount of time the mineral crystal remains buried. When the mineral crystal is recovered, it is stored away from heat or light until analysis under controlled laboratory conditions. During analysis, the mineral crystal is exposed once again to heat or coloured light, which stimulates the electrons to escape from the crystal. As they escape, the electrons emit light that is measured to provide an estimate of the total number of trapped electrons in the crystal.

The minerals thus act as memory devices, storing the record of when radiation (such as light or heat) last struck the mineral crystal. While the mineral grains are being transported by wind or water, they are exposed to sunlight and heat, and their energy signal is set (or reset) to zero. Once buried, the mineral crystal captures energy from surrounding radiation and stores this energy in the form of solitary electrons trapped in the imperfections of the crystal. The solitary electrons accumulate in the mineral crystal for as long as it is buried.

The amount of radioactivity must also be measured within the mineral and in its immediate environment. This makes it possible to calculate the rate at which the solitary electrons are generated. From two parameters, the total solitary electrons in the mineral and the rate at which they are generated, it is possible to calculate the amount of time since the mineral was buried.

Both optically stimulated luminescence and thermoluminescence are useful for dating the last exposure of the minerals to heat or light and the occurrence of mineralisation. Therefore, they can provide an estimate of the time of burial in sedimentary rock. However, they cannot generally provide an estimate for the absolute age of the original mineral that was deposited. The technique is suitable for ages up to 100 000 years and is often used to estimate the age of archaeological artefacts associated with modern humans.

Electron spin resonance

Electron spin resonance also measures the properties of electrons trapped in mineral crystals. In contrast to luminescence techniques, the electrons do not need to be released to be measured.

When a magnetic field is applied to the mineral, the solitary electrons respond as though they are tiny magnets aligning in the magnetic field. A signal is recorded and its strength indicates how many solitary electrons are inside the mineral. The amount of radioactivity is then measured within the mineral and in its immediate environment, which makes it possible to calculate the rate at which the solitary electrons are generated. The total solitary electrons and the rate at which they are generated are used to calculate the amount of time since the mineral was formed.

As with luminescence techniques, it is necessary also to measure how much radioactivity the sediment grains are exposed to, to gauge the rate at which solitary electrons are generated. The measurements for both the total solitary electrons and the rate of their production are used to calculate the time since the minerals were last exposed to light or heat.

The sensitivity of electron spin resonance depends strongly on the nature of the sample and the environment it has experienced but it can be used to measure ages from a few thousand years up to about 2 million years. An advantage of electron spin resonance is that it can be applied directly to minerals produced by organisms, such as teeth and shells.

KEY CONCEPTS

» Fossils are preserved remains of organisms or traces of their existence. The conditions for fossilisation occur rarely and this can cause a bias in the fossil record.

» The fossil record is delineated by consecutive layers of rocks called strata. The youngest fossils are found in the uppermost strata and the oldest are found at the bottom.

» Comparative dating can determine the age of a fossil or fossil-bearing rock in relation to the surrounding rock, but it does not give a numerical age.

» Index fossils are used to correlate the ages of strata that occur in deposits far from each other.

» Absolute dating gives numerical ages for the time of fossil formation and includes radiometric dating, luminescence and electron spin resonance.

Concept questions 10.1

1 Describe the stages of the fossilisation process.

2 Most of our knowledge of the evolution of sharks is based on the remains of fossilised shark teeth. Suggest why other fossilised body parts of sharks have not been found in abundance.

3 Palaeontologists have found tracing the evolution of sea jellies ('jellyfish') to be very challenging. With your knowledge of fossils and the process of fossilisation, suggest why this may be the case.

4 Suggest what process might explain how fossils that died on the sea floor could be found high in the Himalayan mountains.

5 Recount two modern techniques for studying fossils and the kind of information that can be obtained from each.

6 List the methods used to determine the age of fossils and give the pros and cons of each.

7 What features make an index fossil?

8 Which isotopes would be measured to radiodate a fossil that comparative dating suggests is approximately 50 000 years old?

HOT Challenge

9 Radiometric dating is based on the concept of half-life.
  a What does this mean?
  b What is the half-life of ^{14}C?
  c Why is carbon dating of no value in dating fossils that are millions of years old?

10.2 Patterns in evolution

The fossil record provides evidence to trace the evolutionary descent of many broad groups and species of animals and plants over time. Emerging through phases of extinction followed by repopulation, the evolution of organisms is shaped by natural selection over vast amounts of time. The two most striking patterns are divergent evolution and convergent evolution.

Mass extinctions

The collection of fossil animals and plants in a stratum sometimes gives way abruptly to a completely different collection of fossils in the next stratum. Near the top of the older stratum, substantial changes occurred in global environmental conditions that forced the life forms in that stratum to come to an end. The boundary between the two strata marks a **mass extinction** event.

Mass extinctions help to explain major episodes of succession. The interpretation of these boundaries is that large and diverse collections of organisms were rapidly wiped out by sudden and extreme changes in environmental conditions. The organisms' demise left many **niches** (ecological roles) vacant. Descendants of the survivors of the mass extinction evolved relatively rapidly, filled the vacant niches and created novel ones in the emerging new world. The extinction survivors and their evolving descendants ultimately form the collection of organisms that populates the subsequent stratum.

The fossil record thus shows evidence of rapid and diverse evolution of many species from a single (or just a few) initial ancestral species. Each of the descendent species has unique anatomical **adaptations** that allow the organism to exploit specific ecological niches.

WB
10.2.1
TRANSITIONAL
FOSSILS
PAGE 209

Transitional fossils

The fossil record demonstrates many phases of evolutionary succession. As new species replace older ones, there are lines of continuity between ancestral and descendant forms. Some fossils bear features of both an older ancestral life form and a younger descendant. These intermediate forms are called **transitional fossils**. Transitional fossils provide copious evidence for evolution, documenting changes between groups of organisms over time.

From fish to amphibians

Many transitional fossils between lobed-fin fish and amphibians have been recovered from the period when land-based **tetrapods** (vertebrate animals with four legs) first appear in the fossil record. Fossils, such as *Acanthostega* species, show the early evolutionary progress of tetrapods (Figure 10.10). These animals were about 1 metre long and had a tail and gills typical of fish. However, they also had relatively well-developed limbs with 6–8 digits like tetrapods. Unlike fish, they had a strengthened rib cage and shoulder blades separate from the skull, accommodating a muscular neck that allowed the head to turn relatively freely. They were mainly aquatic but showed the skeletal and morphological adaptations to land that indicate they were the probable ancestors of modern tetrapods.

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Figure 10.10 a A fossil of *Acanthostega* and **b** a digital reconstruction of an *Acanthostega* species from 380–365 million years ago

From dinosaurs to birds

The most well-known mass extinction in the fossil record was the one that ended the age of the dinosaurs. Among the survivors of the extinction were small, ground-dwelling feathered dinosaurs called *Archaeopteryx* (Figure 10.11). About the size of a modern crow, *Archaeopteryx* had the uniform teeth, bony tail and claws typical of dinosaurs. However, it also had the forked wishbone characteristic of birds and the sediment around the animal bears the imprint of feathers. Therefore, *Archaeopteryx* is interpreted as a transitional fossil showing that modern birds descended from dinosaurs. Palaeontologists currently know of five feathered dinosaur species from the fossil record that survived the extinction of dinosaurs. Those ancestors have given rise to more than 10 000 bird species today.

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Figure 10.11 a An *Archaeopteryx* fossil and **b** a reconstructed model of the bird-like dinosaur

WB
10.2.2
DIVERGENT
EVOLUTION
PAGE 211

Divergent evolution

Divergence is a pattern of evolution where groups of organisms become so different from each other that a new species forms. This is called **speciation**. This is usually the result of the dispersal of a single species to different environments; that is, groups from the same species become isolated from each other. The isolation stops the gene flow between these separated populations and different selection pressures work on each isolated population. Over many generations, members of the population develop adaptations to the different selection pressures, and they eventually become new species. This is described as **divergent evolution**.

For example, koalas (tree-dwelling herbivores), Tasmanian devils (ground-dwelling carnivores) and marsupial moles (dune-burrowing insectivores) have a common ancestor (Figure 10.12). However, they have quite different feeding structures that adapt them to different diets. These animals have evolved by divergent evolution.

Adaptive radiation

Adaptive radiation is a pattern of divergent evolution in which organisms rapidly diversify into many new forms. The fossil record shows that every mass extinction event was followed by adaptive radiation. Adaptive radiation occurs when environmental changes trigger the availability of new resources and environmental niches. A clear example of this can be found in Australia's fossil record, which indicates that

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Figure 10.12 a Koalas, b Tasmanian devils and c marsupial moles evolved from a common ancestor that probably lived during the Eocene epoch. These are examples of divergent evolution.

during the Middle Miocene epoch (approximately 15 mya), dense tropical forests covered central Australia where the Simpson Desert is now.

Forests, lakes and permanent rivers provided a lush habitat for marsupials such as giant koala-like possums, shrewish insectivores and sheep-sized browsers. Flamingos, crocodiles, turtles and dolphins flourished in the waterways. The range of habitats allowed the extensive radiation of animal species that adapted to the available resources and which is an example of adaptive radiation.

Slowly, the tropical centre of Australia began to dry out during the Pliocene epoch (approximately 5 mya). This brought an end to the tropical habitat, which gave way to broad grasslands. Large browsing mammals called diprotodontids (Figure 10.13) and a variety of possums could not survive with the reduction of trees and the subsequent limited food available.

As the tropical forests retreated from central Australia, the animals they once supported were forced to compete for diminishing resources and became vulnerable to extinction. Remnants of these forests and their inhabitants are now confined to Papua New Guinea and pockets of northern Queensland. The grasslands that replaced the forests provided new habitats that allowed for adaptive radiation of other Australian mammals: the kangaroos and wallabies. A summary of the adaptive radiation of marsupials is shown in Figure 10.14.

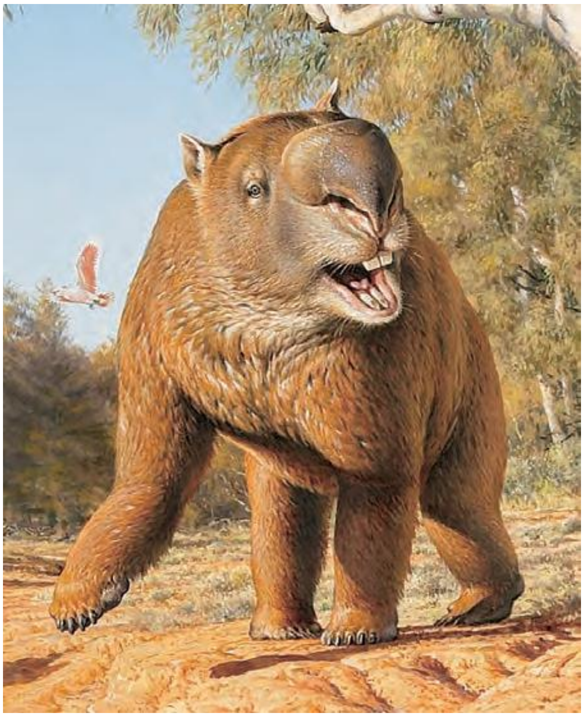

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Figure 10.13 The giant *Diprotodon optatum* was a type of megafauna that browsed on leaves.

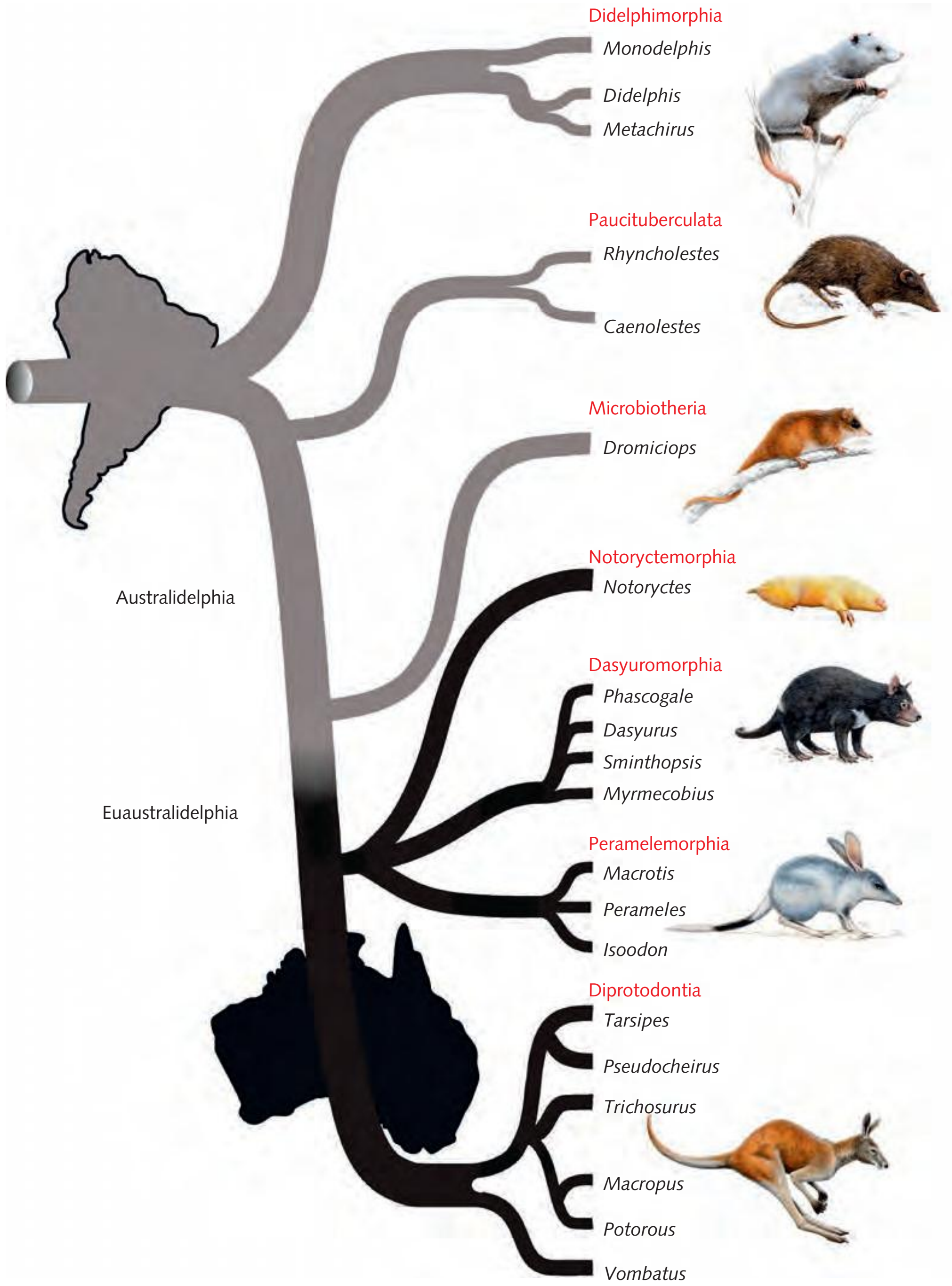

Figure 10.14 **Adaptive radiation of marsupials began in South America, which was joined to Antarctica and Australia in the supercontinent Gondwana. The Australian continent detached and began drifting north about 45 mya. Most surviving marsupials are now restricted to the Australian continent.**

WB
10.2.3
CONVERGENT
EVOLUTION
PAGE 212

Convergent evolution

Convergent evolution occurs when unrelated organisms evolve similar adaptations in response to similar selection pressures. Ant-eating mammals are an example of convergent evolution. Many animals eat ants and termites and have developed similar structures, but they are not closely related.

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Figure 10.15 **Ant-eating mammals, including a echidnas (monotremes), b numbats (marsupials) and c pangolins (placental mammals) show convergent evolution with ant-eating structures.**

CONNECT

See Chapter 11 for more on monotremes, marsupials and placental mammals.

Modern ant-eating mammals include echidnas, which are monotremes; numbats, which are marsupials; and aardvarks and pangolins, which are placental mammals (Figure 10.15). All of these species have an elongated snout for smelling and digging, a long, extendible tongue that can extract ants from crevices, and powerful claws that can dig up ant and termite nests.

The different species of ant-eating mammals do not share a common ancestor. They have developed ant-eating habits independently and coincidentally under the same selection pressure to survive by sourcing ants for food.

KEY CONCEPTS

- The fossil record demonstrates that mass extinction events have occurred many times in the past. After each mass extinction, a collection of new species replaces the extinct ones.
- Transitional fossils provide evidence for evolutionary relationships between groups of organisms and document change in organisms over time.
- Divergent evolution occurs when different selection pressures apply to different populations of an ancestral species. The different populations accumulate many changes, becoming new species with characteristics that differ from those of the ancestral species. When this occurs on a large scale it is called adaptive radiation.
- Convergent evolution occurs when unrelated organisms (or organisms with a very distant common ancestor) evolve similar structures or adaptations to perform a similar function in response to the same selection pressures.

Concept questions 10.2

1. How do strata provide evidence for mass extinction events?
2. Explain what a transitional fossil is and how it shows evidence of the descent of a later group of organisms from an earlier group of organisms.
3. Give an example of a transitional fossil, the ancestral and descendant groups it 'transitions' between, and the features of the fossil that serve as evidence for the transition.
4. Draw an annotated diagram contrasting the patterns of divergent and convergent evolution. Include ancestral and descendent species with arrows connecting the ancestral species to the descendent species.
5. From your knowledge of the fossil record, give an example of a situation that leads to adaptive radiation of species.

HOT Challenge

6. **a** What type of evolution might exist in the following groupings?
   - **i** Dolphin and whale
   - **ii** Shark and whale
   - **iii** Dog, fox and wolf
   - **iv** Hummingbird and hummingbird moth

   b How is adaptive radiation of a species different from divergent evolution?

   c Is the example of Darwin's Galapagos tortoises an example of adaptive radiation or divergent evolution?

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Figure 10.16 a The famous Galapagos tortoises are similar to **b** the much smaller Chaco tortoise (*Chelonoidis chilensis*), found in South America.

10.3 Emergence of new species

The Galapagos Islands lie about 1000 km west of Ecuador (South America) in the Pacific Ocean. Charles Darwin drew inspiration for his proposal for the origin of species from observations and collections he made at the islands in 1835. During his famous voyage on the HMS *Beagle*, he realised that these islands were geologically quite young. They were teeming with life but the animals and plants on the islands were of recent origin. Many of these appeared to be related to similar species on the South American mainland but were also clearly different from them. One of the most famous groups of animals on the Galapagos Islands are the 15 or so species of giant tortoise, whose closest living relative, the Chaco tortoise, is found in mainland Argentina (Figure 10.16). Darwin wondered how the tortoises had got to the islands, and how there could be so many different species. He hypothesised that the tortoises on the Islands originally came from the mainland population but had changed over time to become better suited to the environment of the Galapagos in the process of speciation. How this occurred was a key aspect of Darwin's theory.

The species concept

Species can be defined in a variety of ways. In 1940, Ernst Mayer proposed that species are groups of actual, or potentially, interbreeding natural populations that are reproductively isolated from other such populations. This is called the **biological species concept**. According to this definition, individuals from different species are unable to produce viable offspring under natural conditions. The biological species concept is the most widely used in evolutionary biology. It relates directly to the concept of a species as a genetically isolated group, which can only breed within itself. In this sense, a species is represented by a totally isolated gene pool.

Sometimes, the only evidence that a species existed is its fossil. The **morphological species concept** identifies different species based on their physical characteristics but is limited to what can be observed in the fossil record. For example, kangaroos are well represented in the fossil record. Twenty-five million years ago the ancestors of modern kangaroos lived in rainforests and fed on fruit. Kangaroo species of today are connected to these distant ancestors through an unbroken line of descent.

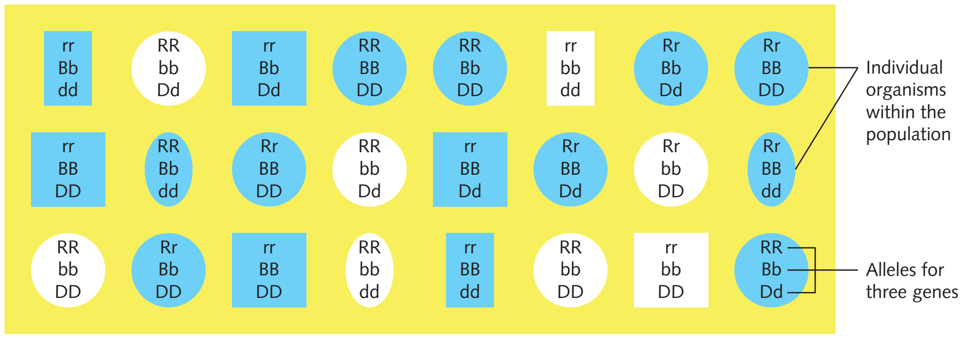
Allopatric speciation

Allopatric (from the Greek *allo* meaning other, and *patric* meaning home) speciation is regarded as the most common mechanism by which new species emerge. **Allopatric speciation** occurs when members of an initial population become geographically separated and each isolated population develops into a new species. The mechanism builds on the principles of natural selection introduced in the previous chapter. The key stages of allopatric speciation are summarised in Figure 10.17 and are as follows:

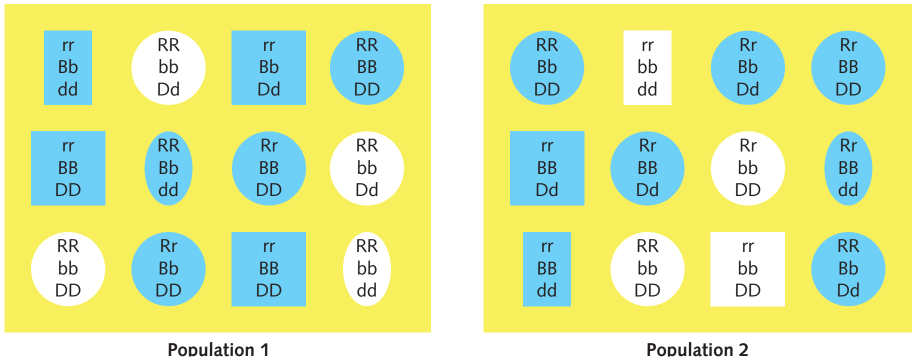
1. The process commences with a population of individuals that contribute variation to the initial gene pool (Figure 10.17a).
2. The population becomes divided by a geographical barrier such as a river or a canyon. The geographical barrier prevents gene flow between the gene pools of the two isolated populations (Figure 10.17b).
3. Different selection pressures act on each isolated population, favouring different variants in each gene pool (Figure 10.17c).

EXAM TIP
A common misconception is that evolution occurs because it benefits the organisms, as though it is intentional. More accurately, evolution occurs as a result of natural selection acting on the variation in a population.

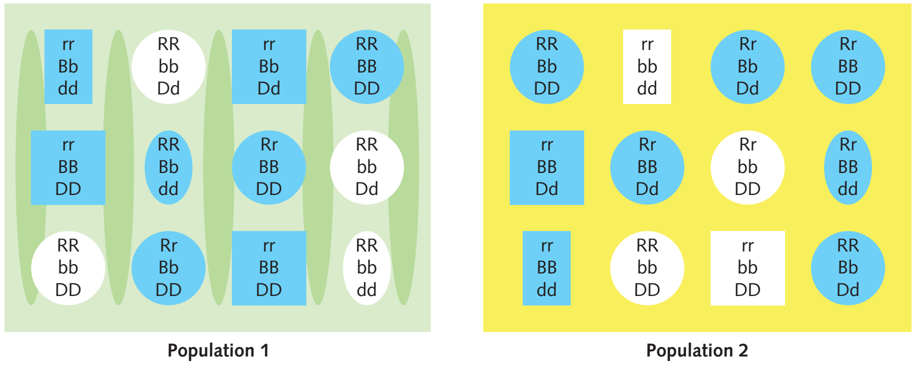
a An initial population shows variation



b The population is divided by a geographical barrier.



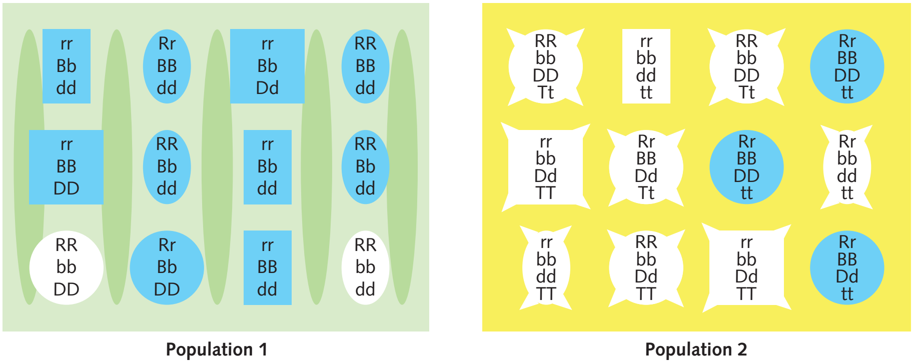
c Different selection pressures act on each separate population.



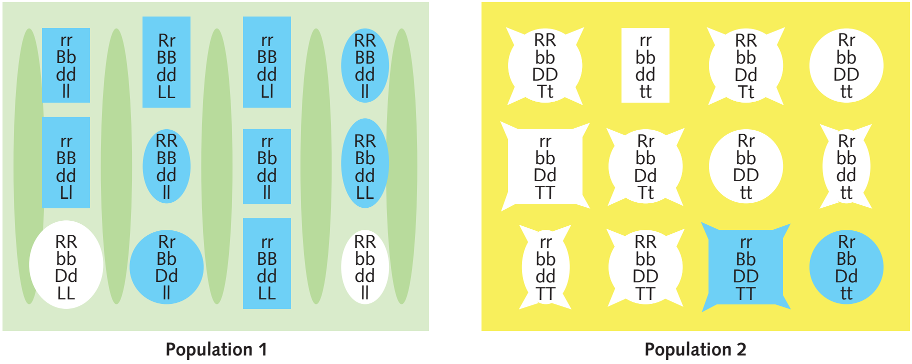
4 Over time, point and block mutations accumulate independently in the gene pool of each isolated population. This implies that new genes, as well as new alleles, are appearing. The gene pool of each isolated population acquires unique mutations that are favoured by local selection pressures, some of which become fixed (Figure 10.17d and e). Note that once an allele is fixed, it no longer contributes variation to the population.

5 Eventually, after enough time has elapsed, the two isolated populations are distinctly different. Members of one population are genetically incompatible with those of the other so they are no longer capable of interbreeding, even if they encounter each other. Each population is now a distinct new species (Figure 10.17f).

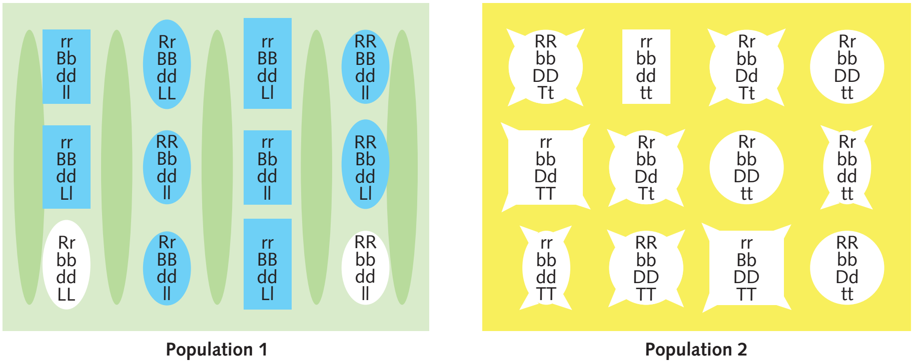
d New mutations accumulate independently in each separate population. A new gene for thorns has appeared in Population 2



e A new gene for size has appeared in Population 1.



f After sufficient time, each population has become a distinct species. The two species have different genes for different characteristics and are genetically incompatible with one another: members of Species 1 cannot interbreed with members of Species 2 and vice versa. Note that allele d has become fixed in Species 1, and allele b has become fixed in Species 2.



CONNECT
See Chapter 9 for more on gene duplication and mutation.

Figure 10.17 a–f The stages of allopatric speciation. Alleles: R = round, r = square; B = blue, b = white; D = broad, d = narrow; T = thorns, t = no thorns; L = long, l = short.

Allopatric speciation and Galapagos finches

WB
10.3.1
ALLOPATRIC SPECIATION AND GALAPAGOS FINCHES
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The Galapagos finches are 14 songbird species native to the Galapagos and Cocos islands. Specimens were first collected by Charles Darwin, who was particularly fascinated by the birds' beaks. The beaks differ in size and shape depending on how the birds obtain their food (Figure 10.18). In 1845, reflecting on the finches' morphologies, Darwin wrote that it was as though 'one species had been taken and modified for different ends'.

Morphological and genetic evidence suggests the Galapagos finches are a closely related group of bird species. They are more closely related to each other than to any other bird species. The finches are found on the Galapagos and Cocos islands but nowhere else in the world. A couple of the finch species are located only on one or two islands but most species co-inhabit a number of different islands. Field observation over multiple generations shows the fundamental ingredients for speciation occur in the finch populations (Figure 10.19). Each population exhibits variations in beak characteristics, as well as other traits, and the traits are heritable. The genetic evidence suggests they share an evolutionary history extending to approximately 3 million years ago.

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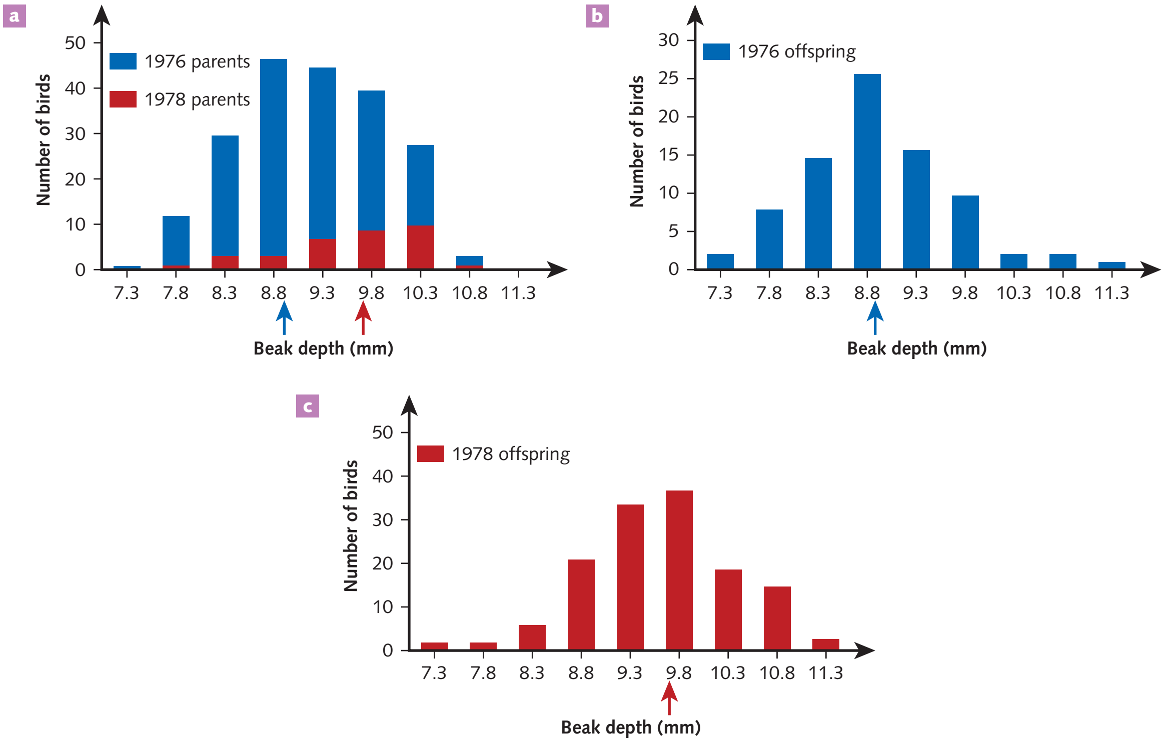
Figure 10.18 Galapagos finch species show variations in beak shape depending on the type of food they eat. **a** The green warbler finch (*Certhidea olivacea*) feeds mainly on small insects and spiders. **b** The sharp-beaked ground finch (*Geospiza difficilis*) has a varied diet but feeds primarily on small seeds. **c** The vegetarian finch (*Platyspiza crassirostris*) feeds mainly on plant leaves, flowers and fruit. **d** The large ground finch (*Geospiza magnirostris*) has a varied diet that includes large woody seeds, cactus fruits and large insects.



Adapted from R.B. Grant and Grant, P.R. (2003) *BioScience* 53: 965–975.

Figure 10.19 **Evolutionary change in beak depth in the population of the medium ground finch (*Geospiza fortis*) on the Galapagos island of Daphne Major. a The distribution of beak depths in the breeding population in 1976 (blue) and 1978 (red). The 1978 breeding population comprises the survivors of a drought that affected the island in 1977. Large ground seeds were the main food source available during the drought. The difference between the means, indicated by the arrows, is a measure of the strength of natural selection. b The distributions of beak depths of fully grown offspring hatched from the 1976 parents. c The distributions of beak depths of fully grown offspring hatched from the 1978 parents. Evolutionary change between generations is measured by the difference in mean between the 1976 offspring before the effect of the selection pressure and the 1978 offspring afterwards.**

The Galapagos Islands are 18 main islands and countless small islands and rocky outcrops spread over an area of 45 000 km^2, about 1000 km west of Ecuador (Figure 10.20). They are a geologically recent volcanic island chain (archipelago) that emerged between approximately 4.2 and 0.7 mya, although the submerged portions of the islands are millions of years older. Cocos Island is a separate volcanic island formed approximately 2 mya about 840 km north of the Galapagos Islands. The global and local climate changed continuously throughout the islands' formation up to the present. These include cycles between glacial and interglacial periods every 100 000 years. Glacial periods were associated with lower sea levels so the area and distance between islands changed throughout the archipelago's history. Ongoing volcanism combined with climatic variations probably resulted in dynamic variations in the vegetation and food types available across the archipelago.

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Figure 10.20 **The location and arrangement of the Galapagos Islands**

Evolution of Galapagos finches

The most straightforward interpretation of these observations is that the Galapagos finches evolved by allopatric speciation. The scenario implies that the ancestral finches arrived from the South American mainland possibly approximately 3 mya at a time when the Galapagos archipelago consisted of five or six islands. The founder population probably entered an environment that was warmer and wetter than at present. The islands were already colonised by plants and insects and were probably covered in rainforest. The founding finches were likely to be generalist feeders of insects, flower nectar and pollen.

Conditions did not remain static. Continuing volcanism, changing climate, and differing patterns of colonisation by plants and insects gradually created new islands with new environments. In time, members of the original finch population dispersed to settle new island habitats. Competition among individuals for the available food presumably exerted selection pressures on resettled finch populations. One of the habitats occupied by resettled finches would have been relatively dry and dominated by bushland. Individuals with sharp beaks were better able to manipulate and crush the small nutritious seeds that fell to the ground. These finches had the best chance of surviving and reproducing, and they passed their alleles for sharp beaks on to their progeny. The finches with the least suitably shaped beaks for feeding on such seeds were more prone to die without leaving offspring. Over many generations, individuals in the population predominantly had sharp beaks. Together with the effects of additional new mutations, alleles that had become fixed by genetic drift and isolation from gene flow, this finch population diverged to become a species distinct from other populations.

Similar selection pressures probably occurred in other island habitats (Figure 10.21). One finch population may have occupied a dry habitat dominated by *Opuntia* cactus. This environment favoured individuals with long slender beaks better able to probe cactus flowers for pollen and nectar. Another population may have occupied a moist upland forest dominated by trees in which individuals with strong large beaks may have had an advantage. These finches were capable of tearing bark from the trees to expose insects upon which they could feed.

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Figure 10.21 **Examples of different environments in the Galapagos Islands: a bushland on Isabella Island; b dry landscape dominated by *Opuntia* cactus on Santa Cruz Island; c lush forest on the highlands of Santa Cruz Island.**

This model of allopatric speciation assumes that geographically separated finch populations adapted under natural selection to local conditions and gradually evolved in isolation into separate species. Allopatric speciation helps us to understand the evolution of Galapagos finches but it is sometimes inadequate to explain other cases of speciation.

Sympatric speciation and *Howea* palms

Lord Howe Island is a remote island in the Tasman Sea located 580 km from the east coast of Australia, which is the island's nearest land mass (Figure 10.22a). It was formed by volcanic activity between 6.4 and 6.9 million years ago. Steadily eroded by oceanic forces, it is now only a minute fraction of its original size and it is likely to become submerged in the next few hundred thousand years.

WB
10.3.2
SYMPATRIC SPECIATION AND *HOWEA* PALMS
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Nature Picture Library/Oriol Alamany

Nature Picture Library/Oriol Alamany

Figure 10.22 a The location of Lord Howe Island. b Curly palm (*Howea belmoreana*) and c Kentia palm (*Howea forsteriana*).

There are approximately 240 plant species on Lord Howe Island, almost half of which are endemic to the island. Among them are four palm species, including two closely related endemic species, *Howea belmoreana* (the curly palm) and *Howea forsteriana* (the kentia palm) (Figure 10.22b and c). The kentia palm was first brought to Europe and propagated more than 150 years ago and it has become one of the world's most popular commercial palm species. Genetic studies suggest the two *Howea* species diverged from their common ancestor about 1 mya, implying the speciation occurred on Lord Howe Island. However, both palms are wind pollinators often found growing near one another, and the island is so small (about 12 km^2) that allopatric speciation on the island is practically impossible.

The two *Howea* palms have evolved through a process of **sympatric speciation** (from the Greek *sym* meaning same, and *patric* meaning home). This occurs when two species evolve from an ancestral population while still inhabiting the same geographical area. Mechanisms other than geographical isolation must be at work for sympatric speciation to occur. Some members of the population must become **reproductively isolated** from all the others so that sexual reproduction can no longer occur freely in the population. Each individual is able to reproduce with only a limited number of other individuals in the population. The two reproductively isolated populations thus form separate gene pools that independently accumulate unique mutations over many generations, eventually becoming separate species.

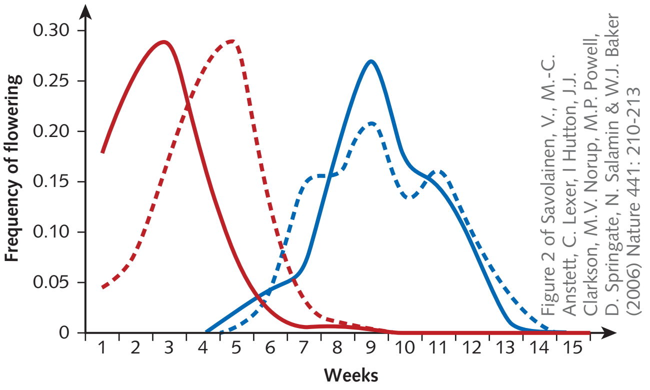

Figure 2 of Savolainen, V., M.-C. Anstett, C. Lexer, I Hutton, J.J. Clarkson, M.V. Norup, M.P. Powell, D. Springate, N. Salamin & W.J. Baker (2006) Nature 441: 210-213

Figure 10.23 The differing flowering times of the two *Howea* species. The blue lines represent 198 curly palms (*Howea belmoreana*) and the red lines represent 177 kentia palms (*Howea forsteriana*) showing female (dashed) and male (solid) flowering phases.

Reproductive isolation between the two palm species came about because of a difference in the timing of their flowering (Figure 10.23). Kentia and curly palms have both female and male flowers but the kentia palms flower about 6 weeks before the curly palms. Kentia palms are also androgenic, which means the male flowers open before the female flowers (Figure 10.23). Flowering timing ensures that kentia palms reproduce mainly with other kentia palms, and curly palms reproduce mainly with other curly palms. Temporal reproductive isolation rather than geographical separation was the mechanism that resulted in two *Howea* species.

Evolution of the *Howea* species

It is reasonable to ask what caused the difference in flower timing within the original population of *Howea* palms. Most of the curly palms grow on the fertile, relatively acidic volcanic soils located throughout the island.

By contrast, the kentia palms tend to grow on the more alkaline and nutrient-poor calcareous soils on the lowlands of the island. Geological evidence suggests the calcareous deposits began accumulating about 1 mya, probably with the emergence of the island's coral reefs. As the coral weathered, the chalky calcium carbonate washed up and accumulated in pockets throughout the island's lowlands. The timing of these geological events coincides with the estimated speciation of the *Howea* palms about 1 mya based on genetic evidence.

Putting together the geological, physiological and genetic evidence suggests the following evolutionary sequence. The ancestor of the *Howea* palms arrived on Lord Howe Island probably about 5 mya. These *Howea* palms grew on volcanic soils throughout the island. Around 1 mya, coral reefs developed, leading to the accumulation of calcareous deposits on the island. *Howea* palms growing on those deposits purely by chance were physiologically stressed. As a stress response, these palms flowered earlier than usual. Under the circumstances, the earlier flowering *Howea* palms were able to reproduce with each other but not with the *Howea* palms growing on the volcanic soils. After the flowering season was over for the *Howea* palms growing on calcareous soils, flowering commenced for the *Howea* palms growing on volcanic soils. These palms could reproduce with one another but were too late to reproduce with the palms growing on the calcareous soils. This pattern repeated over many years and led to eventual speciation of the two *Howea* palms.

It has also been observed that curly palms are less tolerant of calcareous soils, while kentia palms grown on nutrient-rich volcanic soils flower at about the same time as curly palms. Some first and second generation hybrids of the two *Howea* palm species have been identified but they are rare and it remains uncertain whether they are fertile beyond the second or third generation.

KEY CONCEPTS

- Allopatric speciation is the process by which new species diverge from members of an ancestral species that have become geographically isolated for long periods.
- The evolution of Galapagos and Cocos Island finches is an example of allopatric speciation.
- The evolution of *Howea* palms is an example of sympatric speciation in which new species emerge from an existing population while inhabiting the same geographical area.

Concept questions 10.3

1 Define 'speciation'.

2 Construct a table summarising the fundamental stages of allopatric speciation.

3 Describe the difference between the biological and the morphological species concepts.

4 What selection pressures acted on Galapagos and Cocos Island finches and what effect did they have on the morphology of the finches?

5 What is meant by 'reproductive isolation' and how did this occur in the speciation of *Howea* palms?

HOT Challenge

6 Forty per cent of the world's species of fruit fly are found on the islands of the Hawaiian archipelago.

- a Propose why the Hawaiian archipelago might provide a suitable habitat for so many different species of fruit flies.
- b Explain how adaptive radiation may have been involved in the evolution of Hawaiian fruit flies.
- c Describe three ways that ancestral fruit fly genes may have been transported from one island to another.

10.4 Determining the relatedness of species

The fossil record demonstrates how older ancestral organisms have evolved into later descendant forms. This implies many species today are related by lines of descent, rather like a branching tree, as first proposed by Darwin. The fossil record for many modern organisms is scant, or even absent, and evaluating the evolutionary relationships between them requires other lines of evidence; for example, anatomical, physiological and molecular evidence that can be observed directly from the modern organisms.

Weblink
Evidence for evolution

Online Worksheet
Evidence for evolution

Classifying relatedness

Biologists have traditionally used a variety of features to categorise organisms. The goal is to group organisms according to the degree to which they share similarities. This is the basis of **taxonomy**, the system of scientific conventions for naming and classifying organisms. Organisms are classified in a hierarchy from phylum (the broadest), class, order and family through to genus and species. The term 'division' in plant taxonomy is equivalent to phylum in animal taxonomy. The scientific name of an organism is derived from its genus and species name.

In modern biology, taxonomy serves as a tool to describe hypotheses about the **phylogeny**, or evolutionary relatedness of organisms. If two species are classified in the same genus (grey wolf and coyote) we are hypothesising that they are more closely related to each other than either is to a third species (puma) that is classified in a different genus. By classifying organisms in taxonomic schemes, we are recognising that organisms that are more closely related have more features in common. Some examples are shown in Table 10.2.

Table 10.2 Taxonomic classification of five predators

Common name	Scientific name	Phylum	Class	Order	Family	Genus	Species
Great white shark	*Carcharodon carcharias*	Chordata	Chondrichthyes	Lamniformes	Lamnidae	*Carcharodon*	*carcharias*
Killer whale	*Orcinus orca*	Chordata	Mammalia	Cetacea	Delphinidae	*Orcinus*	*orca*
Puma	*Puma concolor*	Chordata	Mammalia	Carnivora	Felidae	*Puma*	*concolor*
Coyote	*Canis latrans*	Chordata	Mammalia	Carnivora	Canidae	*Canis*	*latrans*
Grey wolf	*Canis lupis*	Chordata	Mammalia	Carnivora	Canidae	*Canis*	*lupis*

As you navigate through the taxonomic ranks from phylum (or division) to species, you are proceeding from the ancestors towards more closely related descendants. Essentially, the taxonomy provides a guide to the progress of evolution over time. The higher ranks, such as phylum or class, represent very ancient divergences, whereas the lower ones, such as genus and species, represent relatively recent divergences between organisms.

Structural morphology

Some species can appear very different, with very few obvious similarities to each other, while others appear so similar that their shared common ancestor must have existed relatively recently. Closer examination of the physical structure and form of species, that is their **structural morphology**, at both the embryonic and adult stages reveals further evidence for evolution.

Embryology

Structural morphology is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of embryos. For example, all members of the phylum Chordata (or 'vertebrates') have, at some stage of their development, a dorsal notochord (a cartilaginous rod running along the back), pharyngeal slits (which turn into gill slits in fish), a dorsal nerve chord and a tail that extends past the anus. Sea squirts are the most unlikely members of this phylum; the adults look more like marine invertebrates than the more closely related vertebrates (Figure 10.24a). However, sea squirt larvae have all the chordate characteristics, including a notochord (Figure 10.24b). Vertebrates have lost the notochord and it is replaced with vertebrae.

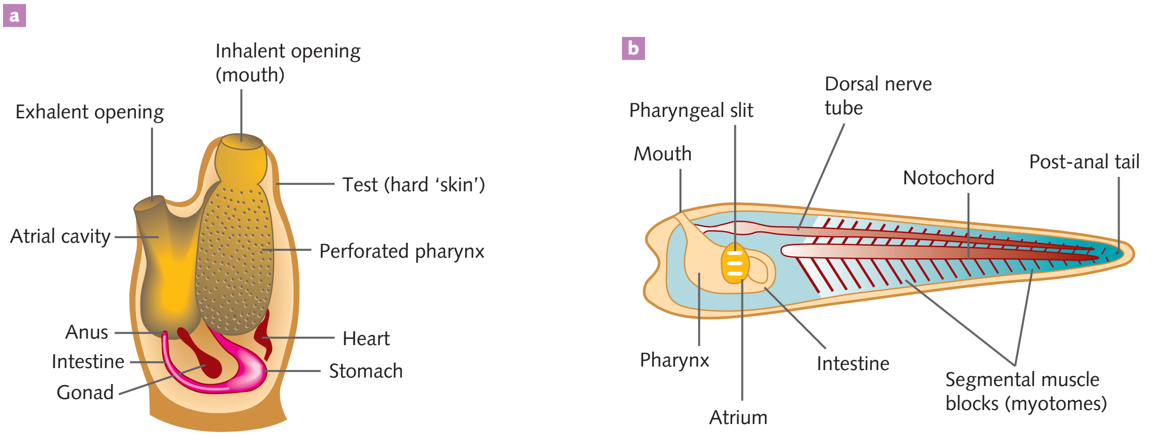

Figure 10.24 a **Adult sea squirts show few characteristics of chordates. b The free-swimming larvae of sea squirts show the characteristic features of chordates, revealing sea squirts' evolutionary affinity with chordates.**

The similarities between embryos of fish, humans and many other organisms suggest a shared ancestor from which all these species have evolved (Figure 10.25). No theory other than evolution can adequately explain why the same structures occur in all chordate embryos, whose adult forms are so diverse.

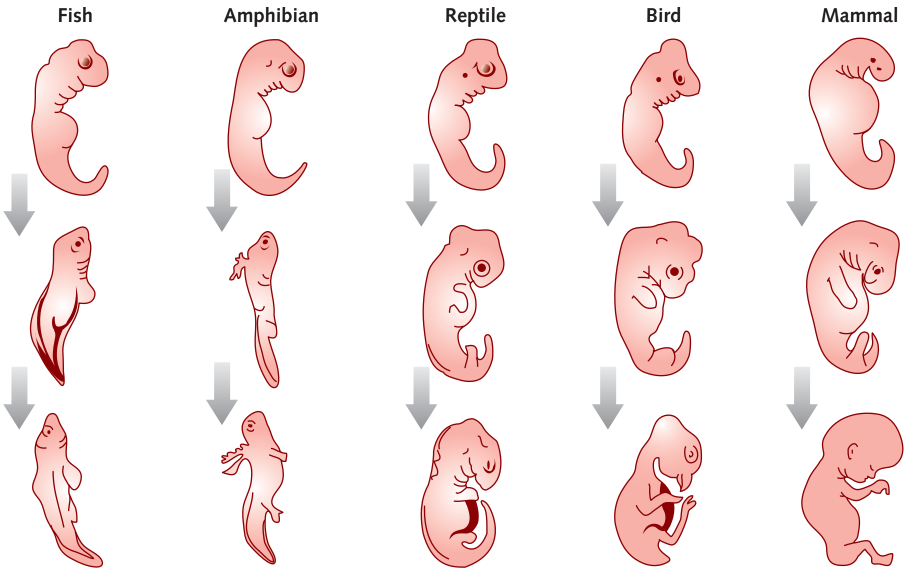

Figure 10.25 **Similarities between chordate embryos suggest a common ancestor.**

Homologous structures

WB
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HOMOLOGOUS STRUCTURES
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Homologous structures are common anatomical structures shared by different organisms that stem from their descent from a common evolutionary ancestor. When an adaptive radiation occurs, organisms retain the same basic structures because they have the same genetic history. For example, all lizards have scaly skin; this is a defining characteristic of their classification. However, the scales can differ in colour, hardness and shape depending on the habitat that they occupy, and may function in defence, temperature maintenance or camouflage. The different types of scales are examples of homologous structures.

Some homologous structures have evolved very different functions. The wing of a bird, the wing of a bat, the leg of a crocodile, the flipper of a whale and the arm of a human all have different functions and appear superficially different. However, they all have the same basic skeletal structure: the pentadactyl limb, a hand or foot with five fingers or toes (Figure 10.26). In each species the limb has become modified to suit the organism's way of life, demonstrated by the different bone lengths and coverings of the limbs. For example, as birds evolved and their forelimb became adapted for flight, the bones of the hand reduced to three fingers. The fact that the bone structures of all these organisms have the same pentadactyl pattern implies they all descended from the same evolutionary ancestor, which also had the pentadactyl limb.

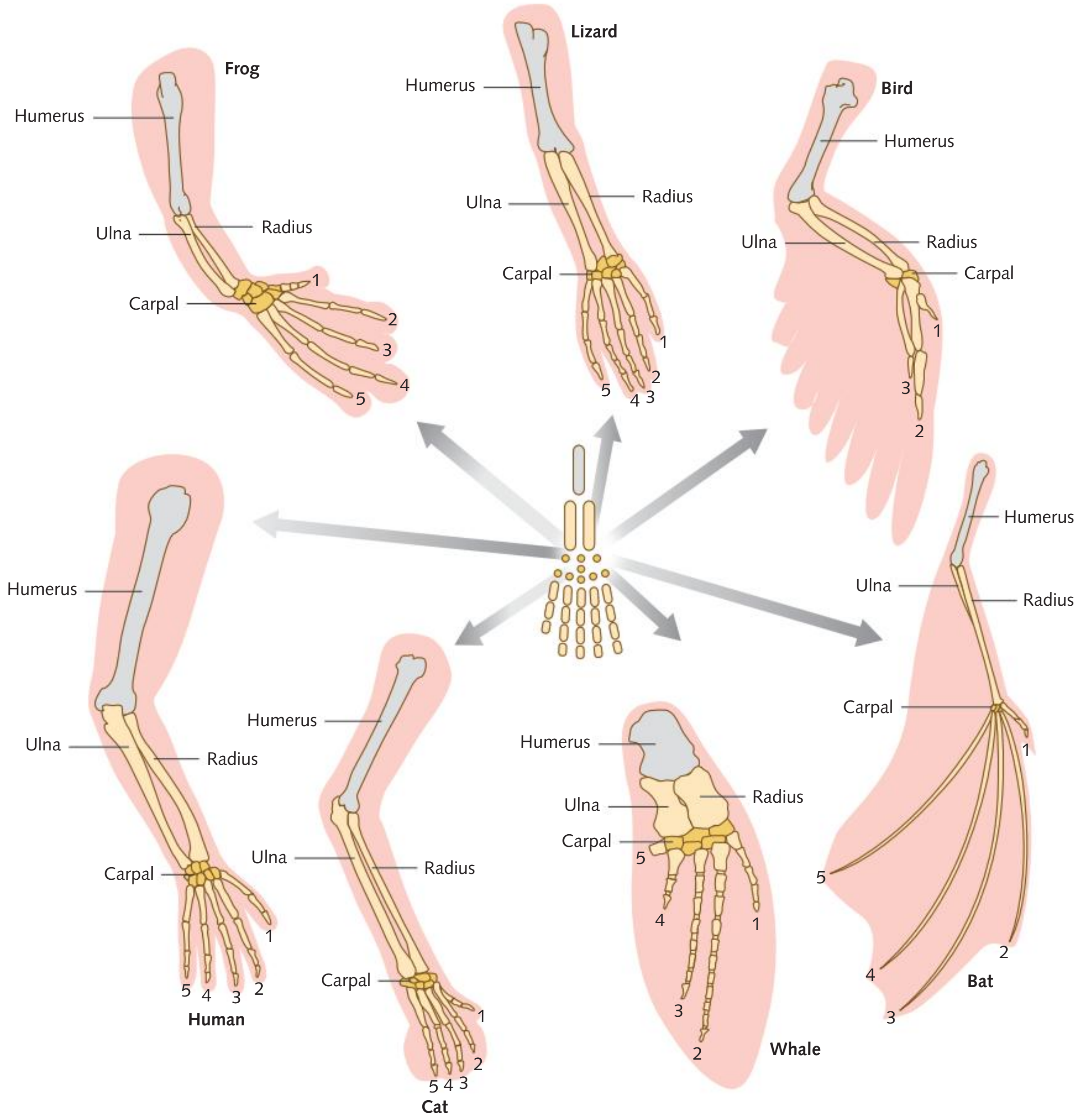

Figure 10.26 The principle of homologous structures can be illustrated by the adaptive radiation of the forelimb of a selection of vertebrates, which all show the basic pentadactyl pattern modified for different uses.

The leaves of land and aquatic plants all have the same basic components, but the structure shows enormous variety in size, shape, colour and function. Some leaves function as coloured petals, some as support structures in buds and others as defensive spines or fleshy water stores (Figure 10.27).

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Figure 10.27 **Homologous structures derived from leaves. a The spines of a cactus and b the coloured bracts that enclose the flowers of *Heliconia* are derived from the same basic leaf structure but now have different forms. In this case, they are homologous structures but serve different functions. In other examples, homologous structures can share functions, but different environments can influence how these functions are necessarily performed.**

Homologous structures can be used to infer phylogenetic (i.e. evolutionary) relationships because only organisms with a common ancestor can have the structures with the same fundamental arrangement.

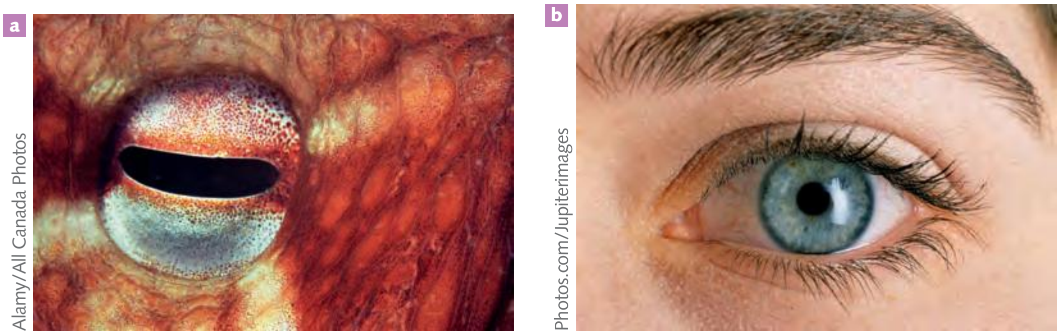
Vestigial homologous structures

In some cases, homologous structures stemming from a common descent can eventually cease to provide a functional use for an organism; the structure may not necessarily impede a particular adaptation of an organism, but at the same time the structure no longer serves a 'useful' purpose. These structures are called **vestigial structures**. Vestigial structures can take a variety of forms, including skeletal structures on vertebrates, soft tissue such as organs, or even features at the cellular and molecular levels.

Wherever vestigial structures may be found, they are usually either rudimentary or atrophied. Vestigial structures are quite common and are yet another line of evidence that points to shared ancestry. Among humans, features that are thought to be vestigial include the coccyx (tailbone), the muscles that allow some people to wiggle their ears, and the palmar grasp reflex that causes babies to grip tightly onto something placed in the palm of their hand. Ostriches still have small wings even though they cannot fly and some cave animals have remnant eyes and optic nerves even though there is no light in a cave and they cannot see.

Analogous structures

Analogous structures are features of organisms that have the same function but not the same basic structure. The eyes of octopuses and vertebrates are remarkably similar, even down to fine points of detail, and an observer could conclude that they are homologous structures (Figure 10.28). However, there is one important difference. In the vertebrate eye, the nerve fibres lie in front of the sensory cells of the retina, whereas in the octopus eye they lie behind them. Because of this, the vertebrate eye has a blind spot where the optic nerve emerges from it, whereas the octopus eye has no blind spot. The reason for this difference lies in the ways the two eyes developed, which indicates that they are the products of two distinct lines of convergent evolution. The same selection pressures probably resulted in the evolution of similar eyes in such unrelated organisms.



Alamy/All Canada Photos

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Figure 10.28 a Octopus eyes and **b** human eyes are the solution to the same problem with similar adaptations.

KEY CONCEPTS

- Taxonomy is a naming system that categorises organisms based on hypotheses about evolutionary relationships.
- Comparing the development and anatomy of organisms can provide evidence that organisms evolved from a common ancestor.
- Homologous structures evolved from the same ancestral form but have different forms or functions due to different selection pressures.
- Analogous structures evolved under the same selection pressures from different ancestral forms so they have a common function but show some fundamental differences.

Concept questions 10.4

1 Which two species are more closely related among *Solenopsis fugax*, *Carebara castania* and *Solenopsis invicta*? How can you deduce this from their scientific names alone?

2 Why does embryology provide evidence for a shared common ancestor of all chordate organisms?

3 Name two homologous structures and two analogous structures.

4 In a group of species that arose from a common ancestor through divergent evolution, would you expect to see homologous structures or analogous structures?

5 The pentadactyl limb is a key structural piece of evidence used when classifying organisms into taxa.
   a Which groupings of organisms demonstrate a pentadactyl limb?
   b Why is the pentadactyl limb evidence for evolution?
   c Is the pentadactyl limb an analogous structure or a homologous structure?
   d How do pentadactyl limb differences relate to different function?

HOT Challenge

6 Fish are thought to be the ancestors of terrestrial animals. The pentadactyl limb exists in terrestrial animals but not in modern fish. Why do you think this might be?

10.5 Molecular evidence for relatedness of species

Almost as soon as the amino acid sequences of proteins were being determined in the 1960s, it became clear that proteins could be used to infer evolutionary relatedness. For any given protein, the numbers of amino acids that differ between a pair of species provided a guide for the **evolutionary distance** between the two species. For example, the mitochondrial protein cytochrome c is a vital protein in the electron transport chain of cellular respiration. The cytochrome c sequences have been determined for the species shown in Figure 10.29. Compared with the human, the number of differences increases in order from the rhesus monkey (another primate), to the rabbit (another mammal), the rattlesnake (a reptile), the bullfrog (an amphibian), the fruit fly (an insect) and wheat (a plant). Interpreted this way, the number of amino acid differences in the cytochrome c sequence reflects the evolutionary distance between humans and each of the other organisms. Humans are most closely related to rhesus monkeys and least related to wheat.

CONNECT

The Electron Transport Chain of cellular respiration was discussed on page 144.

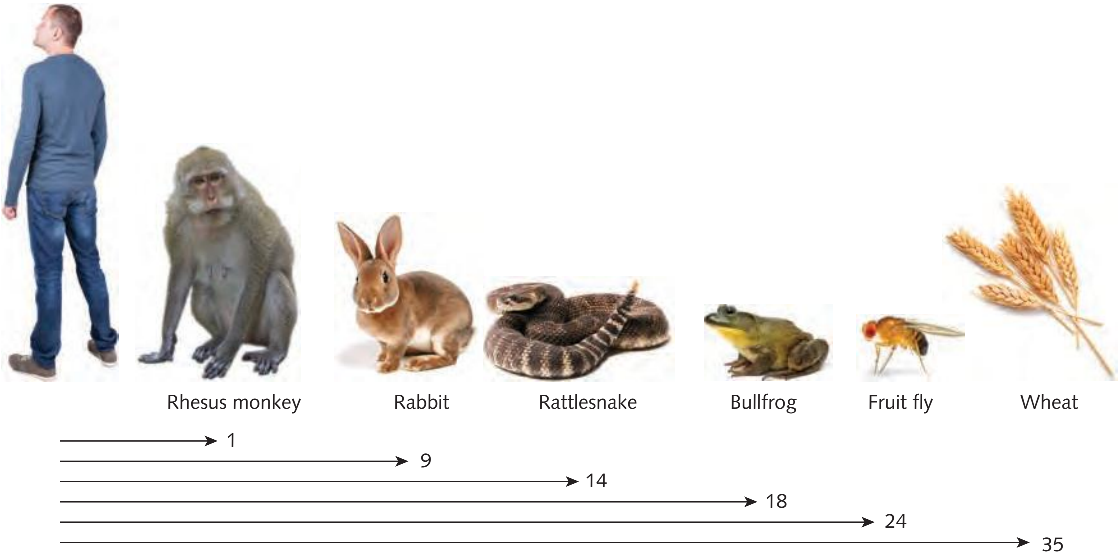

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Figure 10.29 The number of amino acid differences between the cytochrome c of humans and that of other organisms.

The concept of molecular homology

In the study of structural morphology, 'homology' refers to the similarity in patterns of anatomical structures between different organisms. The more similar the structural pattern, the closer the evolutionary relationship between the organisms. Molecular biologists have applied the concept of homology to molecules. The term **molecular homology** refers to the similarity of patterns in the nucleotide sequences of DNA or the amino acid sequences of polypeptides from different organisms as evidence for a common evolutionary origin. Genes or polypeptides from different species that exhibit molecular homology are described as **homologous**.

WB

10.5.1
THE CONCEPT OF MOLECULAR HOMOLOGY
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DNA and proteins suit studies of molecular homology

There are two key reasons why DNA and proteins are suited to the study of evolution.

The first reason relates to their structure. DNA and proteins are unbranched polymers. They are very long, linear molecules composed of a limited number of possible building blocks. DNA is made up of four possible nucleotides in different orders. Proteins consist of polypeptides, which are made up of the

CONNECT

See Chapter 2 for more on the transcription and translation of genes into polypeptides.

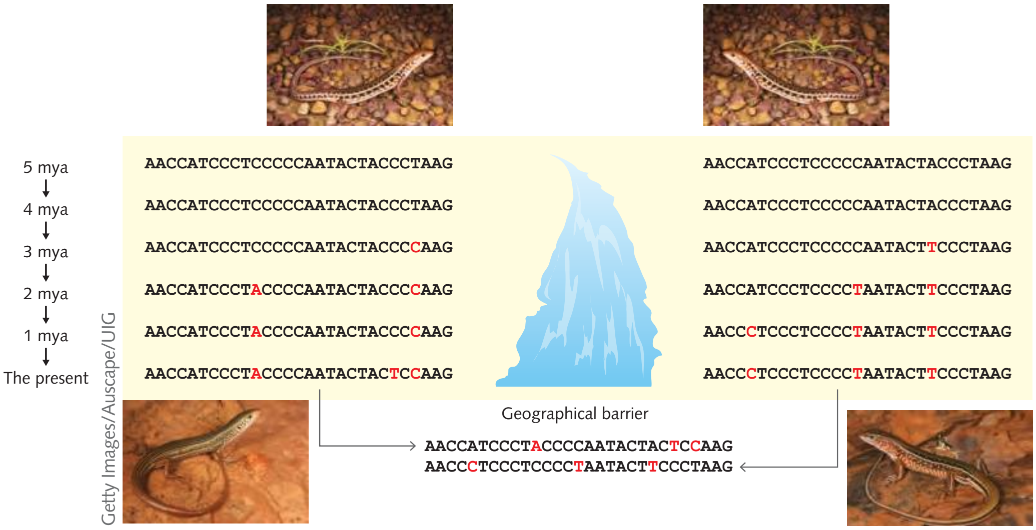
20 amino acids in different orders. This makes it straightforward to align and compare the sequences of DNA or proteins from different organisms. Similarities in sequences can be identified readily, and differences, corresponding to changes over time, can be easily calculated.

The second reason relates to technological developments in the field of molecular biology, which reflect advances in both the efficiency of chemical analysis of the molecules and the speed and power of computer processing. One outcome of this is automated, high-throughput sequencing to generate large amounts of DNA sequence data. Complementing this is the enhanced capacity to manage and explore the large amounts of data produced. A relatively new branch of science called **bioinformatics** has emerged from the application of computer science to storing, retrieving and analysing large volumes of biological data. Bioinformatics is an interdisciplinary field combining mathematics, computer science, engineering, chemistry and biology. There are many different aspects to the field of bioinformatics, one of which is that it can be used to determine how closely related different species are.

A model to explain molecular homology

Figure 10.30 illustrates how speciation results in sequence variation between species. Originally (5 mya) there was a single interbreeding population of skinks. Assume there is a gene with a nucleotide sequence that is characteristic for the population. In reality, there may be very few individual variations (alleles) in this gene sequence among members of the population, but it is broadly representative of the whole population. After some geological time, the original population has separated to become two reproductively isolated populations (Figure 10.30). The separation may be because a geographical barrier has formed to divide the original population, or because of physiological or behavioural differences that segregate members of the original population.

The example illustrates how a single ancestral species diverges, or splits, to eventually become two descendant species. Two **lineages**, or two populations that represent separate lines of descent, emerge from the point in time when the split occurs. Over time, the descendant lineages evolve different morphological, physiological and behavioural features and become recognisably distinct from one another as separate species. It is possible to infer speciation events that have occurred in the past by comparing DNA sequences of different species that are alive today.



Getty Images/Auscape/UIG

Figure 10.30 A representative DNA sequence in a population of skinks from 5 mya to the present. After the original population becomes divided by a geographical barrier (such as a body of water), mutations accumulate independently in the DNA sequences of each isolated population over time. Today, the two independent populations are separate species. The representative DNA sequences of the two species are aligned to show their homology (similarities in the sequence). The differences between the two sequences provide a measure of the evolutionary distance between the species.

Sequence alignments as a tool for investigating molecular homology

The DNA sequences in the present-day skink species illustrated in Figure 10.30 are homologous. They are related to each other by descent from a common ancestral DNA sequence. If we make a **sequence alignment** of those sequences today we can identify the original pattern in the gene by the similarities (homology) in the nucleotide sequences (Figure 10.30). Comparing this alignment with the mechanism depicted in Figure 10.30 highlights three key observations.

1 The nucleotides that are identical in both sequences are presumably ancestral. That is, these nucleotides were in the gene of their common ancestor and both descendant species inherited those nucleotides. These nucleotides are conserved nucleotides. They were retained during the course of evolution in the sequences of the descendant species.

2 The number of differences between the gene sequences of the two species – the independent mutations in each sequence – provide a measure of how related the two species are. This is the measure of evolutionary distance. Generally, for a given gene, the greater the differences in the sequence, the less related the two species are.

3 The degree of difference between the sequences indicates the time of divergence from the last common ancestor. Generally, for a given gene, the more differences there are between the two sequences, the more time has elapsed since the species last shared a common ancestor.

The last point explains the concept behind the **molecular clock**. The rate of the molecular clock describes the number of point mutations that occur in a specified polypeptide or gene over a defined period of time (Figure 10.31). In principle, the 'ticking' of the molecular clock is used to estimate the time since two species diverged from their last common ancestor. The rate of the molecular clock varies for different genes and proteins and must be calibrated against the fossil record.

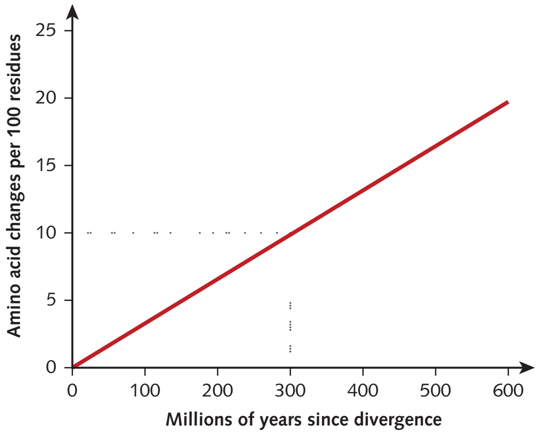


Figure 10.31 The rate of the molecular clock for cytochrome c. The dotted lines show how the number of amino acid differences in the sequence from two organisms (in this case, 10 per 100 residues) can be used to estimate the time since they diverged from a common ancestor (approximately 300 mya).

Mitochondrial DNA

The mitochondrial genome is contained entirely on a double-stranded, circular chromosome (Figure 10.32) and possesses a suite of genes for many of the proteins involved in cellular respiration. So it can express these genes inside the organelle, the mitochondrial genome also has genes for ribosomal RNA (rRNA) and transfer RNA (tRNA) molecules. Polypeptide synthesis is achieved by ribosomes within the mitochondrion.

Mitochondrial DNA (mtDNA) is often used in evolutionary studies because it is found in essentially all eukaryotic organisms, it is abundant, it has sufficiently variable DNA sequences, and its inheritance is easily traced. The mtDNA is useful for studying the evolutionary relationships of recently extinct organisms up to 100 000 years old.

Eukaryotic cells have many identical copies of the mitochondrial chromosome. This is because there are many copies of the chromosome inside each mitochondrion, as well as many mitochondria inside each cell (Figure 10.32). A given eukaryotic cell will contain only a diploid set (two copies) of each nuclear chromosome but thousands of copies of the mitochondrial chromosome. Furthermore,

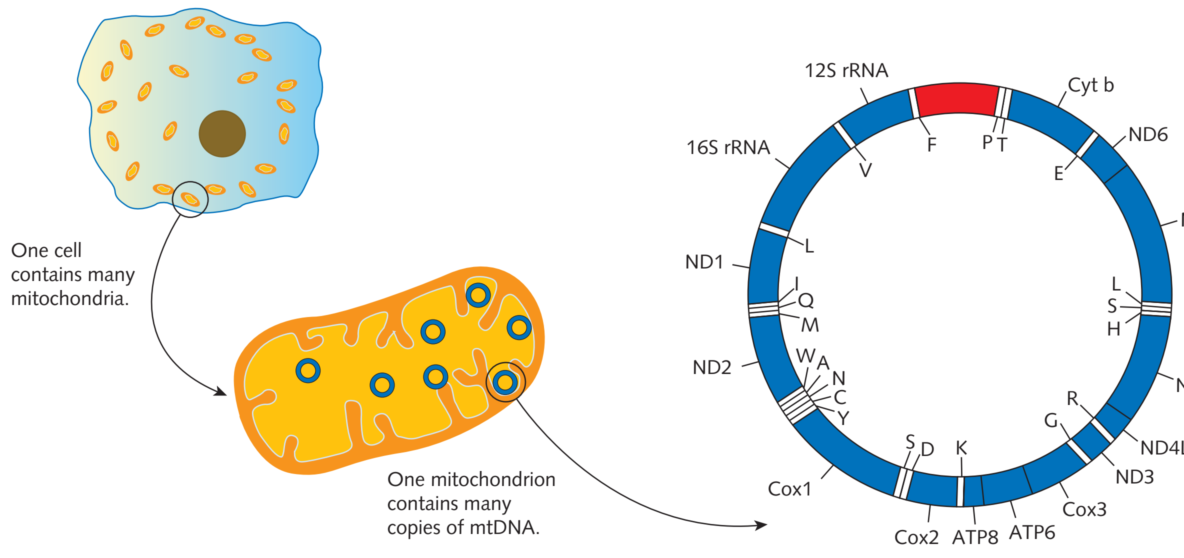


Figure 10.32 Eukaryotic cells contain many copies of the mitochondrial genome. Genes for mitochondrial proteins and rRNA are labelled on the outside of the chromosome; genes for mitochondrial tRNA are labelled with single letters on the inside.

the mtDNA is a very compact genome. For example, in humans the mitochondrial chromosome comprises about 16 600 base pairs coding for just 37 genes (Figure 10.32). This contrasts with the nuclear genome, which comprises about 3.1 billion base pairs encoding between 21 000 and 25 000 genes. The very high number of relatively small mtDNA molecules makes it comparatively easy to extract and manipulate for sequencing. It also means that, compared with nuclear DNA, there is a better chance of recovering an intact mitochondrial genome than a nuclear genome from fossil specimens.

Nuclear DNA is inherited equally from both parents, but mitochondrial chromosomes are inherited independently of nuclear chromosomes. mtDNA is **maternally inherited**. Offspring inherit their mtDNA only from their mother because essentially all the cytoplasm in the fertilised zygote is derived from the egg and not the sperm. This ensures the zygote contains an almost uniform population of mitochondria derived from the mother. Furthermore, mtDNA does not undergo independent assortment or crossing over in the way nuclear chromosomes do during meiosis. Consequently, while the ancestral history for a mutation in nuclear DNA is quickly obscured or lost after just a few generations of recombination and random fertilisation, the ancestry of mtDNA variation can be simply and continuously traced through the female line of inheritance, from mother to offspring, or vice versa (Figure 10.33). This aspect of mtDNA favours its use for identifying individuals long deceased, as well as for exploring patterns of ancestry and migration within and between populations.

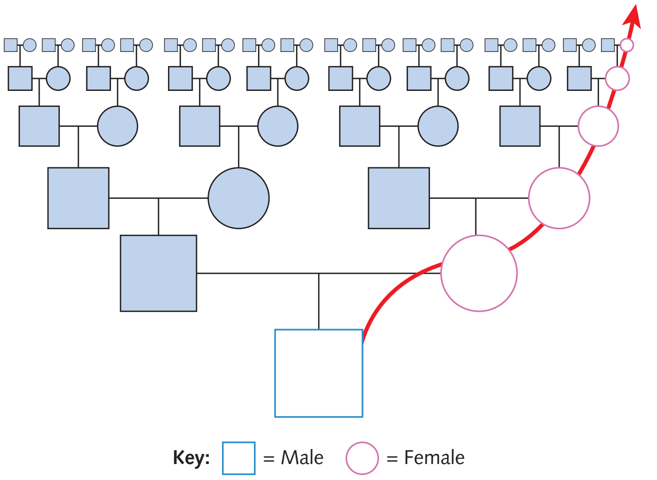
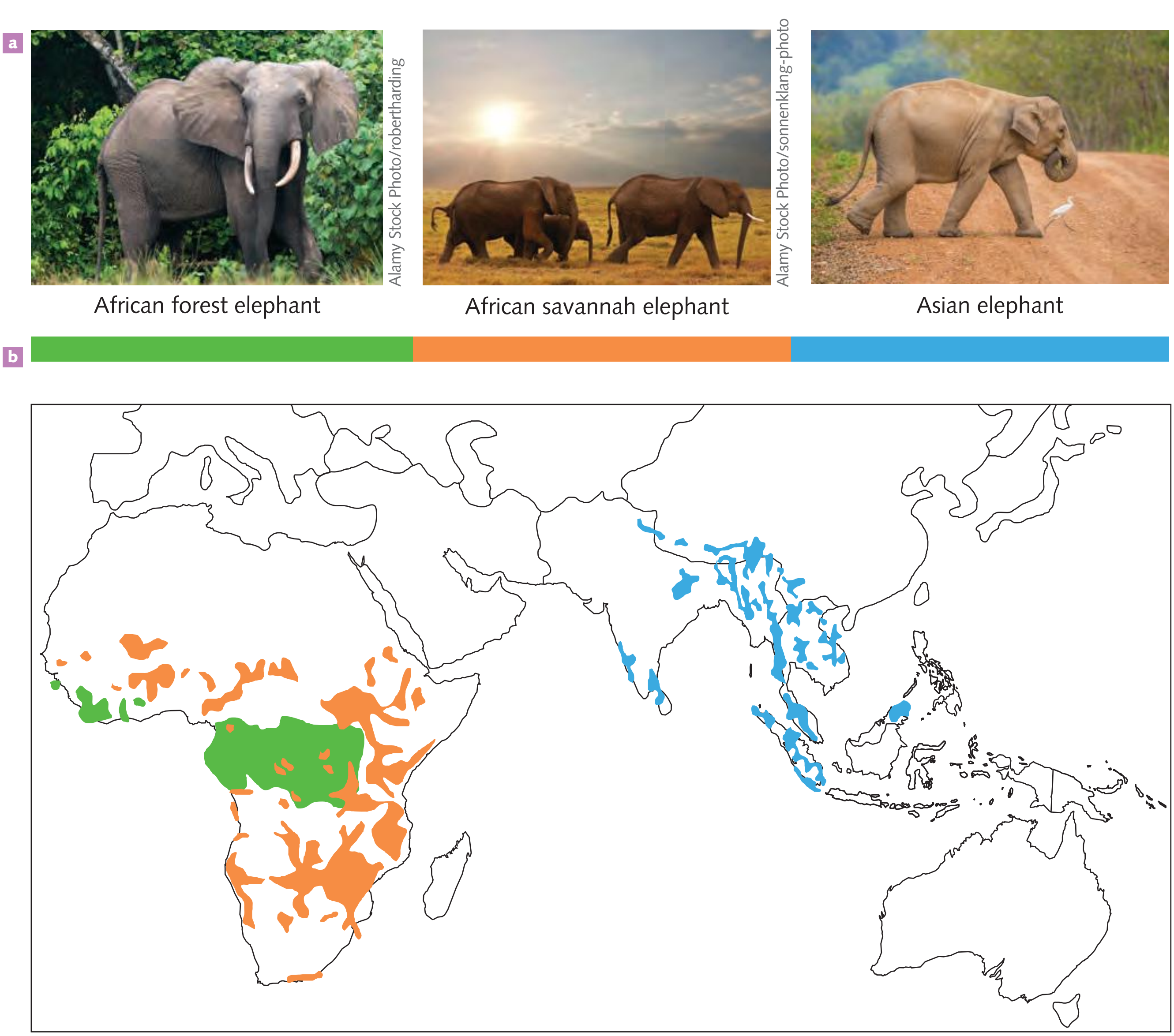


Figure 10.33 Inheritance of mitochondrial DNA can be traced simply through the female line of descent.

Reconstructing evolution from gene sequences

Figure 10.34 illustrates how DNA sequences of organisms alive today can be used to reconstruct evolution. The Asian elephant, African savannah elephant and African forest elephant live in different locations and are different in size and morphology. In Figure 10.34c, a 100-nucleotide segment of the ND4 gene in the mtDNA from each of the three elephant species is aligned by the conserved nucleotides – the nucleotides that are identical in each gene. While most of the nucleotides are conserved, some nucleotides differ between each sequence. These differences have arisen by mutation in one of the sequences.



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c

```
Forest    TATTATAATAATAGCCTGATTTATTATCCACTTAAATTCCTGAGAGTTTCAACAAATCTTCTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGT
Savannah  TATTATAATAATAGCCTGATTTACTATCCATTTAAATTCTTGAGAGTTTCAACAAATCTTCTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGT
Asian     CATTATAATAATAGCCTGATTTATTATCCATTTAAACTCCTGAGAATTTCAACAAATCTTTTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGC
           ********************** ****** ***** ** ***** ************** ************************************** 
```

Figure 10.34 a Three elephant species and **b** their corresponding global ranges. **c** A sequence alignment of 100 nucleotides from the mitochondrial ND4 gene of each elephant species. Asterisks indicate conserved nucleotides in the sequences of the three species.

It is possible to determine how similar the sequences are. For example, a **pairwise comparison** of the sequences of the African forest elephant and African savannah elephant shows that 97 of the 100 nucleotides are identical (Table 10.3). The mtDNA sequences of the African forest and African savannah elephants are 97% conserved.

Table 10.3 Pairwise comparisons of mtDNA sequences between three elephant species

Pairwise comparison	Number of nucleotides in the DNA segment	Number of conserved nucleotides	Number of nucleotide differences	% sequence conservation
African forest elephant with African savannah elephant	100	97	3	97%
Asian elephant with African forest elephant	100	94	6	94%
Asian elephant with African savannah elephant	100	93	7	93%

Pairwise comparison of the DNA sequences of the Asian elephant with either the African forest or the African savannah elephant gives lower levels of sequence conservation: 94% and 93%, respectively (Table 10.3). Therefore, the mtDNA sequences from the two African species share more nucleotides with each other than either does with the Asian elephant. This suggests the two African species shared a more recent common ancestor than either did with the Asian elephant. In evolutionary terms, the data indicates that the two African species are more closely related. This reasoning is based on the concept of evolutionary distance and is described as the 'distance method' for inferring evolutionary relationships.

The example of the three elephant species can be re-framed in a slightly different way. Rather than asking how conserved the mtDNA sequences are, it is possible to ask how different the mtDNA sequences of each species pair are. Counting the differences between each pair of sequences provides a measure of the evolutionary distance between each species. The more nucleotide differences, the greater the evolutionary distance. The data shows that the Asian elephant is the most distantly related of the three species.

Assembling phylogenetic trees

WB

10.5.2
ASSEMBLING PHYLOGENETIC TREES
PAGE 222

Phylogeny is the study of lines of descent from ancestral organisms and the relationships among descendant organisms. A convenient way to visually represent phylogeny is with a **phylogenetic tree**.

The evolutionary relationships inferred from the data in Table 10.3 can be represented in a phylogenetic tree (Figure 10.35). The tips of the phylogenetic tree are labelled with the organisms under study. Each **node**, or branch point, represents the last common ancestor of the organisms whose lineages emerge from it. The root is the ancestral lineage leading to all the descendants in the tree. The first node encountered nearest the root is the common ancestor of all the organisms in the tree. In these phylogenetic trees, the two African elephant species are the most closely related because they share the most recent common ancestor.

The phylogenetic tree also implies the passage of time. The tips represent the present. To trace back to the root is to journey back in time. The most ancient lineages branch near the base of the tree and the most recently derived ones branch closer to the tips. In the trees shown in Figure 10.35, the lineages leading to the Asian elephant and to the African elephants diverged first. The lineage leading to the two African species diverged more recently. Figure 10.35 depicts two types of phylogenetic trees: cladograms and phylograms.

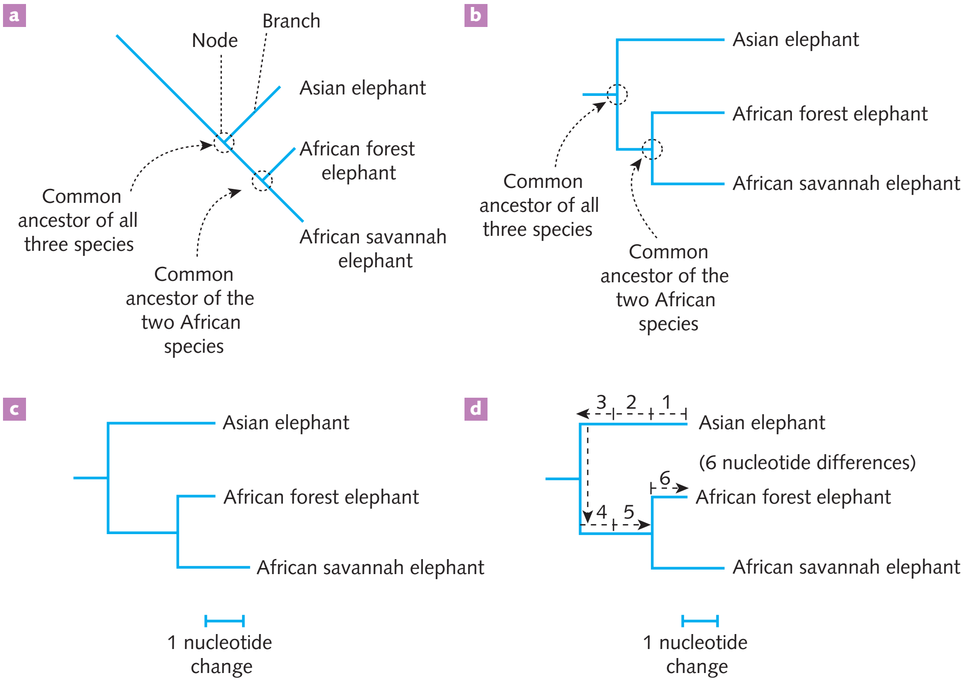

Figure 10.35 Phylogenetic trees depicting the evolutionary relationships of three elephant species. **a, b** Two representations of the same cladogram. The nodes are circled and each indicates a common ancestor to the lineages branching from it. **c** A phylogram with horizontal branch lengths representing the number of nucleotide changes occurring during evolution of the lineage. **d** A demonstration of how the scale of the phylogram is used to measure the evolutionary distance between the Asian and African forest elephants (six nucleotides)

Cladograms

A **cladogram** (Figure 10.35a and b) represents a hypothesis for the evolutionary history leading to the descendant species. It is characterised by **clades**, where each clade is a branch of the cladogram that comprises an ancestor and all its descendants. In each of Figures 10.35a and 10.35b, two clades can be identified. One clade comprises all three elephant species and their common ancestor. The other comprises the two African elephant species and their common ancestor.

The goal is to define each taxonomic group as a clade within the cladogram. A taxonomic group is described as **monophyletic** if all the species in that taxonomic group are descended from the same common ancestor. Therefore, a clade is the representation of a monophyletic group. If the taxonomic group is missing one or more descendants, or if it includes descendants that belong to a different clade, the taxonomic group is not monophyletic and is considered invalid (Figure 10.36).

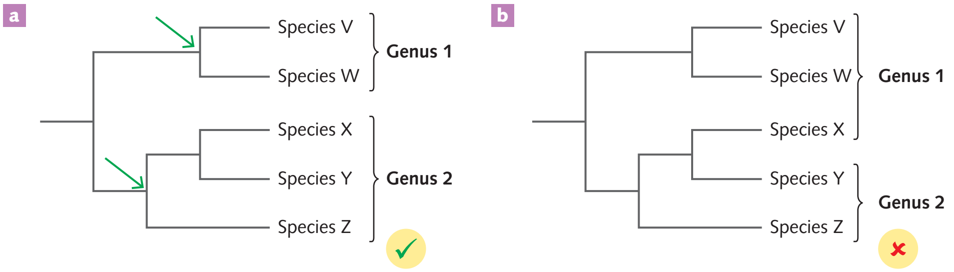

Figure 10.36 Describing monophyletic taxonomic groups. **a** Genus 1 (with species V and W) and Genus 2 (with species X, Y and Z) are both monophyletic groups. The species contained within each genus share a common ancestor (green arrows). **b** In this case, neither Genus 1 (with species V, W and X) nor Genus 2 (with species Y and Z) is monophyletic. Genus 1 contains an additional species (X) which does not share a common ancestor with species V and W. Genus 2 is missing a species (X) that shares a common ancestor with species Y and Z.

Phylograms

A **phylogram** is a scaled, or quantified, version of the phylogenetic tree. The branch lengths are proportional to the amount of inferred evolutionary change or the number of nucleotide changes that have occurred during the evolution of the lineage. The scale bar represents a single nucleotide change (Figure 10.35c and d). The nodes – the points of divergence from a common ancestor – are counted as zero length. For example, the path traced along the horizontal branches from the Asian elephant to the African forest elephant is six times longer than the scale of one nucleotide. Therefore, the difference between the sequences of the two species is six nucleotides.

Representing phylogeny using taxonomy

We can now apply taxonomy to formalise our hypothesis for the evolution of the three elephant species. All three species are classified within the same monophyletic family (Elephantidae). Reflecting the evidence from molecular homology, the Asian elephant is classified in one genus (*Elephas*), and the two African species are classified in another (*Loxodonta*). The placement of the two African species in the same genus formally recognises them as more closely related to each other than either is to the Asian elephant. The genus *Loxodonta* is therefore also monophyletic. The taxonomy of the three species is represented in Figure 10.37.

We still describe the phylogeny as a hypothesis because it is based on an interpretation of the available evidence. Classification schemes may be modified as new evidence emerges or as existing evidence is re-examined and re-interpreted.

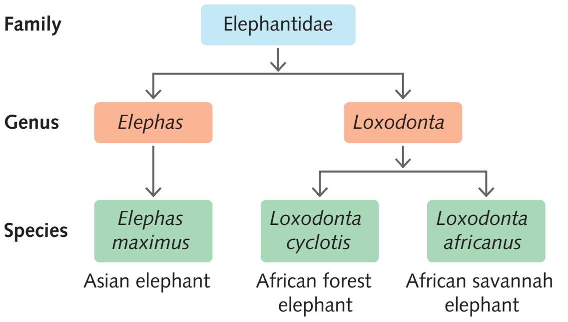

Figure 10.37 The taxonomy of elephant species

INVESTIGATION 10.1

Evidence fit for a mammoth task

The largest living land animals in the world today are the elephants, members of the order Proboscidea. The proboscideans were far more numerous and diverse in the geological past than they are today. There are more than 100 species in the fossil record over some 60 million years but nearly all are now extinct. The more famous extinct proboscideans include mammoths (genus *Mammuthus*) and mastodons (genus *Mammut*).

The woolly mammoth (*Mammuthus primigenius*) and the American mastodon (*Mummut americanum*) (Figure 10.38) occupied the cold arctic and sub-arctic regions of the world. A few preserved specimens have been excavated from permafrost and their ancient DNA has been extracted from their fur. Although still relatively degraded, scientists have managed to sequence the entire mitochondrial genomes (approximately 16 500 base pairs) of the woolly mammoth and the American mastodon.

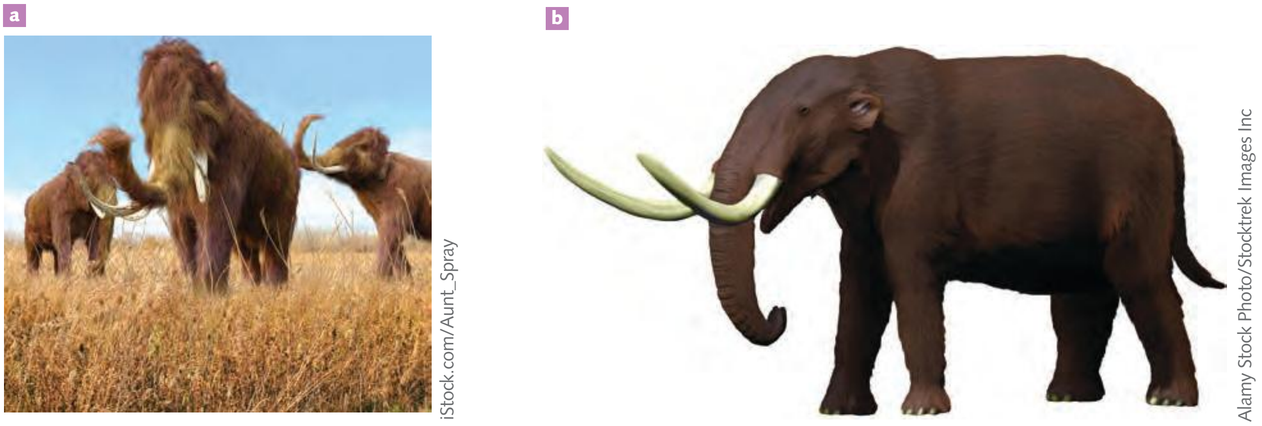

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Figure 10.38 Two extinct members of the order Proboscidea: **a** the woolly mammoth and **b** the American mastodon

The DNA sequences of organisms can be analysed and used to build phylogenetic trees. This approach has provided insights into evolutionary relationships between living species. Sequencing ancient DNA means that phylogenetic trees can be used to explore evolutionary relationships between living and extinct species.

Aim

To use molecular homology to investigate the evolutionary relationships between the woolly mammoth, the American mastodon and members of living proboscideans

Observations

Mammoths appear in the fossil record 4 million years ago and were widespread throughout Europe, Asia and North America. They persisted until about 4000 years ago, disappearing as the climate warmed after the last ice age. There were about 12 mammoth species, including the woolly mammoth (*Mammuthus primigenius*) (Figure 10.38).

The fossils of mastodons (genus *Mammut*) date back at least 40 million years, with discoveries in Africa, Europe and North America. Like woolly mammoths, mastodons were covered by thick, shaggy fur but mastodons were smaller and stockier and their skulls were larger and flatter. The most recent species is the American mastodon (*Mummut americanum*) (Figure 10.38), which died out approximately 10000 years ago.

Fossils suggest modern elephants originated in Africa 4 million years ago and dispersed from there. African savannah elephants (*Loxodonta africana*) inhabit the sub-Saharan regions of Africa as far south as South Africa. They are larger than Asian elephants (*Elephas maximus*), which occur from India in the west to Myanmar in the east. The ranges of elephants are rapidly shrinking and fragmenting due to habitat loss. Elephant species are threatened, with the Asian elephant listed as endangered.

In the Proboscidea, the distinctive tusks evolved from the 'adult' incisors, rather than from canines, as in other tusked mammals such as walruses and wild boars. Teeth are one diagnostic feature used to distinguish between the many different living and extinct members of the order.

Mastodons (from the Greek mastodont meaning nipple tooth) are named for their molars, which have 6–8 cone-shaped cusps that resemble nipples (Figure 10.39b). African savannah elephants also have raised diamond-shaped extensions on their molars. By analogy with modern African elephants, mastodons are believed to have been browsers that ate leaves and twigs.

In contrast, mammoths had teeth with thin, parallel ridges (Figure 10.39a) that resemble those of modern Asian elephants. It is inferred that mammoths, like Asian elephants, were grazers that preferentially ate grass.

Getty Images/Walter Geiersperger

Alamy/Sabena Jane Blackbird

Figure 10.39 Molar teeth of **a** the woolly mammoth and **b** the American mastodon

Predictions

1 Suggest two physical (morphological) features that characterise animals of the order Proboscidea.
2 Which one of the four proboscideans might have evolved earliest? What evidence supports your answer?
3 Which of the four proboscideans are extinct today?
4 Divide the four proboscideans into two most similar pairs (pair 1 and pair 2) based on their fur covering.
5 Divide the four proboscideans into two most similar pairs (pair 1 and pair 2) based on their teeth morphology.
6 Consider the evidence and propose your own hypothesis for proboscidean evolution, which gave rise to the woolly mammoth, the American mastodon and the African and Asian elephants. Copy the evolutionary tree in Figure 10.40 into your logbook and complete it by by adding the names of the four species.

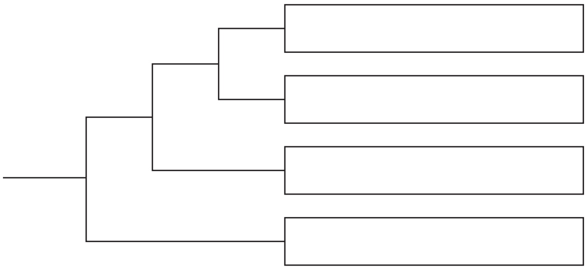


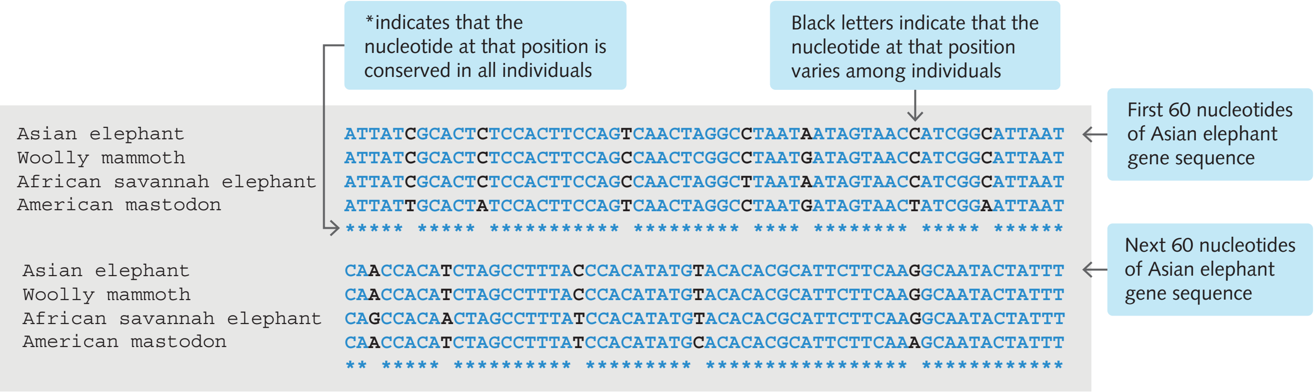
Figure 10.40 Hypothesis for the evolution of four species of the order Proboscidea

Analysis and results

Table 10.4 lists the two living and two extinct proboscidean species used in this study. Figure 10.41 shows a sequence alignment of a segment of the mitochondrial ND5 gene from the four proboscidean species.

Table 10.4 Probiscidean specimens used in this study

Common name	Scientific name	Sample age
Woolly mammoth	*Mammuthus primigenius*	~17 000 years
American mastodon	*Mammut americanum*	~90 000 years
African savannah elephant	*Loxodonta africana*	Present day
Asian elephant	*Elephas maximus*	Present day



*indicates that the nucleotide at that position is conserved in all individuals

Black letters indicate that the nucleotide at that position varies among individuals

First 60 nucleotides of Asian elephant gene sequence

Next 60 nucleotides of Asian elephant gene sequence

```
Asian elephant            ATTATCGCACTCTCCACTTCCAGTCAACTAGGCCTAATAATAGTAACCATCGGCATTAAT
Woolly mammoth            ATTATCGCACTCTCCACTTCCAGCCAACTCGGCCTAATGATAGTAACCATCGGCATTAAT
African savannah elephant ATTATCGCACTCTCCACTTCCAGCCAACTAGGCTTAATAATAGTAACCATCGGCATTAAT
American mastodon         ATTATTGCACTATCCACTTCCAGTCAACTAGGCCTAATGATAGTAACTATCGGAATTAAT
                          ***** ***** *********** ********* **** ******** ***** ******

Asian elephant            CAACCACATCTAGCCTTTACCCACATATGTACACACGCATTCTTCAAGGCAATACTATTT
Woolly mammoth            CAACCACATCTAGCCTTTACCCACATATGTACACACGCATTCTTCAAGGCAATACTATTT
African savannah elephant CAGCCACAACTAGCCTTTATCCACATATGTACACACGCATTCTTCAAGGCAATACTATTT
American mastodon         CAACCACATCTAGCCTTTATCCACATATGCACACACGCATTCTTCAAAGCAATACTATTT
                          ** ***** ********** ********* ***************** ************
```

Figure 10.41 Alignment of 120 nucleotides of the *ND5* gene from four probiscidean species. The alignment is chunked into two blocks of 60 nucleotides. Conserved nucleotides are shown in blue and variable nucleotides are shown in black.

1 Copy Table 10.5 into your logbook and complete it, using the sequence alignment in Figure 10.41.

Table 10.5 Summary of pairwise comparisons between each of the four proboscidean species

Pairwise comparison	Number of nucleotide differences	% sequence conservation
Asian elephant with woolly mammoth		
Asian elephant with African savannah elephant		
Asian elephant with American mastodon		
Woolly mammoth with African savannah elephant		
Woolly mammoth with American mastodon		
African savannah elephant with American mastodon		

2 Explain how you would determine the two most closely related species from the data in Table 10.5.

3 Copy the cladogram in Figure 10.42 into your logbook.

- a Identify the two most closely related species from the data in Table 10.5 and write their names in the appropriate boxes of the cladogram.
- b Identify the next most closely related species and write its name in the appropriate box of the cladogram.
- c Identify the least related species and write its name in the appropriate box of the cladogram.

Discussion

Use your completed cladogram to answer the following questions.

1 Which one of the four species diverged earliest? Explain how you interpreted this from the cladogram.
2 Which species is most closely related to the Asian elephant?
3 Would a taxonomic family Elephantidae, containing only the African and Asian elephants, be a monophyletic group? Explain.
4 Does your cladogram generated from the *ND5* gene sequences (Figure 10.42) support or reject the hypothesis you proposed in Figure 10.40? Explain.
5 Which character – fur covering or teeth morphology – provides evolutionary inferences that agree best with the *ND5* gene? Explain with reference to structural homology.
6 Is molecular homology more, less or equally useful to the character you identified in Question 5 for inferring evolutionary relationships among proboscideans? Give reasons to justify your response.

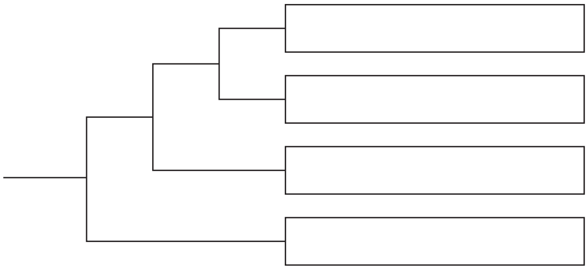


Figure 10.42 A cladogram generated from a 120-nucleotide segment of the mitochondrial *ND5* gene from four species of the order Proboscidea

Conclusion

Write a brief conclusion stating the outcomes of your analysis and summarising your evaluation of the value of molecular homology for inferring evolutionary relationships.

KEY CONCEPTS

- Molecular homology is the similarity of patterns in the nucleotide sequences of DNA or the amino acid sequences of polypeptides from different organisms. It is explained by divergent evolution from a common ancestor.
- The evolutionary distance between two species is estimated by the number of amino acids differing in a homologous polypeptide, or the number of nucleotides differing in a homologous gene, between the two species. Evolutionary relationships between species can be constructed based on evolutionary distances; the more alike the sequences, the more closely related are the organisms.
- Evolutionary relationships among species can be represented by phylogenetic trees that depict the patterns of descent from common ancestors.

Concept questions 10.5

1 Give two reasons why DNA and proteins are suited to studies of molecular homology.
2 What three things does a sequence alignment of homologous genes reveal?
3 Describe the features of mtDNA that make it suitable for evolutionary studies.
4 Explain how sequence conservation can be used to estimate how closely or distantly related species are.
5 Use the alignment presented in Figure 10.30 to estimate how conserved the reptile sequences are.
6 What does a node represent in a phylogenetic tree?
7 Describe a cladogram and a phylogram. Distinguish between them.

HOT Challenge

8 Examine the animals presented in Table 10.2.
  a Identify one pair that is likely to have homologous structures and one pair that is likely to have analogous structures.
  b How does the taxonomy support your interpretation?
  c Construct a cladogram that represents the relationships between the organisms in the table.

BRANCHING OUT

Speciation and conservation: eastern barred bandicoot

Populations with reduced diversity have an increased risk of extinction, so conservation efforts usually focus on maintaining genetic diversity. When large-scale extinctions occur, not all species are lost, and some seem to be at more risk than others. Rapid extinction events tend to lead to the loss of larger organisms at the top of food chains rather than smaller ones. Large populations can be more resilient than small populations, probably because the population has a more diverse gene pool. That is, it holds a greater reserve of different alleles to draw on as the pressures from natural selection change.

The eastern barred bandicoot (*Perameles gunnii*) belongs to the marsupial family Peramelidae. It is small (body about 300 mm, tail 200 mm), grey-brown in colour, with four pale stripes or 'bars' on its hindquarters (Figure 10.43). It has three claws on the front feet, which it uses for digging, while the back feet are long, and similar to those of a kangaroo.

age-fotostock/Photoshot/NHPA

Figure 10.43 The eastern barred bandicoot (*Perameles gunnii*)

Populations of the eastern barred bandicoot were once common over a wide area of south-western Victoria. Numbers reduced dramatically in the 1900s and now there are fewer than 200 eastern barred bandicoots isolated to a small area around Hamilton. This resulted from a change in environmental conditions (e.g. clearing of woodlands, growing exotic pasture grasses, grazing by domestic stock, introduction of rabbits and foxes), which severely reduced its available habitat in Victoria. However, the eastern barred bandicoot is still widespread throughout most of Tasmania.

Conservation plans for the eastern barred bandicoot depend heavily on how populations are classified. A subspecies is a level of classification below species, referring to races of a species that are fairly permanently geographically isolated from each other and may in future diverge to become two different species. Because of the relatively healthy bandicoot populations in Tasmania, the bandicoot is regarded as vulnerable but not endangered. If the Victorian population were identified as a different species, or subspecies, then it could be recognised independently for conservation purposes.

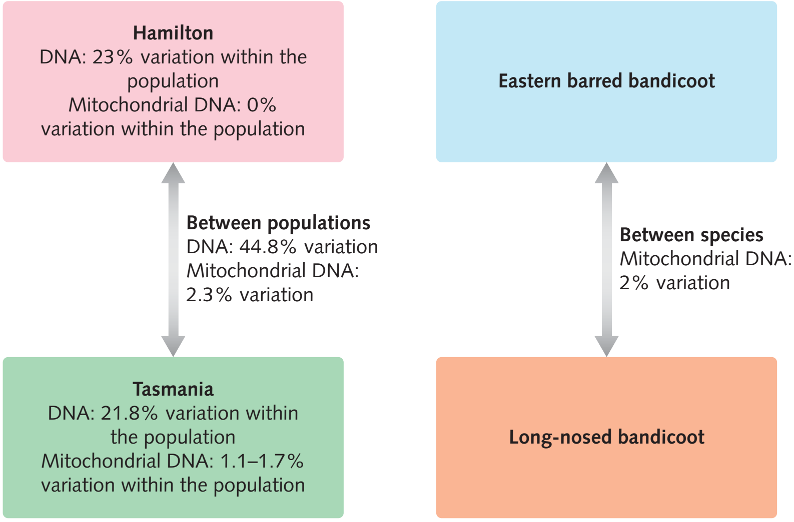


Figure 10.44 **DNA variability in different populations of eastern barred bandicoot. A 2% variation is the average difference between subspecies and closely related species of mammals.**

A number of studies have been conducted on the Victorian and Tasmanian populations in an attempt to protect the Victorian population. The bandicoots were trapped, small blood samples were taken and the animals were released immediately into the same areas. The blood was snap-frozen and later a DNA fingerprint was taken by analysing genomic variable nucleotide tandem repeats. The average percentage difference in variable nucleotide tandem repeats within the populations around Hamilton was about 23%, and for those in Tasmania it was 21.8%. The average percentage difference between the Hamilton and Tasmanian populations was 44.8% (Figure 10.44).

Further testing was done using mitochondrial DNA (mtDNA) restriction fragment length polymorphism analysis. This revealed a 0% nucleotide variation within the Victorian populations and a 1.1–1.7% variation for the Tasmanian populations. The percentage variation between the Victorian and Tasmanian populations was 2.3%. Variation of 2% is the average difference between subspecies of mammals.

There is no doubt that the two populations have diverged to some extent due to geographical isolation. It is important to know whether the two populations are separate subspecies because this affects how the conservation of these two populations of eastern barred bandicoot is managed. Biologists currently use a variety of species concepts, all of which are based on the theory of evolution.

The biological species concept defines a species as a reproductive community of populations that occupies a specific niche in nature. The identification of species often uses data from genetic analysis. 'DNA fingerprinting' is predominantly used to determine which groups are related – that is, share a gene pool – and which are not. A species defined according to this concept would be the smallest group of organisms that share a common ancestor not shared by any other organism.

The Australian Government, through the Department of the Environment, lists two subspecies of *P. gunnii*. The following is an excerpt from the listing for the eastern barred bandicoot.

> Scientific name: *Perameles gunnii* Victorian subspecies
> Common name: Eastern Barred Bandicoot (Mainland)
>
> The genetic diversity, as measured by the variable number of tandem repeat markers and mitochondrial DNA restriction fragment length polymorphisms, among specimens from Hamilton, Victoria, was greater than that found in widespread populations of the Tasmanian subspecies (*Perameles gunnii gunnii*). The justification for considering the mainland form to be distinct is based in part on morphological comparisons of island and mainland forms, and that mtDNA data indicated separation 270000–620000 years ago.

Questions

1. What species definition could be used to justify classifying the two populations as separate subspecies?
2. Does the recognition of two separate subspecies appear to be well accepted by the Australian Government at this stage?
3. What does the DNA evidence suggest about how the populations became separated? To what extent does this example illustrate the concept of allopatric speciation?
4. Would the small genetic variability in the eastern barred bandicoot populations affect their survival? Explain.
5. Explain why the identification of the two possible subspecies of bandicoot is important for their conservation.

Online Key Concepts
Chapter 10: Summary of key concepts

10 Summary of key concepts

10.1 Studying fossils

KEY CONCEPTS

p. 373

- Fossils are preserved remains of organisms or traces of their existence. The conditions for fossilisation occur rarely and this can cause a bias in the fossil record.
- The fossil record is delineated by consecutive layers of rocks called strata. The youngest fossils are found in the uppermost strata and the oldest are found at the bottom.
- Comparative dating can determine the age of a fossil or fossil-bearing rock in relation to the surrounding rock, but it does not give a numerical age.
- Index fossils are used to correlate the ages of strata that occur in deposits far from each other.
- Absolute dating gives numerical ages for the time of fossil formation and includes radiometric dating, luminescence and electron spin resonance.

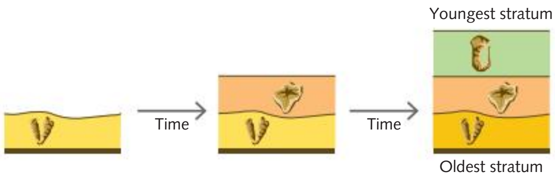


Figure 10.6 Over geological time, younger strata are deposited over older strata. The youngest fossils are found in the uppermost stratum and the oldest are found at the bottom.

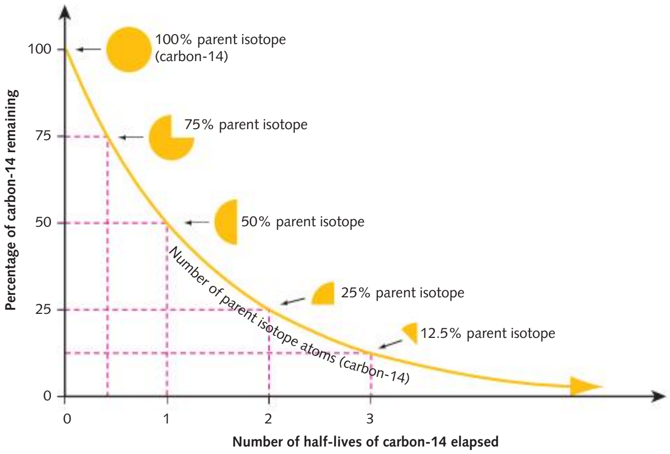


Figure 10.8 A graph of the decay of ^{14}C (half-life 5730 years)

10.2 Patterns in evolution

KEY CONCEPTS

p. 381

- The fossil record demonstrates that mass extinction events have occurred many times in the past. After each mass extinction, a collection of new species replaces the extinct ones.
- Transitional fossils provide evidence for evolutionary relationships between groups of organisms and document change in organisms over time.
- Divergent evolution occurs when different selection pressures apply to different populations of an ancestral species. The different populations accumulate many changes, becoming new species with characteristics that differ from those of the ancestral species. When this occurs on a large scale it is called adaptive radiation.
- Convergent evolution occurs when unrelated organisms (or organisms with a very distant common ancestor) evolve similar structures or adaptations to perform a similar function in response to the same selection pressures.

10.3 Emergence of new species

KEY CONCEPTS

p. 386

- Allopatric speciation is the process by which new species diverge from members of an ancestral species that have become geographically isolated for long periods.
- The evolution of Galapagos and Cocos Island finches is an example of allopatric speciation.
- The evolution of *Howea* palms is an example of sympatric speciation in which new species emerge from an existing population while inhabiting the same geographical area.

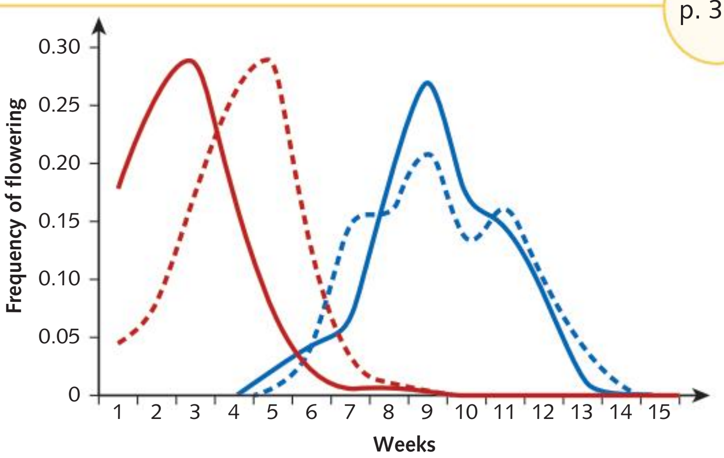


Figure 10.23 The differing flowering times of the two *Howea* species. The blue lines represent 198 curly palms (*Howea belmoreana*) and the red lines represent 177 kentia palms (*Howea forsteriana*) showing female (dashed) and male (solid) flowering phases.

10.4 Determining the relatedness of species

KEY CONCEPTS

p. 393

- Taxonomy is a naming system that categorises organisms based on hypotheses about evolutionary relationships.
- Comparing the development and anatomy of organisms can provide evidence that organisms developed from a common ancestor.
- Homologous structures evolved from the same ancestral form but have developed different forms or functions due to having different selection pressures.
- Analogous structures evolved under the same selection pressures from different ancestral forms so they have a common function but show some fundamental differences.

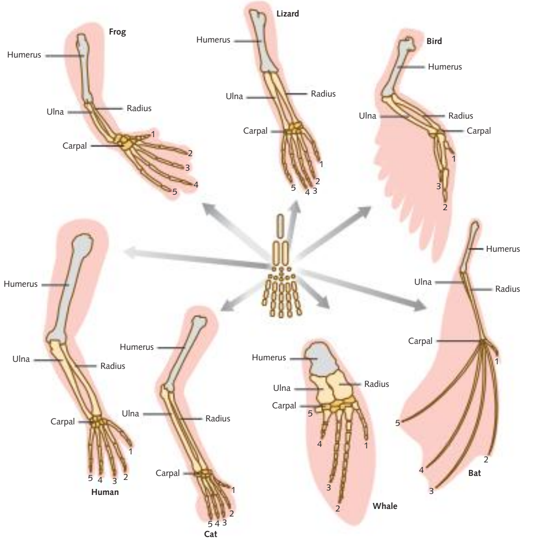


Figure 10.26 The principle of homologous structures can be illustrated by the adaptive radiation of the forelimb of a selection of vertebrates, which all show the basic pentadactyl pattern modified for different uses.

10.5 Molecular evidence for relatedness of species

KEY CONCEPTS

p. 399

- Molecular homology is the similarity of patterns in the nucleotide sequences of DNA or the amino acid sequences of polypeptides from different organisms. It is explained by divergent evolution from a common ancestor.
- The evolutionary distance between two species is estimated by the number of amino acids differing in a homologous polypeptide, or the number of nucleotides differing in a homologous gene, between the two species. Evolutionary relationships between species can be constructed based on evolutionary distances; the more alike the sequences, the more closely related are the organisms.
- Evolutionary relationships among species can be represented by phylogenetic trees that depict the patterns of descent from common ancestors.

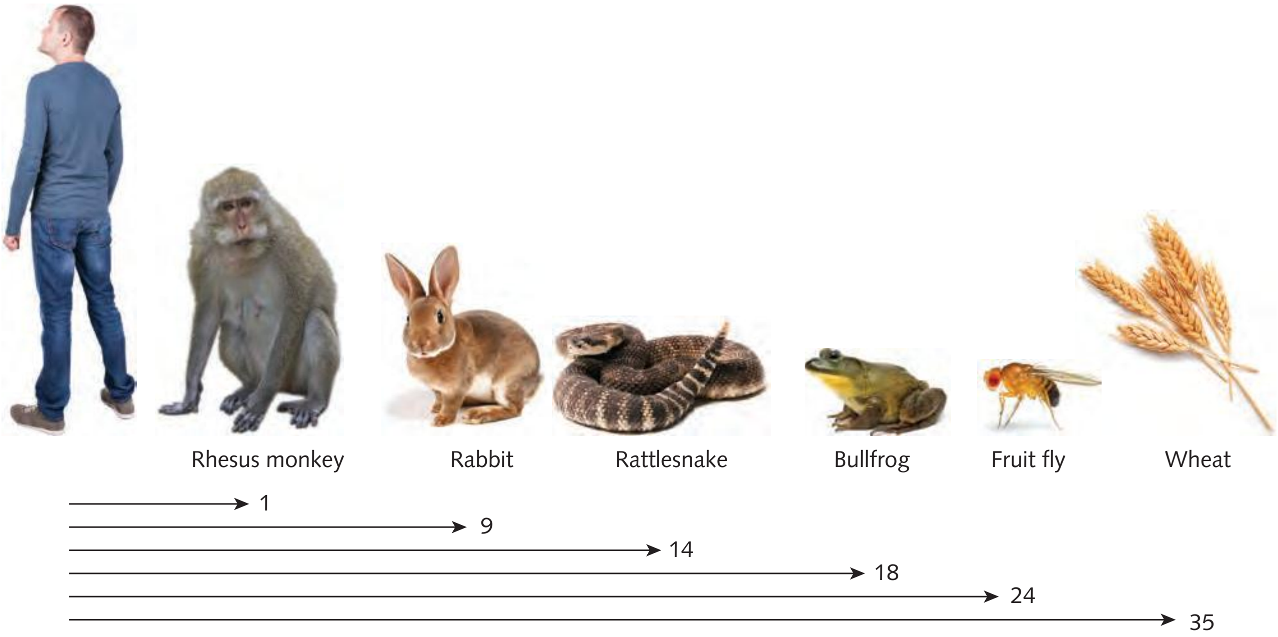


Figure 10.29 The number of amino acid differences between the cytochrome c of humans and that of other organisms.

WB

10.6.1 KEY TERMS PAGE 224

10 Chapter glossary

absolute dating the process of determining the age in years of rocks and the fossils they contain on the basis of the physical or chemical properties of materials in the rock

adaptation an anatomical, behavioural and physiological characteristic that allows an organism to exploit a specific ecological role

adaptive radiation when a single species diversifies relatively rapidly into many new species because of the availability of many different ecological niches

allopatric speciation speciation that occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species

analogous structure an anatomical or morphological feature in different organisms that has the same function but not the same basic underlying structure

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

clade a branch of a cladogram that represents a common ancestor and all of its descendants

cladogram a phylogenetic tree that depicts a hypothesis about the evolution of a group of organisms from a common ancestor

comparative dating *see* **relative dating**

convergent evolution when organisms that are not closely related independently evolve similar traits as a result of having to adapt to similar environments or ecological niches

correlation the inference that rock layers located in distant sites must be of the same age if they have identical mineral and fossil composition

divergent evolution when members of a population develop adaptations to different selection pressures over many successive generations and eventually become new species

electron spin resonance a method for determining the age of a rock or fossil based on the properties of electrons trapped inside the crystals of minerals

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

fossil the preserved remains or traces of an organism

fossil record the worldwide collection of fossils as they occur in the surface layers of Earth

fossil succession when fossils appear in a consistent order in the fossil record from older rock layers to younger overlying rock layers; the same order is found worldwide

homologous refers to genes or polypeptides that have similar sequences and indicate a shared evolutionary ancestry

homologous structure an anatomical feature in different organisms that has the same basic underlying structure but different functions

index fossil a fossil that is representative of a specific geological time

isotope one of two or more atoms of the same element with the same atomic number and number of protons, but different numbers of neutrons and therefore different relative atomic masses

lineage in evolution, a population that represents a separate line of descent from a common ancestor to modern species

luminescence in absolute dating, a method for determining the age of a mineral crystal based on measuring the emission of light by electrons as they are stimulated to escape from the crystal

mass extinction the extinction of many species over a relatively short (geological) period

maternally inherited describes a genotype that is transmitted entirely from the female parent to the offspring

mineralisation the process by which minerals from sediments have replaced the biological matter in a deceased organism, making it prone to become a fossil

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

morphological species concept usually applied to fossils, defines a species by measurable anatomical criteria and characteristics

mya millions of years ago

niche an organism's ecological role; the way the organism lives and functions in its environment

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

optically stimulated luminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is exposed to coloured light

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny the evolutionary relationships that exist between species, often expressed as a tree-like diagram or represented by taxonomic classification

phylogram a type of phylogenetic tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

radioactive decay a process by which the nucleus of an unstable isotope splits and emits energy in the form of radioactivity

radiometric dating a method for determining the age of a rock or fossil based on the predictable rates of decay of naturally occurring radioactive isotopes present

relative dating the process of determining the age of rocks and the fossils they contain relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

reproductively isolated when sexual reproduction can no longer occur freely among any adult members of the population

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

speciation the evolution of one or more new species from an ancestral species

strata (singular: stratum) successive layers of sedimentary rocks; each layer represents a unique age range and contains a unique collection of fossils

structural morphology the study of the physical structure and form of organisms

superposition the principle that strata are deposited in a time sequence, with the oldest at the bottom and the youngest at the top

sympatric speciation when two species evolve from an ancestral population while still inhabiting the same geographical area

taxonomy a system of scientific conventions for naming and classifying organisms

tetrapod a 'four-footed' vertebrate animal; includes amphibians, reptiles and mammals

thermoluminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is heated

trace fossil a fossil produced by an organism's activities, such as fossil footprints or burrows

transitional fossil a fossil that bears features of both an older ancestral life form and a younger descendant

vestigial structure a structure found in organisms that has lost most, if not all, of its original function in the course of evolution; in ancestral organisms, the structures served a purpose, but in their descendants, the structures become atrophied or rudimentary

10.6.2 EXAM PRACTICE PAGE 226

10 Chapter review

Remembering

1 Define:
  a fossil succession
  b transitional fossil
  c vestigial structures
  d homologous structures
  e evolutionary distance.

2 The fossil record is a vital stream of evidence for evolution, but it is patchy and incomplete. Recall why it is incomplete.

3 Describe what makes an ideal index fossil and how it is applied to the principle of correlation.

4 Explain the difference between a phylogram and a cladogram.

Understanding

5 Draw an annotated diagram of an *Archaeopteryx* fossil. Explain why *Archaeopteryx* is an excellent example of a transitional fossil between dinosaurs and modern birds and label the relevant features on your diagram.

6 The phrase 'survival of the fittest' has been used to describe Darwin's concept of natural selection. Outline the ways in which this term could be misleading.

7 In North America, species of fruit fly of the genus *Rhagoletis* are confined to different species of apple trees and hawthorn bushes.
  a Describe how this could lead to speciation.
  b Would this be allopatric or sympatric speciation? Explain.

8 Both birds and bats have wings, while mice and crocodiles do not. Explain if this means that birds and bats are more closely related to one another than to mice and crocodiles.

9 Defend or refute the statement, 'There is nothing more to be learnt from fossils that have already been examined'. Use evidence to support your position.

10 What would it imply if the sequences of homologous genes from two organisms were 100% conserved?

11 You are related to your first cousins because you share two recent 'common ancestors' (your grandparents). The theory of evolution states that all organisms on Earth today have also arisen from a single common ancestor. How are these two usages of this term similar and how are they different?

Applying

12 Provide an example of how an understanding of changing gene pools is important to understanding evolutionary change.

13 Embryological studies show bird embryos develop a fourth finger and a fifth toe that vanish as the foetus develops. This vestigial developmental structure is evidence for common descent.
  a Explain what this evidence explicitly says about the characteristics of the ancestors of birds.
  b Explain whether you would expect a complete fossil skeleton of a common ancestor showing this characteristic to have been found.

14 Stone tools have been found with campfire charcoal. Explain how the technique of carbon dating could be used to determine the time at which the tools were made.

15 Explain the difference between the techniques of electron spin resonance and optically stimulated luminescence.

16 A fossilised fish skeleton is found in sandstone, 1 m below the surface, at location X. A very similar skeleton is found at location Y, 2 m below the surface and 1 km away from location X. Another similar skeleton is found at location Z, 3 m below the surface and 3 km away from location X. Describe what can be inferred about the:

- **a** way in which the rocks were formed
- **b** age of the fossil at location Y.

17 Examine the cladogram of the Galapagos finches in Figure 10.45.

- **a** Which species is most closely related to *Geospiza fortis*?
- **b** Which species is most closely related to *Camarhynchus parvulus*?
- **c** What evidence from the cladogram supports the ground finches being a monophyletic group?
- **d** How is a monophyletic group of ground finches recognised in their taxonomy?

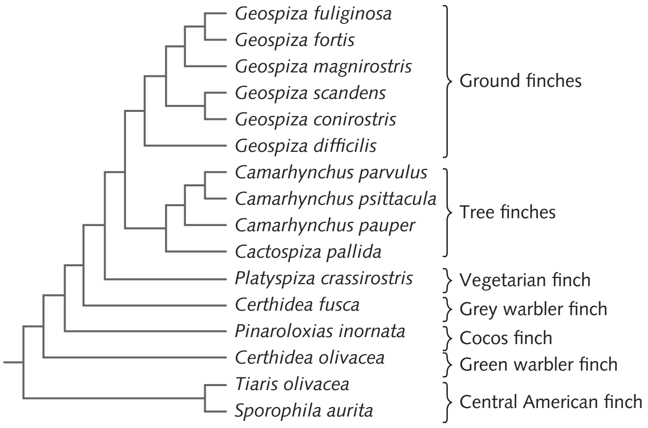


Figure 10.45 A cladogram representing the Galapagos finches

Tebbich, S., Fessl, B. & Blomqvist, D. Exploration and ecology in Darwin's finches. Evol Ecol 23, 591–605 (2009). https://doi.org/10.1007/s10682-008-9257-1

18 The cladogram in Figure 10.46 represents evolutionary relationships among groups of land plants.

- **a** Which group diverged the earliest?
- **b** Which two groups are most closely related?
- **c** A biologist proposed recognising a taxonomic group of 'non-flowering plants' consisting of mosses, lycophytes, ferns and conifers. What is your recommendation for that proposal? Justify your recommendation.

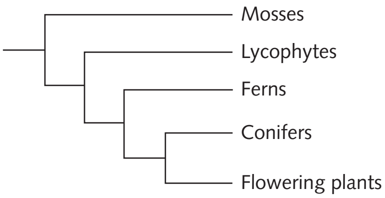


Figure 10.46 A cladogram representing evolution of land plants

Analysing

19 Over the last 30 years many new pre-human fossils have been found, but scientists often find it difficult to agree whether they should be identified as new species or not. Account for this limitation in terms of our current understanding of the species concept.

20 Identify a limitation of luminescence in dating sedimentary rock.

21 There is a variety of types of tortoise on the Galapagos Islands. One species has a domed shell and a short neck and is found on islands with high moisture content. Another species has a long neck and a shell that flares up at the front so that the tortoise can lift its long neck up. The long-necked tortoise is found on the more arid islands. The main food of the tortoises is the prickly pear cactus. On the islands with no tortoises, the prickly pear has a low spreading form with soft spines. On the islands with the long-necked tortoise, the prickly pear has a tall form with hard spines.

- **a** Explain how the tortoises could have first reached the Galapagos Islands.

- **b** Assess and explain if it is likely that the ancestor tortoises would be identical to the modern tortoises.
- **c** Explain why prickly pear would grow in different plant forms on different islands.
- **d** Define and explain what type of evolution is illustrated by the association of the long-necked tortoise and the tall prickly pear.

22 The last known Tasmanian tiger (thylacine) died in the Hobart Zoo in 1936 and the species is now recognised as extinct. Hypothesising a close evolutionary relationship between the Tasmanian devil and the Tasmanian tiger, scientists sought to explore the phylogeny of Australian carnivorous marsupials using DNA. They sequenced the mitochondrial genome from a pelt of the Tasmanian tiger and from tissues of living marsupials. They compared the mitochondrial genomes and constructed the phylogram shown in Figure 10.47.

- **a** Suggest why the scientists chose to sequence the mitochondrial genome rather than the nuclear genome.
- **b** What do the branch lengths represent in the phylogram?
- **c** What has happened during evolution to result in the different branch lengths?
- **d** Which is the most closely related species to the Tasmanian devil?
- **e** Was the scientists' hypothesis supported or rejected?
- **f** Decide whether the following taxonomic families are monophyletic or not and give reasons to justify your decision.
  - **i** Family Dasyuridae, which includes the Tasmanian devil, phascogale and dunnart
  - **ii** Family Thylacinidae, which includes the Tasmanian tiger

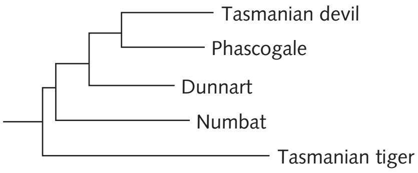


Figure 10.47 **A phylogram of Australian marsupial carnivores based on mitochondrial genome sequences.**

Evaluating

23 The hoatzin (*Opisthocomus hoazin*) is a remarkable bird from South America. It has only one known fossil ancestor, identified from a 10-million-year-old skull fragment found in Colombia. The age of the fossil demonstrates that hoatzins were endemic to South America; the fossil pre-dates the land bridge between North and South America by 8 million years. Genetic analysis of the living hoatzin has shown it to be unique, perhaps because of its extensive history of geographic isolation, and it has its own suborder. Chicks of the hoatzin show a characteristic seen in no other living bird: a pair of claws on their wings, a characteristic similar to those seen on the bird-like dinosaur *Archaeopteryx*, which had three wing claws. From this description, identify lines of evidence for evolution from the disciplines of:

- **a** palaeontology, via the fossil record
- **b** morphology
- **c** genetics.

24 You are invited to construct a phylogenetic tree to represent the relationships between *Eucalyptus* species. You have the resources to copy and sequence the genes listed in Table 10.6.

Consider the merits of using any one of these genes for investigating evolutionary relationships.

- **a** Discuss which features of a gene you would consider important in deciding whether or not to use it for phylogenetic studies.
- **b** Which gene would you choose to explore the phylogenetic relationships of species within the genus *Eucalyptus*? Give reasons to support your choice.
- **c** Which would you choose to explore the phylogenetic relationships between genus *Eucalyptus* and other representatives of kingdom Plantae? Give reasons to support your choice.
- **d** In parts **b** and **c**, would you choose to represent the evolutionary relationships as a cladogram or phylogram? Justify your answer.

Table 10.6 Genes found in *Eucalyptus* and some of their characteristics

Gene	Genomic location	Approximate size (bp)	Notes
his4	Nucleus	500	Codes for a subunit of the proteins around which DNA is wound to form chromatin inside the nucleus
gapdh	Nucleus	2900	Codes for an enzyme involved in glycolysis
rbcL	Chloroplast	1400	Codes for a subunit of Rubisco, an enzyme involved in the light-independent reactions of photosynthesis
ATP8	Mitochondrion	450	Codes for a subunit of mitochondrial ATP synthase
CO1	Mitochondrion	700	Codes for a protein involved in the mitochondrial electron transport chain
ITS1	Nucleus	200	Transcribed but untranslated region of DNA situated between two rRNA genes; may occur in thousands of copies
hmgb1	Nucleus	4000	Non-functional and does not code for protein

25 Defend or refute the statement: 'Gene phylogeny is the same as species phylogeny'. Outline the arguments in support of your position.

Creating

26 Consider the gene sequences shown in Table 10.7.

a Propose a series of operations for using the sequences to infer the evolutionary relationships between the four organisms. Depict your proposal as a flow chart.

b Construct a cladogram from the sequences and outline your rationale for the arrangement of the branches.

Table 10.7 A 100-nucleotide segment of the *CO1* gene from four bird species

Species name	Common name	Locality	DNA sequence
Eclectus roratus	Eclectus parrot	Northern Australia, New Guinea	CTTCGGCGCATGAGCTGGCATAATCGGTACCGCCCTAAGCCTACTTATCCGCGCAGAACTAGGCCAACCTGGAACCCTACTAGGAGACGACCAAATCTAC
Ciconia boyciana	Oriental stork	China, Russia	CTTCGGCGCATGAGCTGGCATAGTTGGAACCGCCCTTAGCCTTCTTATTCGCGCAGAACTTGGTCAACCAGGAACCCTCCTAGGAGACGACCAAATCTAC
Agapornis roseicollis	Rosy-faced lovebird	South-western Africa	CTTCGGCGCATGAGCTGGCATGATTGGTACATCCCTAAGCCTCCTCATCCGCGCAGAACTAGGCCAGCCAGGAACCCTGCTAGGAGACGACCAAATCTAC
Rhynchopsitta terrisi	Maroon-fronted parrot	Mexico	CTTCGGCGCATGAGCAGGCATGGTCGGTACCGCCCTAAGCTTGCTTATTCGTGCAGAGCTCGGTCAACCAGGGACCCTCCTAGGAGACGACCAGATCTAC

Human change over time

11

By the end of this chapter you will have covered the following material.

Key knowledge

Human change over time

- the shared characteristics that define mammals, primates, hominoids and hominins pp. 425–433
- evidence for major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure pp. 433–442
- the human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence of new putative *Homo* species pp. 442–454
- ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place pp. 455–464

Key science skills

Plan and conduct investigations

- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 452–453
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 452–453

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 452–453
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 452–453
- demonstrate ethical conduct when undertaking and reporting investigations pp. 452–453

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 452–453

Analyse and evaluate data and investigation methods

- identify outliers, and contradictory or provisional data pp. 452–453

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 452–453
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 452–453

Online Chapter Map
Chapter 11 Map

11 Human change over time

Humans have evolved under the same constraints as all other species. Human evolution can be traced over millions of years using fossil and molecular evidence.

11.1 The taxonomy of modern humans

p. 425

Humans are placental mammals. Humans are further classified into (order) primates with lemurs, lorises, tarsiers, monkeys and apes; (family) hominoids with the apes and then split off into their own genus and species, *Homo sapiens*.

11.2 Adaptations that define humans

p. 433

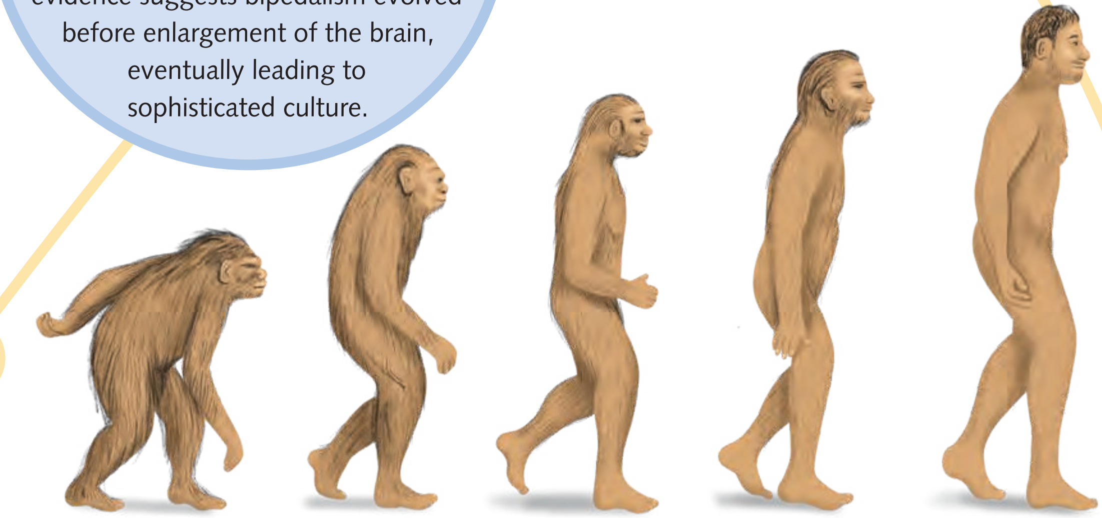
Humans differ from other hominoids by having a skeletal structure fully adapted for walking upright on two legs (bipedalism). Our hands allow us to finely manipulate objects. We communicate with speech, display advanced intellectual abilities, use complex technology, and practise elaborate symbolism.

11.3

Meet the ancestors

p. 442

The fossil record depicting human evolution is patchy and continually being refined or challenged by new fossil discoveries and the application of new technology. The evidence suggests bipedalism evolved before enlargement of the brain, eventually leading to sophisticated culture.

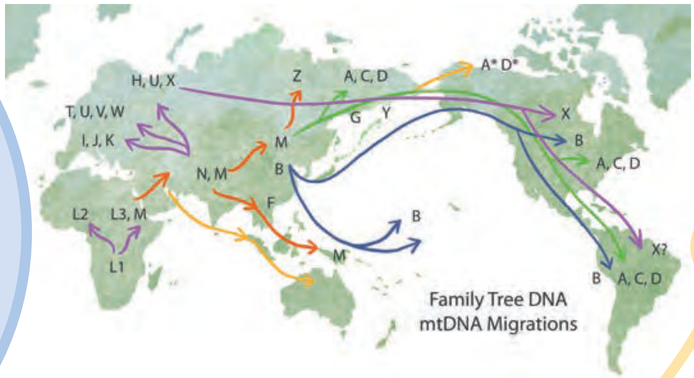


11.4

Modern humans and Neanderthals

p. 451

Fossils, archaeology and mitochondrial and nuclear DNA sequences support the Out of Africa hypothesis. They indicate modern humans' patterns of migration across the world, including the peopling of Australia. DNA evidence shows that modern humans interbred with Neanderthals and other ancient humans as they migrated into Europe and across Asia.



Humans share characteristics with other mammals, primates and hominoids. We are set apart from other hominoids by having certain characteristics that enabled us to take advantage of the natural world and use it for our own advantage. Humans can trace their ancestry to Africa, which they left to populate the rest of the world.

Know your key terms

Online Key Terms
Chapter 11 flashcards

arboreal
australopithecine
bipedalism
brachiation
brain case
brow ridges
carrying angle
cerebral cortex
cognitive capacity
cranial capacity
cranium
cultural evolution
Denisovan
dentition
exocrine gland
foramen magnum
gracile
hallux
haplogroup
haplotype
hominin
hominoid
kya
language
mammal
mammary gland
mandible
midden
Out of Africa hypothesis
oviparous
palaeoanthropology
placental
postcranial
precision grip
prefrontal cortex
prehensile
primate
prognathism
quadrupedalism
recent single origin
robust
sagittal crest
sagittal keel
sebaceous gland
sexual dimorphism
stereoscopic
superfamily
suspensory locomotion
sweat gland
tribe
viviparous

Remember

WB
REMEMBER
PAGE 229

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Allopatric speciation occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species.
2 Sympatric speciation occurs when two species evolve from an ancestral population while still inhabiting the same geographical area.
3 The fossil record is not a complete record of past living things, as it is biased towards living things that fossilise more easily than others.
4 Radioisotope dating takes advantage of the decay of naturally occuring radioactive materials and is used to absolutely date fossils, artefacts or the sediments they come from.
5 All mammals possess hair at some stage in their development and feed their young on milk produced in mammary glands.
6 A phylogenetic tree is a branching diagram showing the evolutionary relationships between species. Groups joined together in the tree are believed to have descended from a common ancestor.
7 A phylogram represents the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage.
8 A cladogram depicts a hypothesis about the evolution of a group of organisms from a common ancestor.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- **Chapter 11 map (p. 422)**

Online Key Terms:
- **Chapter 11 flashcards (p. 424)**

Weblinks:
- **Four billion years of evolution (p. 442)**
- **A brief history of dogs (p. 463)**

Online Worksheets:
- **Myths and misconceptions about evolution (p. 442)**
- **A brief history of dogs (p. 463)**

Online Key Concepts:
- **Chapter 11: Summary of key concepts (p. 466)**


Newspix/Cameron Richardson

Figure 11.1 What has occurred during evolution to enable *Homo sapiens* to rise to prominence?

We, *Homo sapiens*, are the dominant vertebrate animal on Earth, and human activities have a profound impact on other species. However, biologists cannot assume humans are special, separate from nature on Earth, because of some divine plan. Rather, *Homo sapiens* is a species that has evolved under the same constraints and biological principles that apply to all species. In this chapter, we will explore the various lines of evidence that argue for the evolutionary history of *Homo sapiens* and the features that have emerged to make the species unique.

Our understanding of human evolution is incomplete, sometimes contentious, and is continually being reshaped as new evidence arises. The evidence may come from new discoveries that are made by using existing investigative methods or by developing new methods. Consequently, the study of human evolution serves as an illustrative model for how biologists infer evolutionary relationships between organisms and assess how closely related one group of organisms is to another. This chapter therefore discusses the current knowledge of human evolution and some of the gaps in our understanding.

Throughout this chapter, we will look at how evolution has enabled *Homo sapiens* to rise to such prominence (Figure 11.1).

11.1 Shared structural and phsiological characteristics of taxonomic groups of humans

WB

11.1.1
TAXONOMY
PAGE 230

CONNECT

Taxonomy is discussed in more detail in Chapter 10.

Like all other animals, humans can be classified within taxonomic schemes. As a tool of systematics, the taxonomy reflects a hypothesis for the evolutionary relationships between humans and other organisms. Taxonomy organises our current understanding about the evolutionary descent of modern humans and which animals are our most closely related living species.

Humans are mammals

Within formal taxonomic schemes, modern humans are members of the phylum Chordata and the class Mammalia. There are approximately 5000 species of **mammals**. They are a diverse group (Figure 11.2) that includes many of the largest terrestrial and marine carnivores, the largest terrestrial herbivores, burrowing and flying species and many of Australia's most iconic animals.

Features that characterise mammals

Many mammals are covered in hair or fur. However, in some mammals the hair or fur is not immediately recognisable. For example, on the echidna, the hairs are fused into quills. Some aquatic mammals, such as dolphins, have fine whiskers at birth that are quickly lost. Humans have a diminished covering of hair.

Other shared features of mammals are shown in Table 11.1. Mammals have several unique types of **exocrine glands**, which secrete fluids to the outer surface of the body. Most notably are the **mammary glands** from which the class name, Mammalia, is derived. Newborn offspring are nourished by nutrient-rich milk secreted by the mother's mammary glands.

Humans have other types of exocrine glands that function as the adaptations of an endothermic (warm-blooded), fur-covered animal. For example, we have **sebaceous glands**, which secrete oils that lubricate and protect patches of the skin and hair. We also have **sweat glands** that help regulate body temperature.

EXAM TIP

Do not get secretion and excretion mixed up. Secretion is when a required substance has been produced in a cell or a gland and is removed from the cell or gland to perform a function. Excretion is the removal of cellular waste from cells or the body.

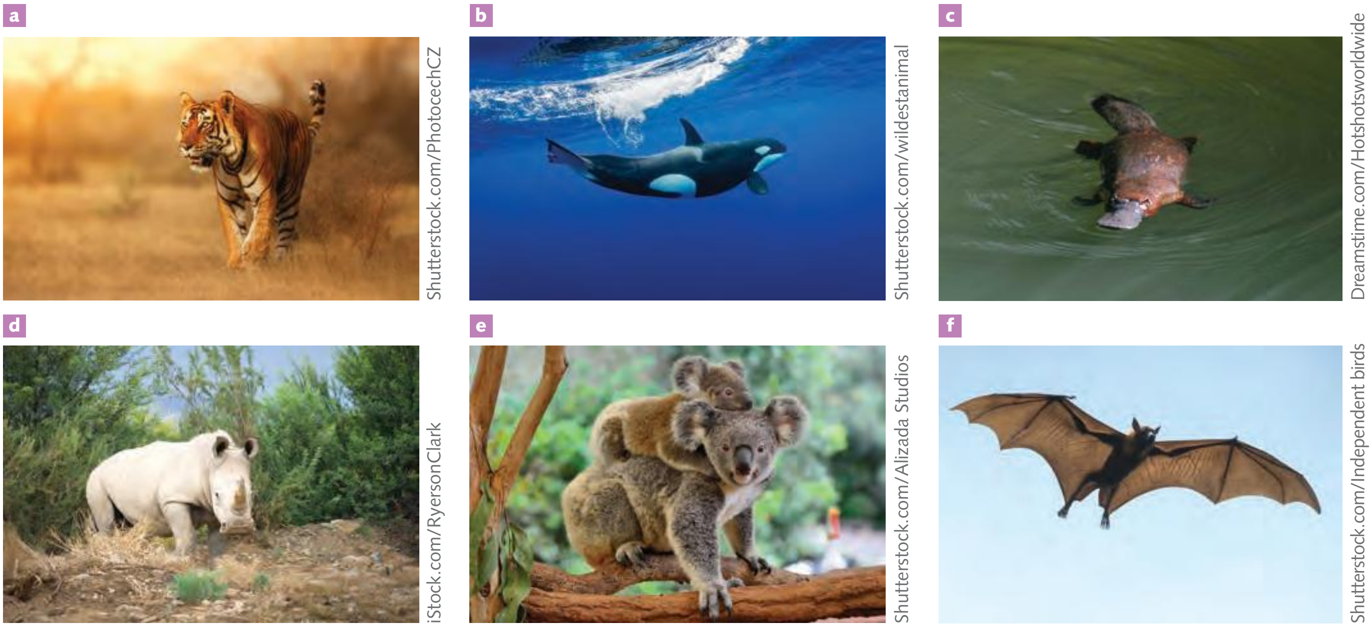


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Figure 11.2 The diversity of mammals: **a** a tiger (*Panthera tigris*); **b** a killer whale (*Orcinus orca*); **c** a platypus (*Ornithorhynchus anatinus*); **d** a white rhinoceros (*Ceratotherium simum*); **e** a koala (*Phascolarctos cinereus*); and **f** a bat (*order Chiroptera*)

Table 11.1 Conspicuous anatomical features that distinguish mammals from other classes in the phylum Chordata

Feature	Mammals	Other chordates
Body covering	Fur or hair	Scales (fish, reptiles), permeable skin (amphibians), feathers (birds)
Temperature regulation	Endothermic	Ectothermic (fish, amphibians and reptiles) or endothermic (birds)
Unique exocrine glands: mammary, sebaceous, sweat and scent glands	Present	Absent
Number of chambers in the heart	Four	Two (bony fishes), three (cartilaginous fish, amphibians and reptiles) or four (birds and crocodiles)
Diaphragm	Present	Absent
Number of bones in the middle ear	Three: malleus, incus and stapes	One: stapes
Structure of lower jaw	Single bone	Multiple bones (fish, amphibians, reptiles) or absent (birds)
Teeth	Specialised into incisors, canines and molars	Uniform (fish, amphibians, reptiles) or absent (birds)

Our heads, like those of other mammals, have conspicuous external ears, a middle ear consisting of three bones, a fused lower jawbone, and teeth that are differentiated by size, shape and position into incisors, canines and molars. Like other mammals, humans have a four-chambered heart with a unidirectional cardiovascular system that circulates oxygen-rich blood independently of the oxygen-poor blood. Humans also have a diaphragm that separates the abdominal and thoracic cavities and acts to inflate and deflate the lungs during rhythmic breathing.

Placental mammals

Most mammals are **viviparous** (bear live young). Other mammals are **oviparous** (lay eggs).

The oviparous subclass Prototheria, or monotremes, constitutes just a few modern egg-laying species, the platypus and the echidnas. Monotremes produce leathery eggs, like those of reptiles. Juvenile monotremes are nourished by the mother's mammary glands, with the milk secreted from a patch of skin, rather than from a discrete teat, enclosed inside a skin fold. Anatomical and DNA evidence suggests that monotremes are the earliest divergence of the mammals.

The viviparous subclass Theria consists of two 'infraclasses'. In the infraclass Metatheria, or marsupials, the embryo is nourished by a yolk-based placenta. The nutrients are soon consumed and the embryo is born at a relatively early stage of development. The embryo moves from the mother's uterus to the pouch, where it attaches to a nipple and suckles for the rest of its development.

Most mammals are members of the infraclass Eutheria, or **placental** mammals. In this group, the placenta is a relatively more complex organ that connects the blood supply of the developing embryo or foetus with that of the mother. The placenta exchanges nutrients and wastes, and provides some immunological protection. By connecting to the mother's blood supply, gestation can proceed for much longer in the uterus and placental mammals are normally born at a substantially more advanced stage than marsupials.

Humans are placental mammals. Humans clearly share an anatomical, physiological and reproductive heritage with thousands of other eutherian mammals that have descended from an ancient, highly successful and highly divergent lineage extending back more than 225 mya.

KEY CONCEPTS

- » Mammals share a range of unique anatomical features, including a body covering of hair or fur, a single jaw with specialised teeth, three bones in the middle ear, specialised exocrine glands, a diaphragm and a four-chambered heart.
- » Mammals are divided into monotremes, marsupials and placental mammals.
- » Humans are placental mammals.

Concept questions 11.1a

1 Describe three types of exocrine glands unique to mammals.

2 Describe the specialisations in mammalian teeth.

3 Distinguish between oviparous and viviparous. Which mammalian subclass is oviparous and which is viviparous?

HOT Challenge

4 Mammals have a four-chambered heart. Mammals have exocrine glands. Mammals have three bones in the middle ear. Referring to Table 11.1, postulate whether or not there is an evolutionary advantage in these characteristics over other chordates.

Humans are primates

Humans have features in common with certain animals. Humans have obvious similarities with apes, monkeys and an even broader group, the order Primata, or **primates**. These similarities inspired Charles Darwin to study primates in the later part of his career. His conclusions about human evolution were publicly ridiculed at the time but are supported today by the evidence collected by modern science.

The primates include lemurs, lorises, tarsiers, New World monkeys, Old World monkeys and apes, as well as modern humans (Figure 11.3). They all have features that unite them as a group.

Features that characterise primates

Many features distinguish primates from other mammals. Primates have hands and feet that bear five digits that include an innermost, opposable digit (Figure 11.4). These are the thumb of the hand and the **hallux** (enlarged toe) of the foot. The hands and feet curl – they are **prehensile**. The tips of the digits have sensitive touch receptors. In contrast to non-primates, the digits typically have flattened nails instead of claws. These adaptations enable primates to grasp, climb and manipulate objects.

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Figure 11.3 Representative primates: **a** a crowned lemur; **b** a pygmy loris; **c** a Siau Island tarsier; **d** a Colombian night monkey (a New World monkey); **e** a mandrill (an Old World monkey); and **f** a western gorilla (an ape).

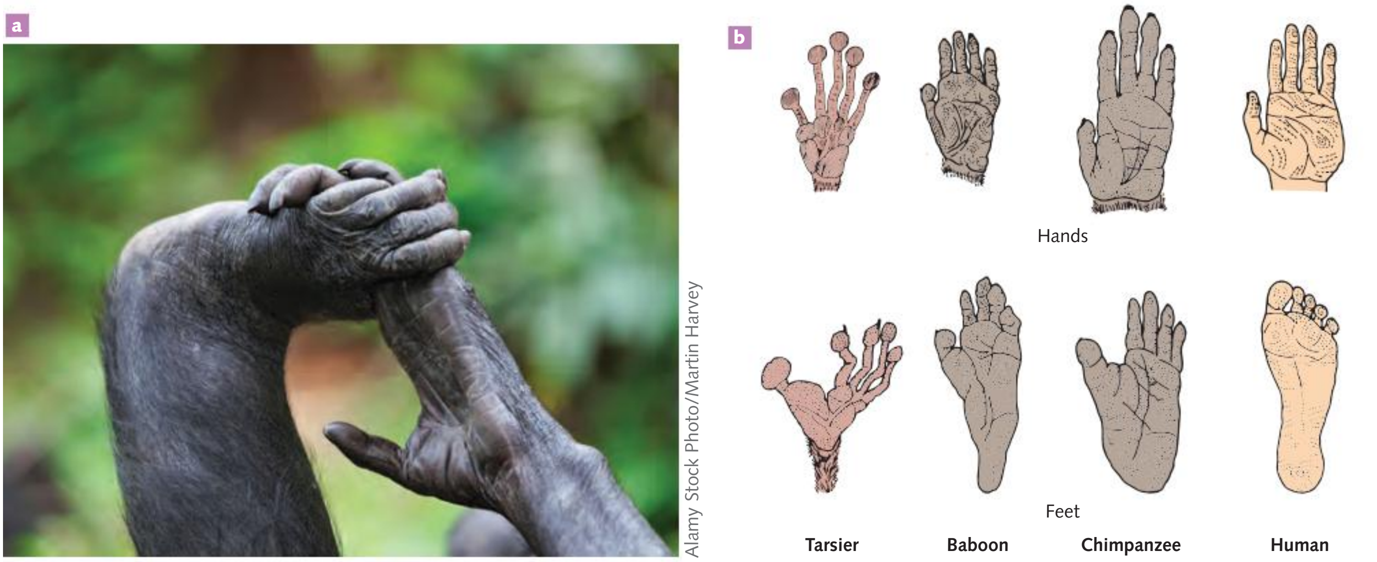

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Figure 11.4 a A chimpanzee has prehensile hands and feet. **b** Hands and feet of four primates. Note that only the human lacks an opposable hallux (big toe).

Primates depend heavily on their sense of vision, for which they have distinctive features. They have forward-facing eyes, which enable **stereoscopic** (3D) vision. This is probably an adaptation for an **arboreal** lifestyle – most primates live in trees. Animals that leap from tree to tree must be able to judge distances. Most primates also have retinas that contain many cone cells (colour photoreceptors), enabling them to see in colour.

Primates tend to have small snouts because they are less reliant on a sense of smell than many other mammals. To accommodate the reduced snout, primates have fewer teeth than other mammals. However, the precise number, structure and arrangement of teeth varies.

Compared with other mammals, primates have a relatively large **cranium** for their body weight. This is due to the expansion of the **brain case**. The outer region of the brain, the **cerebral cortex**, has expanded the most during primate evolution. The cerebral cortex is responsible for the higher functions of the brain, such as processing visual and tactile sensory information, as well as memory and reasoning. It has been proposed that the enlarged cerebral cortex is an adaptation to life in the treetops, which depends heavily on processing visual and tactile input and coordinating it with responses from the body's muscles.

Primates have flexible spines and have considerable rotation about the hips and shoulders, enabling them to move in various ways. Their modes of locomotion include climbing, **brachiation** (swinging through the air between branches), **suspensory locomotion** (using all fours to amble while hanging from a branch), **quadrupedalism** (moving on all four limbs) and **bipedalism** (moving on the hind limbs) (Figure 11.5). Primates tend to shift their body weight onto their hind limbs, whereas other terrestrial mammals shift their body weight onto the forelimbs (e.g. dogs and horses).

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Figure 11.5 **Representative hominoids displaying their chief mode of locomotion. a A Lar gibbon brachiating; b a Bornean orangutan moving by suspensory locomotion; c a western gorilla knuckle-walking (quadrupedal locomotion); d a chimpanzee knuckle-walking; e a human walking upright (bipedal locomotion).**

Primates also have intangible characteristics that are not necessarily useful for classification. For example, many primates are social animals, often living in extended family and social groups. Most give birth to a single offspring at a time, and the juveniles need relatively intense care for an extended period.

It is within the context of these features that modern humans evolved.

Classifying primates

The Primata is divided into two suborders: the Strepsirrhini or 'wet-nosed primates' and the Haplorrhini or 'dry-nosed primates' (Figure 11.6). The Strepsirrhini comprises lemurs and lorises. The Haplorrhini includes all the other primates. This division has not always been clear-cut and the position of the tarsiers is still being debated. Traditionally, tarsiers were considered more closely related to lemurs and lorises based on presumably shared morphological features. The modern classification reflects DNA sequence data. DNA sequence data tends to suggest that tarsiers represent the earliest split within the Haplorrhini.

Humans are classified within the suborder Haplorrhini, along with monkeys, apes and tarsiers, which are regarded as a distant relation within the suborder.

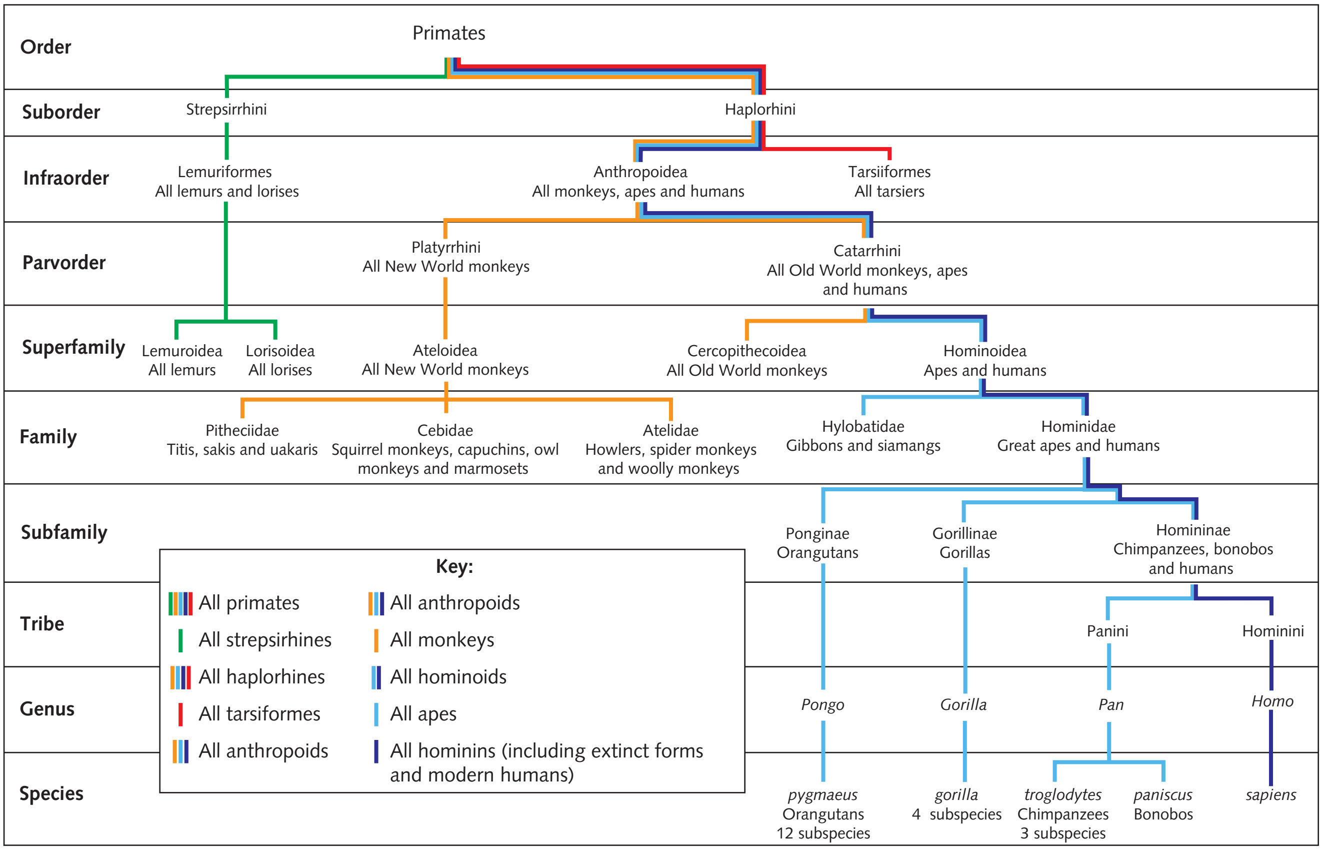

Figure 11.6 Taxonomy for the primates superimposed on a cladogram showing the position of modern humans. Additional levels of classification are introduced (e.g. superfamily, tribe) to better represent the evolutionary relationships within the order.

Classifying monkeys

Monkeys are divided into Old World monkeys and New World monkeys. These common names refer to the continents the monkeys inhabit. Historically, the 'Old World' refers to Europe, Africa and Asia, whereas the 'New World' refers to the Americas. The geographical isolation imposed by continental separation shaped the evolution of the monkeys on each continent and they now have discernibly different anatomy, physiology and behaviour.

The fundamental feature that distinguishes monkeys is their nostrils. New World monkeys have compact noses with nostrils that angle sideways ('platyrrhine'). Old World monkeys, as well as apes, have expanded noses with downward-facing nostrils ('catarrhine', Figure 11.3e). New World monkeys are thus classified in the taxonomic group of Platyrrhini, and Old World monkeys are classified in the Catarrhini (Figure 11.6).

Additional differences relate to the number and pattern of premolar teeth, the form of the bony ring in the ear drum and the type of tail.

On the basis of anatomy, morphology, physiology, biogeography and DNA sequence data, the Old World monkeys are considered most closely related to the apes. Therefore, apes are classified with Old World monkeys in the Catarrhini (Figure 11.6).

KEY CONCEPTS

- Modern humans are classified in the order Primata (primates) within the suborder Catarrhini.
- Key characteristics of primates include opposable digits, stereoscopic colour vision, nails on the digits, and a relatively enlarged brain.

Concept questions 11.1b

1 Define ‘prehensile’.
2 What are four different modes of primate locomotion?
3 How are the key characteristics of primates relevant to organisms that live high in the treetops?
4 What is the fundamental anatomical difference between Old World monkeys and New World monkeys?

HOT Challenge

5 What evolutionary advantage might stereoscopic vision confer on primates?

Humans are hominoids

Humans are **hominoids**, which are classified within the **superfamily** Hominoidea (Figure 11.6). The superfamily is a taxonomic rank immediately above the traditional rank of family. The hominoids include the gibbons, orangutans, gorillas, chimpanzees, bonobos and humans. Hominoids are fundamentally apes. They are distinguished from monkeys by lacking a tail.

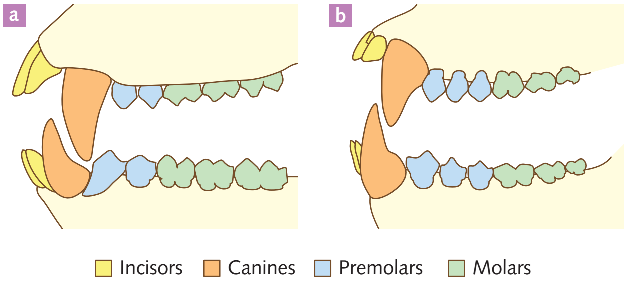

Figure 11.7 The dentition of **a** hominoids and Old World monkeys and **b** New World monkeys

Hominoid dentition

The **dentition** (teeth arrangement) of hominoids has similar features to that of Old World monkeys but differs from those of other primates. For example, adult hominoids and Old World monkeys have eight premolars, whereas New World monkeys have 12 (Figure 11.7). The molars of hominoids and Old World monkeys have five cusps on their molars with a Y-shaped upper valley. By contrast, New World monkeys have four cusps with a cross-shaped valley (Figure 11.8). These observations support a closer evolutionary relationship between Old World monkeys and hominoids.

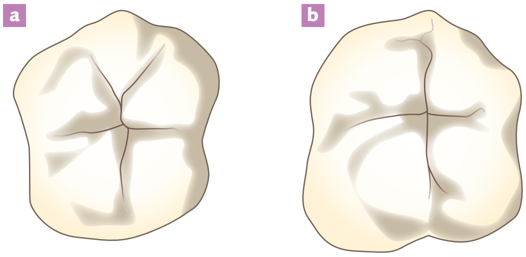

Figure 11.8 **a** Molars of hominoids and Old World monkeys have five cusps. **b** Molars of New World monkeys have four cusps.

Hominoid posture

The rib cage in hominoids is flattened compared to that in monkeys, which gives hominoids a broader chest. In addition, for their body length, hominoids have a comparatively shorter spine between the rib cage and the pelvis. It is inferred that these features enable hominoids to sit comfortably with an upright posture.

The relative length of the forelimbs and hind limbs varies among primates depending on the animal’s lifestyle and mode of locomotion. Most hominoids, except humans, have longer forelimbs (arms) than hind limbs (legs). Most hominoids have flexible shoulder and elbow joints that allow a range of locomotion styles (Figure 11.5, p. 429). The shoulder blades of hominoids sit further back than in other primates. This allows hominoids to move their arms freely around the shoulders. To maintain their balance when moving, hominoids display an ‘orthograde’ form of locomotion. The limbs on the same side of the body move in opposition to each other so that the forelimb swings back as the hind limb swings forward.

By contrast, monkeys have relatively narrow rib cages and comparatively longer spines for their body length. The forelimbs and hind limbs of most monkeys are approximately equal in length, adapting them for quadrupedalism on tree limbs or on the ground. Monkeys are highly dependent on their tails to control their balance. Consequently, when monkeys 'walk' on their hind legs, their forelimbs tend to hang loosely. Some monkeys, such as New World monkeys of the family Cebidae, have a prehensile tail that they use for gripping, swinging and climbing.

Humans are great apes

Hominoids are divided into the lesser apes and the great apes (Figure 11.6). The lesser apes (family Hylobatidae) comprise the gibbons, and great apes (family Hominidae) comprise the orangutans, gorillas, chimpanzees, bonobos and humans.

The division between the lesser apes and the great apes reduces to a key difference in the anatomy of the wrist bones. The wrist of lesser apes (gibbons) has a ball-and-socket joint, which permits the wrist to swivel and facilitates highly agile brachiation. Gibbons move by swinging briskly between tree branches (Figure 11.5a), and they can freely reverse their direction by pivoting on one wrist while in motion.

By contrast, great apes, including humans, have a gliding wrist joint. The joint moves up and down with limited twisting movement. Great apes do not practise brachiation in the same sense as gibbons do. Great apes more commonly use suspensory climbing and knuckle walking. They can also stand and walk on their hind legs to a limited extent. Humans are exceptional among the great apes by being exclusively bipedal.

EXAM TIP
Remember the key features that identify hominoids (absence of a tail) and hominins (bipedalism).

Humans are hominins

A **hominin** is any organism classified in the taxonomic tribe Hominini (Figure 11.6). The **tribe** occupies a taxonomic position between family and genus. The defining feature of hominins is that they are principally bipedal and walk on their hind limbs (Table 11.2). Humans are uniquely classified as hominins for this reason; however, they also have many features that distinguish them from other apes (Section 11.2, p. 433).

Table 11.2 Summary of defining features for taxonomic groups within which *Homo sapiens* is classified

Group	Defining features
Primates (Primata)	Hands and feet with five digits that include an innermost, opposable digit Forward-facing eyes enabling stereoscopic colour vision Enlarged cranium relative to body weight Flexible spine with considerable rotation about the hips and shoulders
Hominoids (apes – Hominoidea)	Distinguished from monkeys by lacking a tail Dentition includes eight premolar teeth Molars with five cusps and grooves in a Y-shaped pattern Broad, flattened rib cage Arms generally longer than legs (except for humans)
Great apes (Hominidae)	Gliding wrist joint with limited twisting movement
Hominins (Hominini)	Bipedal mode of locomotion

Delineating the human lineage

Within the Hominini, modern humans are classified in the genus *Homo* as the species *sapiens* (Figure 11.6). *Homo sapiens* is Latin for 'wise man'.

The scientific name was introduced by scientist Carl Linnaeus in 1758 and highlights a bias of reasoning that confounded taxonomy for centuries. Humans seemed so different from other great apes that they were placed in a separate taxonomic family. During the second half of the 20th century, the taxonomy was progressively amended. Humans were grouped together with the other great apes in a single family. By the mid-1980s, orangutans were regarded as the earliest divergence of the great ape lineage, based on morphology and molecular evidence. However, there was much debate about which two were more closely related among gorillas, chimpanzees and humans. As a compromise, for a time the three were regarded as equally related.

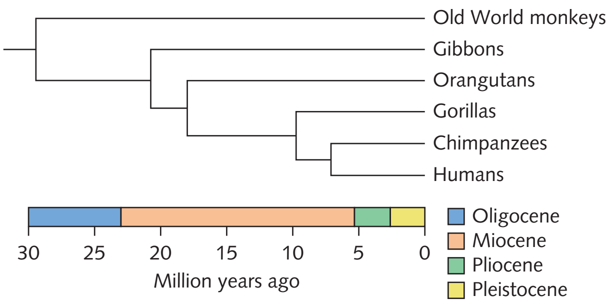

Figure 11.9 A phylogenetic tree depicting molecular clock estimates for divergences between major hominoid lineages

In the late 1980s, a clearer picture of the phylogeny of humans emerged with the first analysis of genetic data. This data showed that the gorilla lineage split first and that the chimpanzee and human lineages diverged later (Figures 11.6 and 11.9). This implies that humans and chimpanzees are the most closely related great apes. This phylogeny is now strongly supported by molecular homology of many gene sequences, as well as whole-genome sequences. Estimates for how long ago the human, chimpanzee and gorilla lineages diverged have been drawn from molecular clock estimates. These are derived from the predicted rate of mutation in a gene sequence. Molecular clock estimates tend to be controversial because they vary with the molecular data, statistical methods and calibration systems used. However, recent molecular clock estimates based on whole-genome analyses (Figure 11.9) indicate that the human and chimpanzee lineages diverged about 7.5 mya. The gorilla lineage is estimated to have diverged about 10 mya. These estimates may be refined in the future.

CONNECT

Refer to Chapter 10 for a discussion about molecular clock estimates.

KEY CONCEPTS

» In contrast to other primates, hominoids lack tails, have eight premolars rather than 12, and molars with five cusps rather than four.

» Hominins are uniquely bipedal.

» Chimpanzees are the living species that are most closely related to humans.

Concept questions 11.1c

1 Explain the difference between a hominoid and a hominin.
2 How many living hominin species are there?
3 Outline the evidence that suggests hominoids are more closely related to Old World monkeys than to New World monkeys.
4 Outline the evidence that suggests chimpanzees and humans are the most closely related great apes.

HOT Challenge

5 A palaeoanthropologist excavated a primate mandible. He observed that it had six premolar teeth and the molars each had four cusps.
  a Has he discovered a hominoid? Provide evidence to support your answer?
  b If he excavated the rest of the skeleton, what other anatomical feature might he find that would indicate it is, or is not, a hominoid?

11.2 Biological adaptations that define humans

Humans have the same basic characteristics as apes, which places them in the hominoid superfamily. Humans also have features that distinguish them from other hominoids, such as the anatomical features that allow humans to stand upright and walk with a fully striding gait. These fundamentally define humans as hominins. Humans are also relatively hairless, have a greatly expanded brain, and have modified teeth and jaws. Furthermore, humans behave very differently from other apes. They communicate with speech, display advanced intellectual abilities, apply sophisticated technology, and practise elaborate symbolism.

Adaptations for bipedalism

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Most hominoids are fundamentally quadrupedal – they use all four limbs for locomotion. Humans' closest living relatives, the gorillas and chimpanzees, mainly knuckle-walk (Figure 11.5c and d).

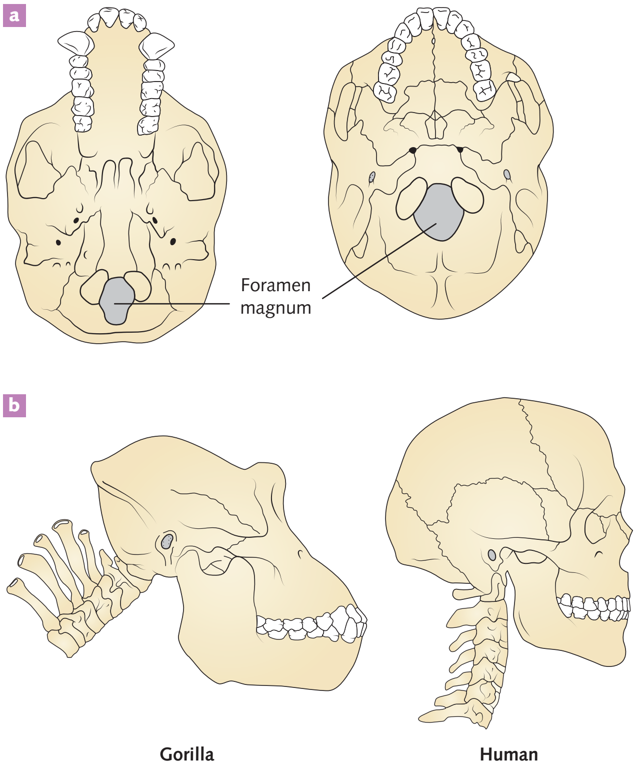

Figure 11.10 a The position of the foramen magnum (shaded) in the gorilla and the human. **b** The corresponding position of the spine and skull in each.

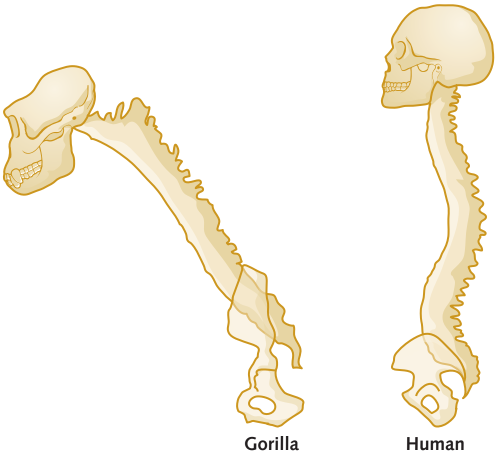

Figure 11.11 A gorilla's spine curves forward, whereas a human's spine is an S shape.

Humans are distinctly different because they stand upright and walk on their hind limbs. They are bipedal (Figure 11.5e). The shift to bipedalism has been accommodated by changes throughout the skeleton. No other animal is as adept as humans at full striding bipedal locomotion. From head to toes, the human body is configured for bipedalism.

Position of foramen magnum

In vertebrate animals, the spinal cord feeds through a hole in the skull called the **foramen magnum** to connect with the brain. The position of the foramen magnum in the base of the skull varies according to how the animal moves. In quadrupedal animals, such as gorillas and chimpanzees, the foramen magnum is towards the back of the cranium (Figure 11.10). This is because the spine is almost horizontal where the spinal cord enters the skull. In the fully bipedal human, the foramen magnum is positioned more centrally at the base of the skull (Figure 11.10). This permits the head to face forward comfortably while resting almost vertically over the spinal cord when upright.

Curvature of spinal column

The posture of modern humans contrasts with that of other apes. In apes such as gorillas and chimpanzees, the spine curves forward (Figure 11.11). Their body weight is evenly distributed by support from the forelimbs during quadrupedal locomotion (Figure 11.5c and d). However, the curve of the human spine follows an S-bend to support the weight vertically (Figure 11.11). The vertebrae in the lumbar (lower back) region of humans are wedge-shaped and pack together with the thin edges pointing backwards. This causes the lower spinal column to adopt a convex (forward) curve.

Pelvis

The lower spinal column connects with the pelvis. In most apes, the pelvis is relatively long and narrow (Figure 11.12a). However, in humans the pelvis is comparatively shallow and bowl-shaped (Figure 11.12b). This shape creates a basin that sustains the weight of the abdomen and provides support for the upper body. The broader hip bones also provide expanded attachment sites for the buttock muscles. The relatively enlarged buttock muscles of humans extend the leg and help steady the pelvis and upper body during walking.

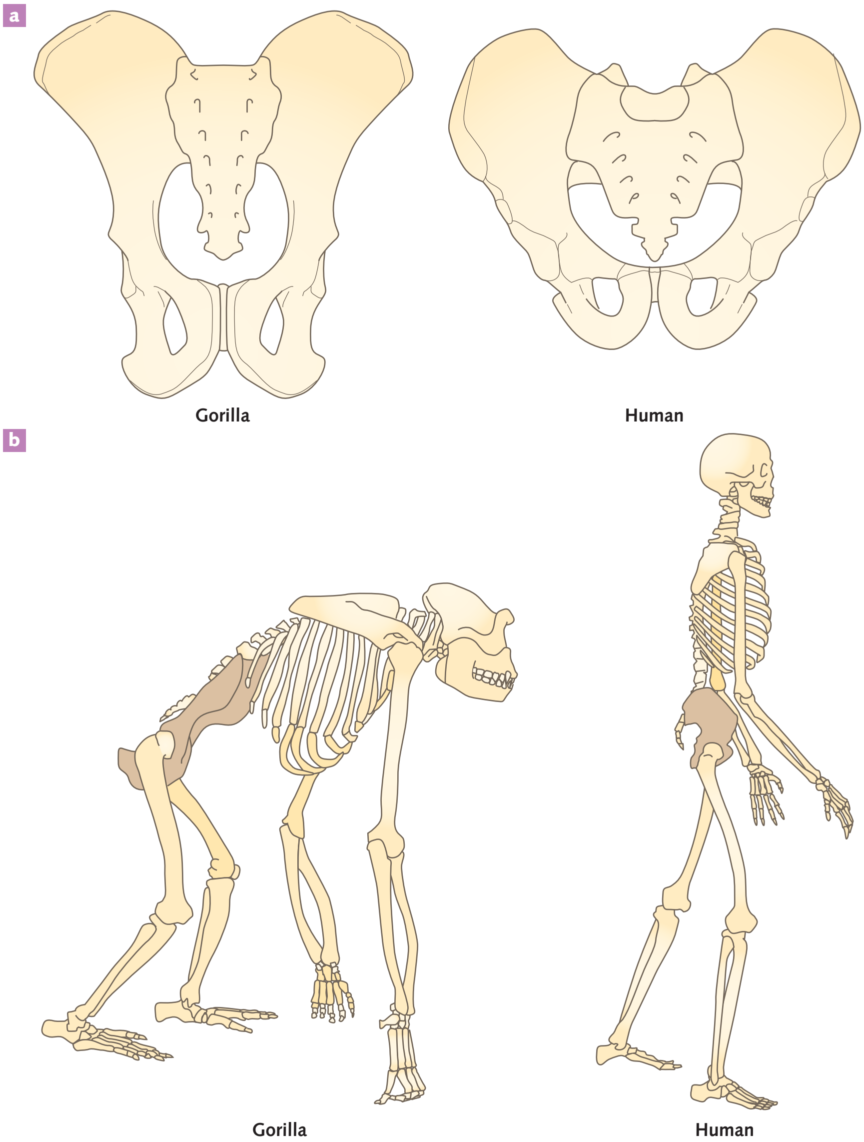

Figure 11.12 a **A comparison of the pelvises of the gorilla and human. b An illustration of the position and orientation of the pelvis during locomotion in gorillas and humans.**

Carrying angle

In humans, the flaring of the pelvis aligns the hip joints directly beneath the head and torso. Therefore, the weight of the upper body is transferred via the pelvis to the legs. However, the broad pelvis spreads the top of the femurs (thigh bones) away from the midline of the body. This is potentially destabilising. The body would have to sway from side to side during walking so one leg could support the body's weight. For this reason, the femurs are angled in towards the knees.

This **carrying angle** relative to the vertical is evident when viewed front-on (Figure 11.13). The angle ensures one knee and foot are directly beneath the body while the other knee and foot are lifted during walking. It allows the body to rotate about the lower leg and foot, and one foot to be set ahead of the other when striding. Orthograde movement of the arms compensates for body rotation. The knees are strengthened to support the weight at the lower end of the femur. This arrangement allows humans to extend the leg fully during walking. The longer legs of humans relative to other great apes increases the length of the stride overall.

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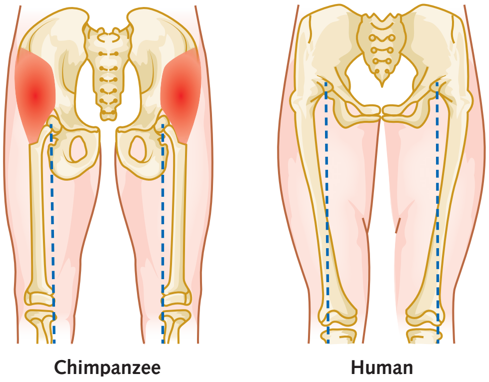


Figure 11.13 A comparison of the pelvis and femur of chimpanzees and humans, showing how the femur of humans is angled inwards towards the knee. The dotted line shows the direction weight is transmitted.

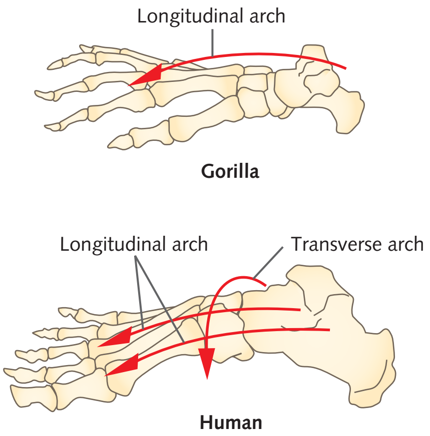


Figure 11.14 A comparison of the arches of a gorilla foot and a human foot

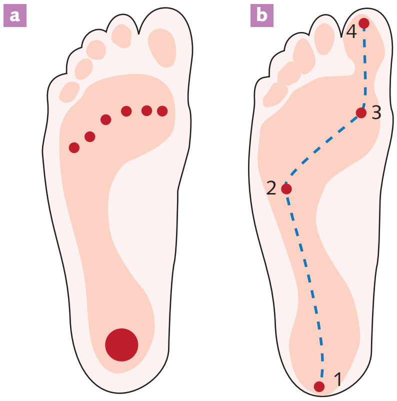


Figure 11.15 How body weight is distributed across the human foot when **a** standing and **b** walking. When walking, the body weight shifts in sequence from position 1 (heel strike) to position 4 (the big toe thrusts off).

Feet

Unlike other apes, humans use their feet mainly for propulsion rather than for grasping or climbing. As a result, the human foot has lost its prehensile capacity and the hallux aligns alongside the other toes. In addition, the human foot has a comparatively wide heel (Figure 11.14) that serves as a shock absorber upon heel strike. Apes' feet have a single longitudinal arch that runs from the back to the front of the foot. By contrast, human feet have two arches, one longitudinal and one transverse, which cross over the foot (Figure 11.14). When a human is standing erect, the foot acts as a pedestal to support the body's weight (Figure 11.15). When the human foot presses to the ground during walking, weight is transferred progressively forward and across the foot via the arches (Figure 11.15). With the weight transmitted to the toes, the big toe pushes off to launch the next step.

KEY CONCEPTS

» Many features of the human skeleton, including the skull, spine, pelvis, femurs and feet, have a range of adaptations for bipedalism.

Concept questions 11.2a

1 Describe the foramen magnum, its function, and what its position indicates about a hominoid's locomotion.
2 How does the shape of the human spine differ from that of other apes? Why is this the case?
3 What advantage is there in the shape of the human pelvis?
4 Define 'carrying angle'. Explain why it is different in humans and other apes.
5 What features does the human foot have that adapt it to walking rather than climbing?

HOT Challenge

6 Examine the following two ideas.
- In the 1980s, Rodman and McHenry, of University of California, Los Angeles, USA, suggested that hominids evolved to walk upright in response to climate change.
- In 2007, researchers studying chimpanzees determined that they required 75% more energy while walking than two-legged humans.

What trait of modern humans do these two ideas support?

Human hands

Bipedalism means human forelimbs do not have to carry the body's weight. Instead, hands have become adapted for manipulation. Compared with other primates, human hands are short and broad, with relatively short, straight fingers and a long, strong thumb. These adaptations allow the human thumb a substantial amount of freedom and the ability to extend to each of the fingertips. The degree of contact between the thumb and forefinger allows humans to grip and manoeuvre small or delicate objects, such as a needle for sewing or a pencil for writing (Figure 11.16). It is the basis of the **precision grip**, which enables humans to grasp and manipulate objects with exquisite dexterity.

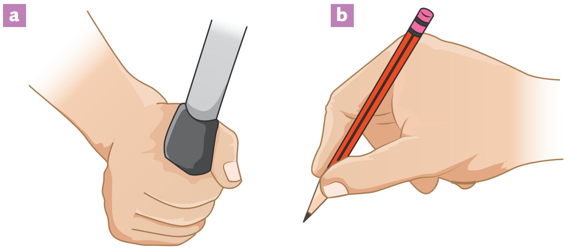


Figure 11.16 A comparison of **a** the power grip and **b** the precision grip

Expansion of cranium

Humans behave very differently from other apes. Humans are very inventive and technologically sophisticated. Humans have an unparalleled aptitude for manipulating and interpreting symbols. They are capable of complex **language**. They indulge in personal ornamentation, art and music. They establish extensive communities and observe societal conventions.

Compared with other apes, humans have an advanced **cognitive capacity**. Cognitive capacity describes an organism's innate intelligence, ability to learn, plan, evaluate, make decisions, and apply new knowledge and skills. Human cognitive capacity is largely due to their relatively big brain (Figure 11.17). Brain volumes of most apes vary between about 350 and 500 cm^3. The volume of human brains varies between 900 and 2200 cm^3 but the average is about 1350 cm^3. Most of the enlargement is associated with expansion of the cerebral cortex, the outermost region of the brain. The surface area of the primate cerebral cortex is further increased by extensive folding, called 'convolutions'. Convolutions are not random. Rather, they form specific patterns in the cerebral cortex of different species. Relative to brain size, the human cerebral cortex is estimated to have about 40% greater surface area than the chimpanzee cerebral cortex.

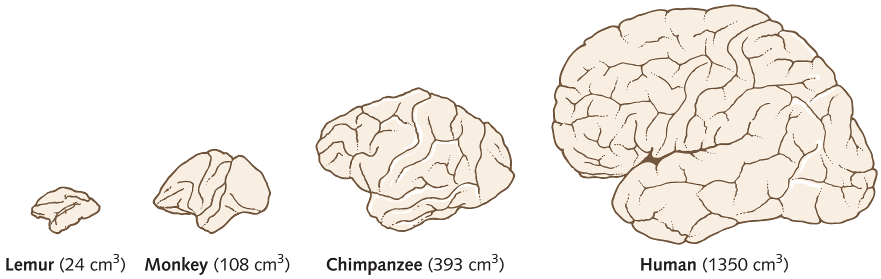
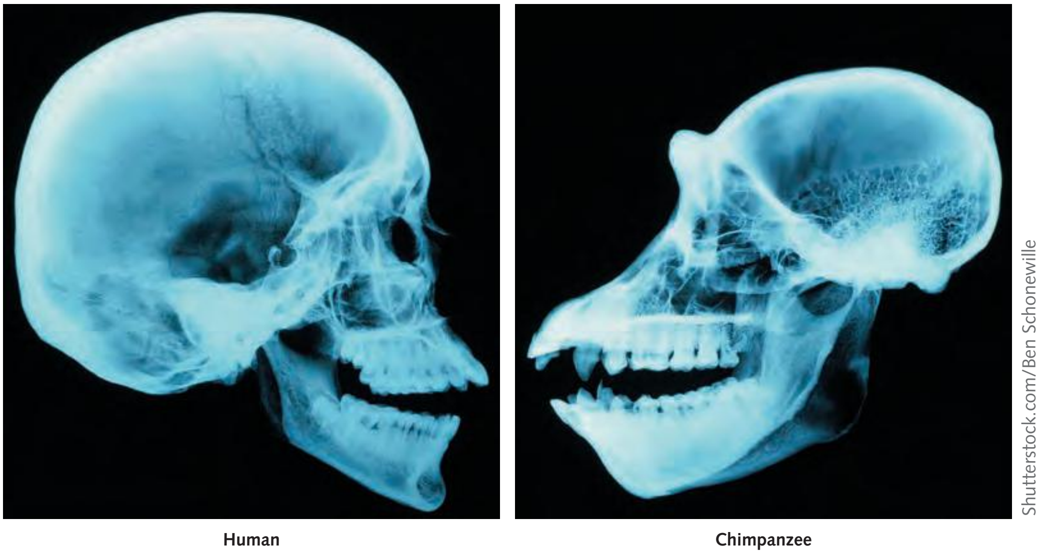


Figure 11.17 The brain sizes of various primates (drawn to scale)

There are also differences in how the brain operates. The cerebral cortex is associated with the executive functions of the brain, including reasoning, planning and judgement. The **prefrontal cortex**, which covers the front part of the brain, is the portion of cerebral cortex that has undergone the greatest expansion. The human prefrontal cortex is about six times larger than that of other apes. The prefrontal cortex governs a variety of functions, including abstract thinking, analysis of conflicting outcomes, and planning and strategising. It is also associated with complex social behaviours, such as impulse control and ethical choices.

The human cerebral cortex consists of more than 50 distinct regions based on cellular staining patterns. The difference between the human and chimpanzee genomes seems small, approximately 1% of total gene sequences. However, it is likely that changes to the genome have affected genes that affect brain development, anatomy and function.



Shutterstock.com/Ben Schonewille

Figure 11.18 Profile images of human and chimpanzee skulls showing differences in cranial capacity, brow ridges, jaws and teeth.

Modification of skull

To adapt to the extraordinary change in brain size, the human cranium has substantially changed during evolution (Figure 11.18).

An enlarged brain case accommodates the brain. **Cranial capacity** is a measure of the volume of the brain case. The shape of the cranium in humans is also altered. In the skulls of most apes, the forehead is sloped back. The **brow ridges** (the bony ridges above the eye sockets) are typically prominent. However, in humans the front of the cranium is higher and more rounded, and the brow ridges are significantly reduced. These changes accommodate the enlarged prefrontal cortex. The effect is the distinctively raised forehead of humans.

Chimpanzees and other apes have protruding jaws (Figure 11.18), a condition known as **prognathism**. In humans, the jaw does not protrude so far. Corresponding to the difference is the shape of the lower jaw, or **mandible**. The chimpanzee mandible has an extended rectangular shape, whereas the human has a shallower, parabolic mandible (Figure 11.19). Most apes have prominent, interlocking canine teeth, whereas human canines are reduced so that they appear similar to the incisors (Figure 11.19). It is proposed that the difference in canines relates to differences in behaviour rather than diet. The canine teeth are most conspicuous in male apes. Male apes exhibit their canines in competitive displays so as to avoid violent aggression.

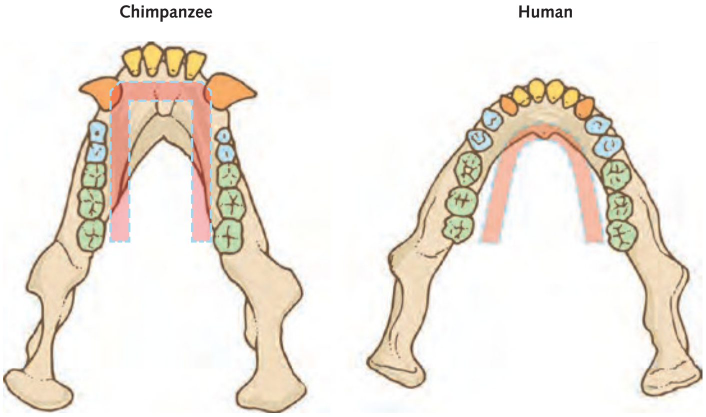


Figure 11.19 The mandible of a chimpanzee and a human. The pink shapes highlight the rectangular shape of the chimpanzee mandible and the parabolic shape of the human mandible.

Overall, the changes to the cranium and jaws have flattened the human face. The chin and prominent nose are distinctly human characteristics.

Table 11.3 summarises the anatomical features that distinguish humans from other hominoids.

Table 11.3 Summary of anatomical features that distinguish *Homo sapiens* from other hominoids (apes)

Feature	*Homo sapiens*	Other hominoids
Body covering	Relatively hairless	Relatively hairy
Mode of locomotion	Bipedal	Quadrupedal
Position of foramen magnum	Closer to the centre of the base of the skull	Closer to the rear of the skull
Spinal curvature	S-bend with convex curve near the base of the spine	C-shape forward curvature
Pelvis	Shallow and bowl-shaped	Long and narrow
Carrying angle of femur	Relatively high	Relatively low
Feet arches	One longitudinal and one transverse	One longitudinal only
Hallux	Not prehensile	Prehensile
Hand	Thumb long compared to fingers for precision grip	Thumb short compared to fingers
Cranial capacity	Relatively large	Relatively small
Brain	Expanded prefrontal cortex, many more convolutions	Smaller prefrontal cortex, fewer convolutions
Brow ridges	Subtle or absent	Prominent
Prognathism (jaw protrusion)	Subtle	Substantial
Mandible shape	Parabolic	Rectangular
Canines	Reduced	Enlarged

KEY CONCEPTS

- Humans have a precision grip that enables manual dexterity.
- The anatomy of the brain, including the enlarged prefrontal cortex and increased convolutions, enhances the cognitive capacity of humans.

Concept questions 11.2b

1 Describe how the anatomy of the human hand differs from that of other apes, and how it adapts the human hand for a precision grip.
2 Define 'cognitive capacity'.
3 Describe three ways the human brain evolved to become different from those of other apes.
4 Describe how the shape of the modern human skull differs from that of other apes, and how these changes accommodate the human brain.

HOT Challenge

5 Why are modern human canines reduced in size when compared with similar structures in other hominoids?

Communication, technology and culture

WB
11.2.3
HUMAN INGENUITY
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The most significant characteristics that result from human evolution are bipedalism and the expansion and development of the brain. As a consequence of bipedalism, the hands are also freed for fine manipulation. The result is an organism that combines an enhanced capacity to imagine and plan with the manual acuity to modify items and reshape its environment. These are the basic biological ingredients for developing tools and utilising technology. Culture is defined as the ideas, customs, and social behaviour of a particular people or society. Humans are the only species to show complex culture. Human culture has evolved as a consequence of human biological change.

Humans are social creatures, like many other primates. However, humans have a unique ability to communicate abstract ideas in detail. This has ensured that knowledge and ideas are transmitted freely between individuals. It is also the foundation for extensive cooperation, which accelerates the pace and scale of innovation. In this section, you will explore some of the behavioural adaptations that distinguish humans from other primates.


Alamy Stock Photo/Steve Bloom Images

Figure 11.20 These common chimpanzees (*Pan troglodytes*) are using sticks to 'fish' for termites.

Language: a mechanism for innovation

Humans are not the only animals to use tools. For example, chimpanzees use a kind of 'toolkit' for capturing termites (Figure 11.20). They use one stick with brush-like leaves to clear the entrance leading into the termite mound. They use a second stick deliberately stripped of leaves for 'fishing' the termites by inserting it into the hole. When the stick is withdrawn, it is covered in termites, which the chimpanzees lick off. Juvenile chimpanzees learn these techniques by watching and imitating the adults.

Humans also learn by imitating others. However, humans have a unique capacity to communicate far more complex and abstract ideas. Humans have the astounding ability to vocalise thousands of sounds, to attach meaning to each, and to reorganise them in expressive new sequences. Humans have the anatomy and neural wiring to coordinate the lungs, mouth, throat and nasal organs to make talking possible. Language provides a functional framework for human speech. As the utterings of infants demonstrate, humans are genetically predisposed to learn vocabulary and to order words according to strict grammatical rules. The language learned by any individual human is influenced by their environment. So if Chinese parents in China adopt a native-born German baby, the infant learns Chinese as his or her principal language.

Humans can write. They record symbols to represent spoken language. Writing allows humans to formalise, store and reference abstract ideas. The ability to manipulate and interpret symbols also allows humans to express meaning in fields such as music or mathematics (Figure 11.21). Spoken language is immediate. It transmits knowledge directly among people at a particular time. Written language preserves ideas for transmission to wider audiences, between generations, and over extended periods of time (Figure 11.21). For example, Charles Darwin's 19th century publications can still inspire biologists more than a century after his death. The result is a collective knowledge that accumulates over time. New ideas and technology can be developed by people today that are based on those of people who lived in another time and place.

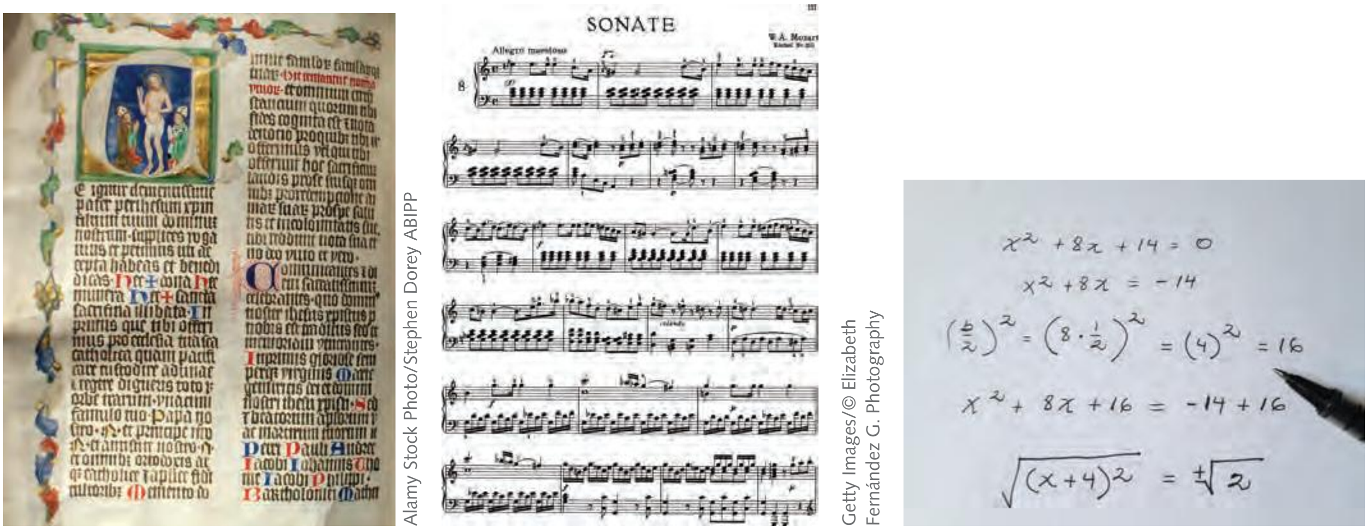

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Figure 11.21 Examples of written communication

Other animals, including chimpanzees, can recognise and communicate using sounds or abstract symbols. A famous case is that of Washoe, a common chimpanzee, who learnt to communicate with up to 200 symbols of American Sign Language. Washoe could string up to three signs into short expressions, and she set about teaching her adopted infant chimp the language. Chimpanzees in the wild also produce scores of vocalised sounds to communicate with one another.

The difference between humans and other animals is a matter of scale and sophistication. Vocabulary size varies from one person to the next, but it has been estimated that, by age 4, most humans know about 4000 words. For adults, it is suggested to be between 15 000 and 25 000 words. A standard English dictionary contains more than 170 000 definitions. The size and versatility of human language means we have an exceptional ability to convey abstract ideas. It enables us to describe models for concepts that are beyond our personal experience. The structure of the atom, principles in electromagnetic theory, supermassive black holes and evolution on geological time scales are all examples.

Cultural evolution

Humans' aptitude for communication has enabled knowledge to spread rapidly between individuals, throughout populations and between different populations. It also enabled individuals to organise themselves and work cooperatively in ever larger groups. Our physical, cognitive and communicative characteristics underpin the evolution of human culture. **Cultural evolution** describes the way human beliefs, social practices, skills and technology change over time.

Cultural evolution contrasts with biological evolution in the speed and means by which it is transmitted. The significant differences between cultural and biological evolution are summarised in Table 11.4. Biological characteristics are exclusively transmitted from parent to offspring. It normally takes many generations to observe biological changes in a population. However, culture can be transmitted rapidly between unrelated individuals of the same generation or of different generations. Cultural characteristics can be communicated informally (e.g. spoken word), formally (e.g. education) and even over long distances without the individuals concerned ever meeting each other (e.g. via books or the Internet). Consequently, cultural evolution occurs rapidly within the lifetime of an individual.

Table 11.4 Summary of differences between features of biological and cultural evolution

Feature	Biological	Cultural
Data coding	Genetic	In written, spoken or symbolic language
Transmission of traits	Inherited from parents. No choice in traits acquired	Communicated from unrelated individuals. Taught and learnt. Choice in traits acquired
Generation	From one generation to the next	Within or between generations
Speed of dispersal	Slow. Many generations required to spread trait in population	Fast. Spreads rapidly in population by immediate learning
Intent	None. Unplanned, resulting from random processes	Deliberate, result of conscious action

KEY CONCEPTS

- Written and spoken language enables humans to convey abstract concepts.
- Cultural evolution is distinguished from biological evolution by the speed, the means and the choice exercised in its dispersal.

Concept questions 11.2c

1 Define 'cultural evolution'.
2 List five ways cultural evolution contrasts with biological evolution.
3 Explain whether the following represent cultural or biological evolution.
  a Invention and subsequent miniaturisation of mobile phones
  b Changes in hairstyles in a population
  c Increasing uptake of the vegan diet in a population
  d Increasing resistance to malaria over many generations
4 How does written language contribute to cultural evolution?

HOT Challenge

5 Gunditjmara country is found around Lake Condah in south-west Victoria. In 1841, the Chief Protector of Aborigines, George Augustus Robinson found an extensive area of channels purposely built by the local Indigenous people. He surmised that the channels were used for catching eels. Shortly after this, other settlers determined that this area was only a swamp that needed to be drained and used for sheep grazing. In the 1970s, archaeological surveys revealed the actual complexity of the constructions and the efficiency of the channels' operations. This implied that the channels were an advanced fish-trapping system that supplied the people with a year long supply of food. In 2019, the area was recognised as a UNESCO World Heritage site to be listed exclusively for its Aboriginal cultural values as one of the finest examples of ancient aquaculture and hydraulic engineering. Three hundred stone houses have also been found at the site.
Is this an example of cultural evolution? Justify your response.

11.3 Meet the ancestors

Video
Four billion years of evolution

Online Worksheet
Myths and misconceptions about evolution

When examining fossils, scientists are often trying to deduce the morphology, lifestyle and behaviour of an extinct organism from a few fragments. To do this, they compare fossils with the skeletal structures of living organisms. Knowing the living organism's appearance and lifestyle allows scientists to infer some things about the appearance and behaviour of the extinct organism. The biological classification scheme of human evolution is based on the human fossil record. As humans are terrestrial, there are few complete skeletons, as there is no rapid burial in sediment, decomposition of body remains is high, and bones are often scattered. Excavation by paleontologists is slow and many fossils are yet to be discovered. This has resulted in different interpretations of human evolution, and changes being made based on more recent evidence from fossil discoveries, as well as from evidence from new molecular biology techniques now employed for studying DNA.

Hominin classification includes species of fossil great apes that have similarities with modern humans. Therefore, as a taxonomic group, hominins are modern humans and their extinct bipedal ancestors. The hominin fossil record (Figure 11.22) demonstrates that human evolution was not a simple, linear progression from one species to the next. Instead, many human species coexisted at one time. Some may have been competitors; others may have occupied different niches. Some species persisted for a long time; others less so.

Palaeoanthropologists draw lines that connect these species into an evolutionary tree. What that tree looks like is inevitably contentious because interpretations vary among scientists. New fossil discoveries occasionally challenge existing hypotheses. However, it is evident that the hominin evolutionary tree has many branches, and all but one of those branches terminates in extinction. Modern humans are the last living legacy of this rich evolutionary history.

In this section we review specific hominins and what they reveal about the course of human evolution. To establish the trends, we will consider fossil hominins mostly in the chronological order of their appearance in the geological record. The oldest of these were recovered in Africa but many more recent ones have been found across Europe and Asia (Figure 11.23). This observation reveals an African origin for the hominins, followed by subsequent migration.

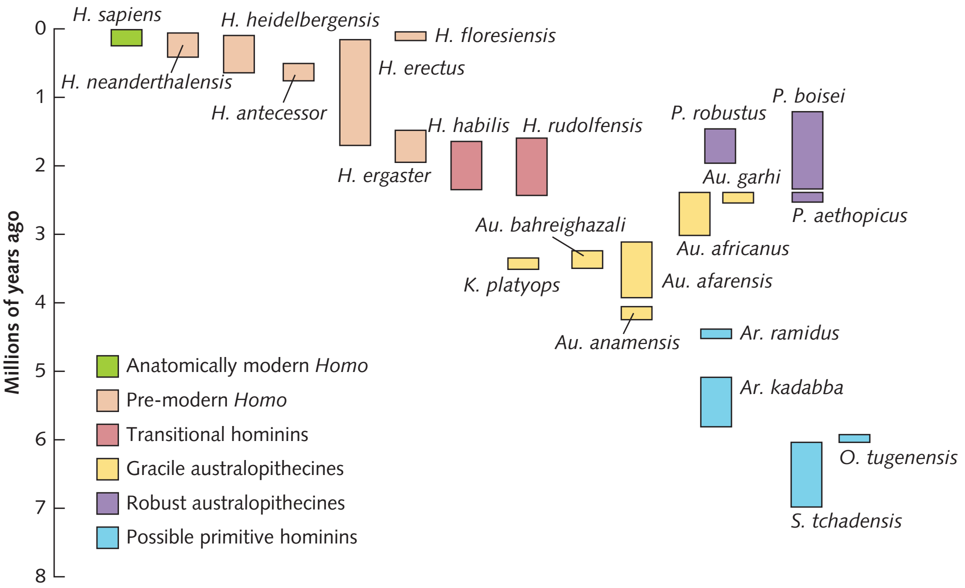


Figure 11.22 Species recognised in the hominin fossil record under the genera *Homo* (H.), *Ardipithecus* (Ar.), *Australopithecus* (Au.), *Kenyanthropus* (K.), *Orrorin* (O.), *Paranthropus* (P.) and *Sahelanthropus* (S.). The rectangles represent an estimate of the periods in the geological record during which the species are presumed to have lived, based on fossil evidence.

Australopithecines

The fossil record for hominins older than about 4.2 million years is limited. There are fossils of possible hominins that date to about 7 mya (Figure 11.22) but their identity and significance as hominins are frequently debated. The earliest universally accepted hominin fossils, those from which modern humans evolved, are the **australopithecines**. These are a varied group of small, bipedal apes that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya. They were evolving during a time of climatic change accompanied by a shift from forests to wooded grasslands. During that period, the australopithecines flourished and diversified into a number of species. The species are distinguished by their morphology as slender **gracile** forms or stocky **robust** forms. There are five generally recognised gracile species, which are classified in the genus *Australopithecus*. The robust forms are placed in the genus

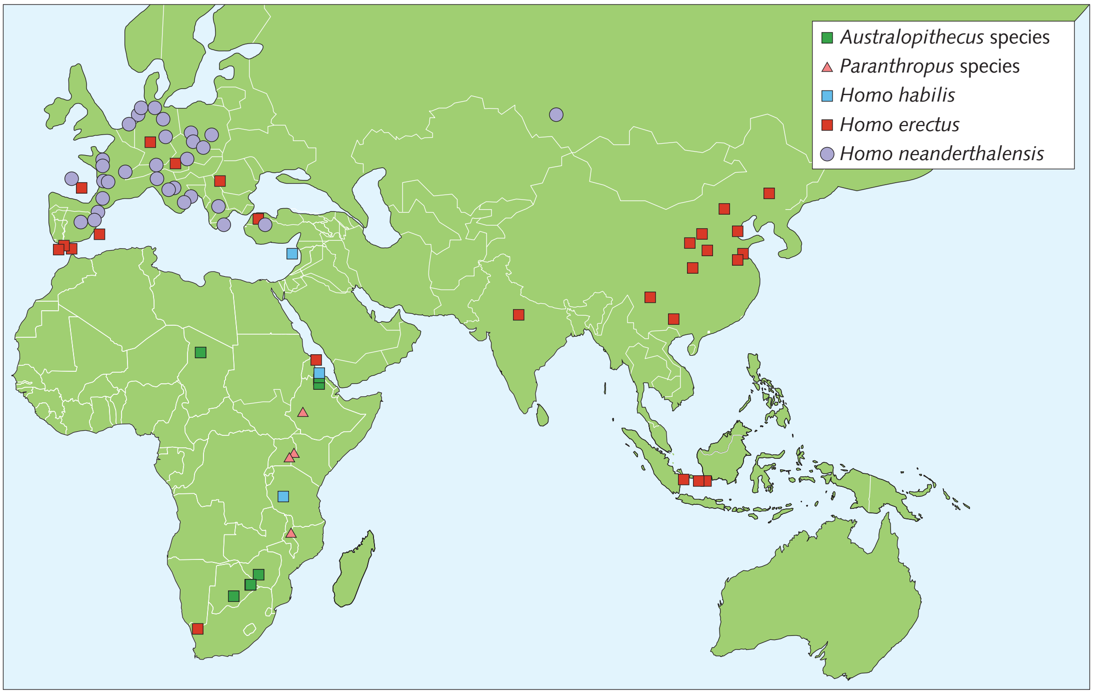
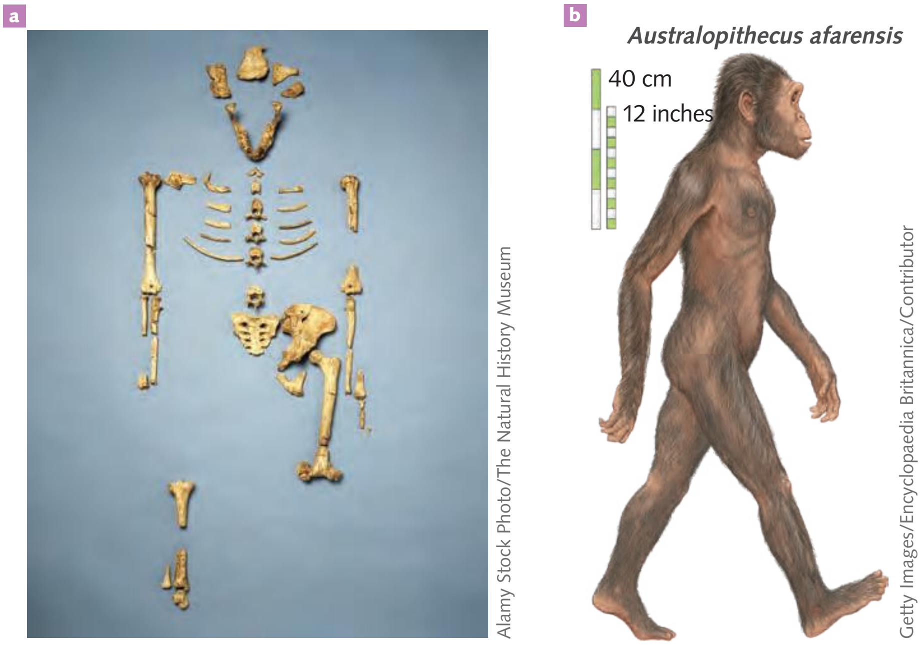


Figure 11.23 Location of major hominin fossil finds

Paranthropus, which contains three accepted species (Figure 11.22). Some australopithecines, such as *Australopithecus afarensis*, are widely regarded as direct ancestors of modern humans. A number of others are considered evolutionary dead-ends.

Archetype for genus *Australopithecus: Au. afarensis*

Australopithecus afarensis was discovered by chance in 1974 when paleoanthropologists were surveying potential excavation sites in the Afar region in Ethiopia. The specimen eventually unearthed and reconstructed to 40% completion was nicknamed 'Lucy' (Figure 11.24). Now known from hundreds of fossils collected in eastern Africa, *Au. afarensis* is considered a key australopithecine. The species persisted 3.8–2.9 mya and is believed to be a direct ancestor of modern humans. Like other australopithecines, the species displayed **sexual dimorphism**, with adult males significantly bigger than females. Males bore a **sagittal crest** at the top of the skull. Male gorillas also have a sagittal crest, which provides expanded surface area for the uppermost attachment of their powerful jaw muscles. By implication, the male *Au. afarensis* had a strong bite.



Alamy Stock Photo/The Natural History Museum

Getty Images/Encyclopaedia Britannica/Contributor

Figure 11.24 ***Australopithecus afarensis*: a the specimen nicknamed 'Lucy'; b an artist's impression of the organism.**

As a model for the genus, *Au. afarensis* clearly showed bipedal features (Figure 11.25). It had a relatively wide and shallow pelvis, femurs angled in towards the knees, strengthened weight-bearing knees, arched feet, wide heels, and a hallux aligned with the other toes. The interpretation was substantiated by a discovery of a trace fossil in 1978 near Laetoli in Tanzania. This was a set of fossilised footprint impressions in a volcanic ash bed laid down some 3.6 mya (Figure 11.26). Attributed to *Au. afarensis*, they show the tracks left by two adults walking one in front of the other and a juvenile walking beside them. These provided direct evidence that *Au. afarensis* was capable of bipedal locomotion. Yet *Au. afarensis* also had relatively long forearms, long curling fingers and toes, and shoulder blades akin to those of other great apes rather than modern humans. These adaptations indicate *Au. afarensis* was a proficient tree climber. The collections of features suggests this species lived in mixed habitats that included forest and grassland, and it both climbed and walked. Microanalysis of fossil teeth indicates its diet consisted mainly of leaves and fruits.

For its body size, *Au. afarensis* had a relatively small cranial capacity (about 430 cm^3). The cranium of its successor, *Au. africanus*, was only marginally larger (about 480 cm^3). In fact, relative to their estimated body masses, the brains of australopithecines were comparable in size to those of modern chimpanzees.

The australopithecines demonstrate that bipedalism preceded expansion of the cranium during hominin evolution.

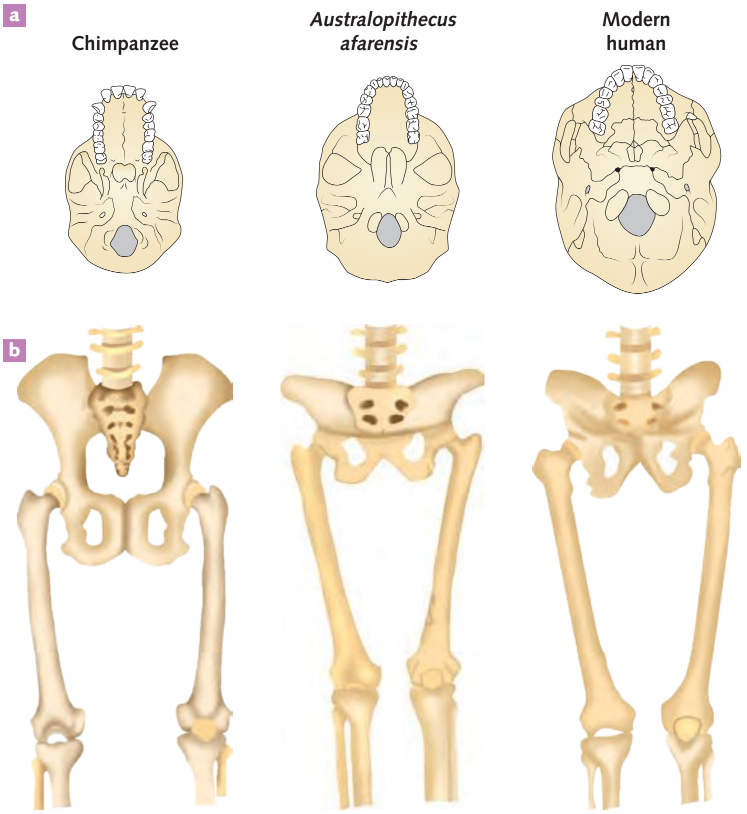


Figure 11.25 *Australopithecus afarensis* showed bipedal features. **a** The position of the foramen magnum was intermediate between that of the chimpanzee and modern human. **b** It had a bowl-shaped pelvis and carrying angle more like those of the modern human.

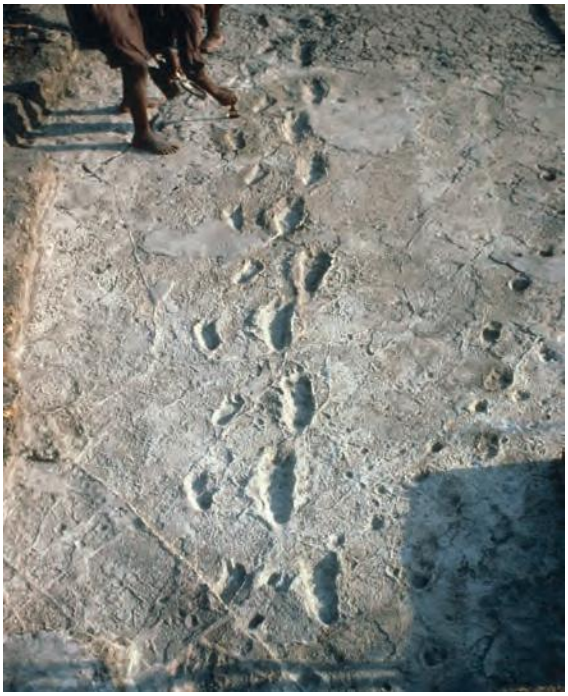

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Figure 11.26 The Laetoli footprints in Tanzania provide evidence that australopithecines were bipedal.

EXAM TIP

For written responses, make comparative statements when describing trends in human evolution. For example, write ‘the foramen magnum is more centrally located than in other hominoids’ rather than ‘position of the foramen magnum’.

Genus *Paranthropus*

The name *Paranthropus* is derived from two Greek words that mean 'beside human'. The 'robust' australopithecines are classified in *Paranthropus*. 'Robust' in this case refers not only to their heavier build but also to their extremely large jaws, premolars and molar teeth (Figure 11.27). One species, *P. boisei*, discovered in Ethiopia in 1959, was nicknamed 'nutcracker man' because it was assumed their strong jaws and large molars were used for crushing and grinding hard, fibrous foods, such as nuts. More recent microwear evidence of fossil teeth suggests the diet of *P. boisei* was much more varied, and nuts were not a staple food item. Despite the striking fossil evidence for *Paranthropus* skulls, there is limited evidence for the remainder of the skeleton. The little that has been recovered for *P. boisei* shows the pelvis and hip joint to be similar to members of genus *Australopithecus*. This suggests it was bipedal but does not prove that walking was its main mode of locomotion.

Paranthropus species persist in the fossil record from around 2.5 to 1 mya. From their age and anatomical features, scientists consider the accepted *Paranthropus* species to be descendants of *Australopithecus* or an undiscovered *Paranthropus* species. Whatever the origin, scientists agree that *Paranthropus* is an extinct side branch to the direct line from which modern humans evolved.

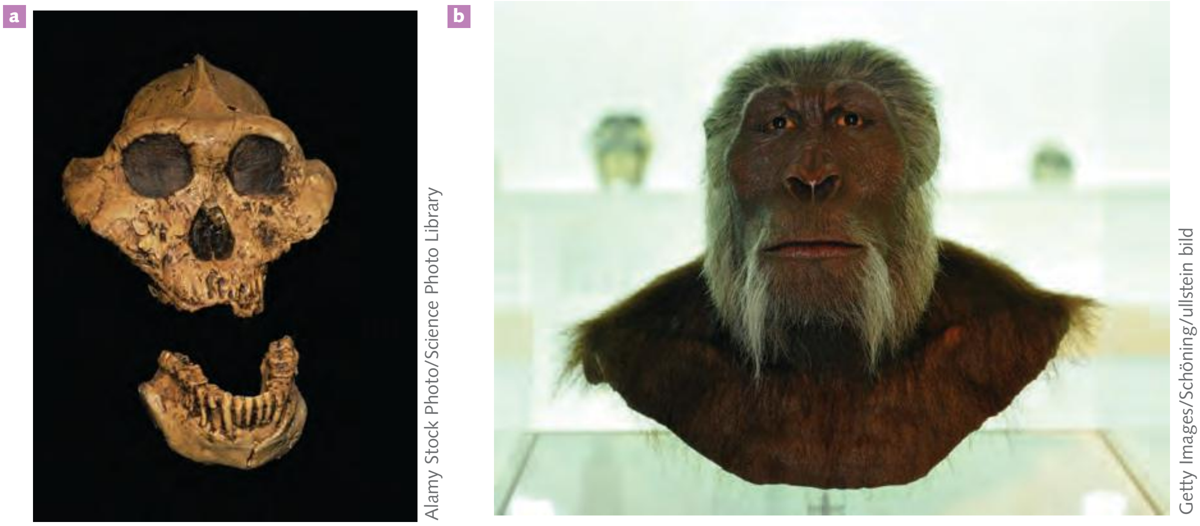

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Figure 11.27 ***Paranthropus boisei*****: a a fossil skull showing the prominent mandible and the sagittal crest along the midline of the cranium; b an artist's reconstruction.**

Science Photo Library/Javier Trueba

Figure 11.28 Samples of Oldowan tools associated with australopithecines

Oldowan technology

Australopithecines apparently occupied home bases from which they ventured to forage for food. There is no evidence for fire use among australopithecines. Stone tools, such as choppers, scrapers and chisels, have been recovered in areas where australopithecine fossils are found. These relatively simple tools are known as Oldowan technology, named for the site in Tanzania where they were first discovered. Most implements are about the size of a tennis ball or smaller (Figure 11.28). Oldowan tools were sculpted to shape by striking the tool stone with a 'hammerstone' to chip off flakes. Although relatively simple, Oldowan tools demonstrate that their makers were fashioning materials towards an imagined end product. They were also using precision grip to carry out the work. Oldowan tools represent the first stage of technological evolution in hominins, presumably enabling australopithecines to exploit their environment more effectively.

Oldowan tools date from around 2.5 to 1.2 mya. They are found along the east coast of Africa and throughout the Old World. These observations suggest Oldowan technology was migrating with early hominins. They also show that cultural evolution was underway. The technology was transmitted across massive geographical areas over many generations.

KEY CONCEPTS

- Hominin evolution is represented by a 'bushy' evolutionary tree. Interpretations about the fossil record are subject to ongoing debate and refinement as new discoveries are made.
- Australopithecines were relatively small, bipedal apes.
- Australopithecine species are grouped into the genera *Australopithecus* (gracile) and *Paranthropus* (robust).

Concept questions 11.3a

1. What does it mean if a species is sexually dimorphic?
2. What was the evolutionary fate of genus *Paranthropus*?
3. *Australopithecus afarensis* shows a range of features that suggests it was both arboreal and bipedal. Defend this statement with evidence.
4. What does the craftsmanship and distribution of Oldowan stone tools indicate about hominin evolution?

HOT Challenge

5. What evidence is there that *Paranthropus* is an evolutionary side branch and not a direct ancestor of modern humans?

Evolution of genus *Homo*

The evolution of genus *Homo* is associated not only with refinements to bipedalism but also with expansion of the cranium (Figure 11.29). There are 11 species classified in genus *Homo*, including *H. sapiens*. The composition of genus *Homo* is continually being revised. For example, a new species, *H. luzonensis*, was added in 2019 after the discovery of ancient skeletal remains in the Philippines. In addition, the validity of a few revisions is often questioned. Some argue that one or other of the existing species should be split into more species. Three species highlight what is understood and what is debatable about the course of hominin evolution.

A transitional fossil: *Homo habilis*

Homo habilis is described mainly from fragments of skull (Figure 11.30a), hand and arm bones discovered in Tanzania in 1960. Dating to 1.8 mya, *H. habilis* is one of the earliest fossil hominins verified to show an increased cranial capacity (Figure 11.29), although its total volume was less than half that of modern humans. It was also the earliest hominin to be found unequivocally associated with Oldowan stone tools, providing direct evidence for the use of technology. These features were used to justify its placement in genus *Homo* and it was accordingly named *H. habilis*, or 'handy man'. However, some scientists contest this placement because selected fossils assigned as *H. habilis* have arm and leg dimensions resembling those of australopithecines.

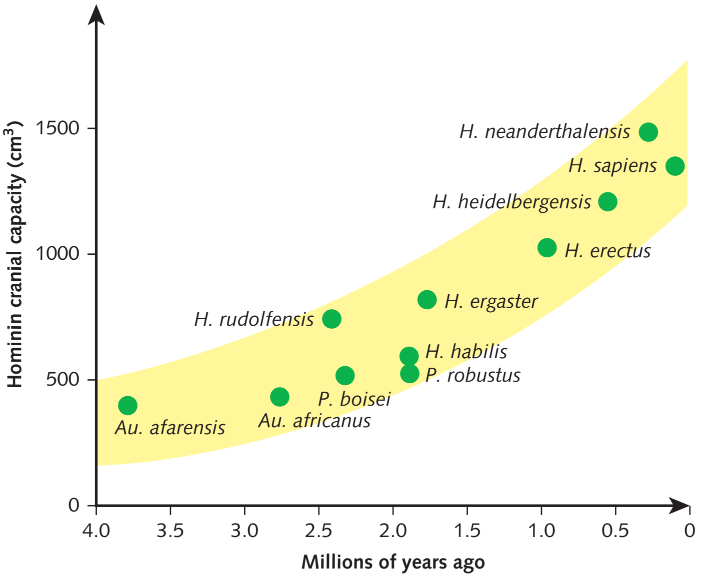
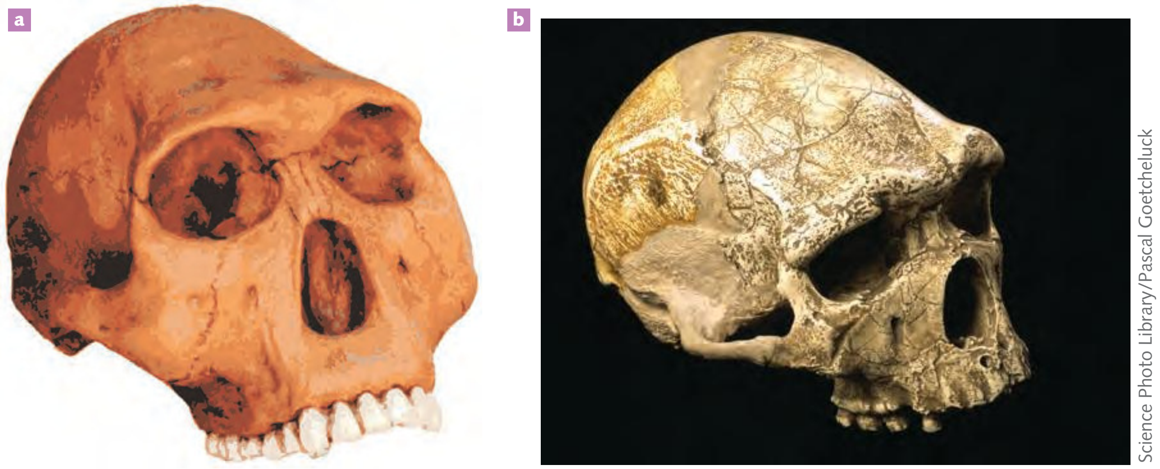


Figure 11.29 The gradual increase in cranial capacity of hominins over time



Science Photo Library/Pascal Goetcheluck

Figure 11.30 Detail of skulls from **a** *Homo habilis* and **b** *Homo erectus*. Note the sagittal keel (thickened midline) on the *Homo erectus* skull.

Alamy/Sabena Jane Blackbird

Figure 11.31 A relatively complete skeleton of *Homo erectus*

H. habilis is currently interpreted as a transitional fossil that shows features of both the australopithecines and the genus *Homo*. *H. habilis* used stone tools, which indicates a significant advance in the cognitive abilities of hominins.

Homo erectus

H. erectus (Figure 11.30b) is the first *Homo* species to resemble modern humans. *H. erectus* is the earliest hominin that combines modern human dentition, fully upright posture, obligatory long-range bipedalism, and at least a middle-sized brain.

The **postcranial** (all the skeleton except the skull) anatomy (Figure 11.31) indicates that *H. erectus* was a dedicated biped capable of walking long distances and running, if necessary. Its fossils are found throughout Africa, Europe and predominantly in Indonesia and China. This indicates *H. erectus* is the earliest known hominin to migrate out of Africa, dispersing across the Old World by about 1.5 mya. In China, repeated cooling and drying through multiple glacial periods encouraged the establishment of grasslands, attracting large grazing animals that *H. erectus* might have hunted.

The cranial capacity of *H. erectus* was greater than that of the australopithecines and *H. habilis* (Figure 11.29), suggesting further evolution of cognitive abilities. Skulls had thickened midlines referred to as a **sagittal keel**. Hands were no longer used for climbing but had become more refined for manipulating objects. Stone tools have been found with *H. erectus* fossils in western Asia, Europe and Africa. Their smaller teeth indicate that their diet had changed in some way compared with that of the australopithecines. *Homo* species were possibly eating different foods or preparing the same foods differently; for example, by cooking them before eating them. Deer, antelope, boar and fish bones found at various sites indicate some of the prey items of *H. erectus*. Burnt stone and animal bones, charcoal and ash deposits dating to about 0.5 mya suggest *H. erectus* used fire (Figure 11.32), but it is difficult to prove that they could control fire.

Acheulean technology

The stone tools found with *H. erectus* fossils are characterised mainly by tear-drop or pear-shaped hand axes. The tools are known as Acheulean technology, after the site in France (St Acheul) where they were first discovered. The axes are 12–20 cm long and are crafted by chipping on both faces of the stone (Figure 11.33). These tools appear in the fossil record from around 1.6 mya to 200 000 years ago (200 kya) (**kya** = thousands of years ago).

Science Source/Publiphoto

Figure 11.32 An artist's impression of a *Homo erectus* campsite. Males in the foreground use fire to fashion spears, while a female and a male in the background skin an animal.

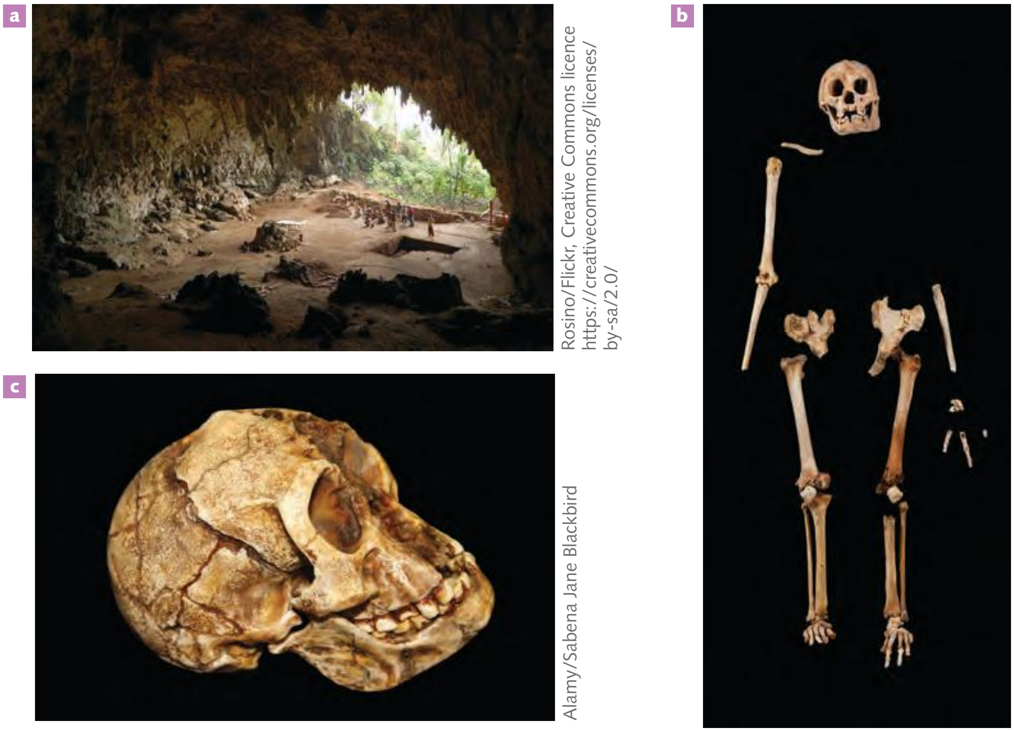
Getty Images/Prisma/UIG

Figure 11.33 Acheulean hand axes associated with *Homo erectus*

Homo floresiensis

WB
11.3.1 FROM *AUSTRALOPITHECINES* TO *HOMO SAPIENS*
PAGE 239

Occasionally, a new fossil discovery challenges existing assumptions about hominin evolution. For example, *Homo floresiensis* is widely regarded as the most surprising fossil hominin find in decades. *H. floresiensis* was discovered in 2003 by a joint Australian–Indonesian team. Skeletal remains unearthed in Liang Bua cave on the island of Flores in Indonesia (Figure 11.34a) were dated to 100–60 kya. The most important specimen, dubbed 'the Hobbit', is the unfossilised skeletal remains of an adult female (Figure 11.34b,c). At just 1.1 metre tall, the Hobbit was a diminutive hominin. Associated with ample stone tool artefacts, *H. floresiensis* evidently hunted and processed island animals. Charred bones demonstrate that *H. floresiensis* used fire for cooking.



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Alamy/Sabena Jane Blackbird

Chip Clark, Smithsonian, Human Origins Program

Figure 11.34 a Liang Bua cave on the island of Flores, the excavation site where *Homo floresiensis* was unearthed. **b** The skeleton and **c** detail of the skull of the *H. floresiensis* specimen dubbed 'the Hobbit'.

It is not known how *H. floresiensis* arrived on Flores. Even with the sea level changes occurring in the last million years, Flores was never connected to mainland Asia and is separated by tens of kilometres of sea. Chance colonisation by drift rafting is a possibility. Archaeological evidence indicates *H. floresiensis* occupied Liang Bua cave from at least 190 kya until 50 kya. Stone tools older than 800 kya have also been found on Flores and may belong to *H. floresiensis* or an earlier hominin. The disappearance of *H. floresiensis* broadly correlates with the timing of modern humans' arrival on Flores. However, there is no direct evidence that the two species interacted.

The discovery of *H. floresiensis* upset the field of **palaeoanthropology** (the study of fossil hominins) for a couple of reasons. First, the remains were originally dated to 38–18 kya, suggesting that *H. floresiensis* coexisted with modern humans until relatively recently. That suggestion was rejected in 2016 after the dating evidence for the remains and surrounding deposits was re-examined. The remains are now considered to be older, 100–60 kya.

Second, and more compelling, the origin of *H. floresiensis* is mysterious. One possibility is that *H. floresiensis* evolved from *H. erectus* that had migrated into Asia. This interpretation recognises similarities in the shapes of their skulls, particularly the brow ridges and sagittal keel. If true, *H. floresiensis* must have evolved to become smaller after its ancestors settled on Flores. Examples of 'insular dwarfism' have occurred on other islands around the world. Dwarfism presumably evolves in colonising species that experience long-term isolation with a restricted food supply and limited predators. The smaller cranium of *H. floresiensis* (about 400 cm^3) (Figure 11.34c) may be such an evolutionary adaptation to reduce the brain's energy demand. This proposal is supported by fossils of other extinct miniature species on Flores, such as those of *Stegodon*, an unusual form of pygmy elephant.

An alternative proposal for the origin of *H. floresiensis* is drawn from the primitive features of its body. *H. floresiensis* had relatively long arms and short legs with long feet, which are more like those of australopithecines. This interpretation is supported by australopithecine features of the wrist, hip and collar bones. It may be that *H. floresiensis* descended from an australopithecine or a *H. habilis*-like ancestor and always was of a comparable size to them. If this were the case, *H. floresiensis* could have initiated the earliest independent migration of hominins out of Africa. Whatever its origin, *H. floresiensis* ultimately represents an extinct side branch to the direct line of human evolution.

KEY CONCEPTS

» Evolution of the genus *Homo* is associated with expansion of the cranium, as well as enhancements in bipedal evolution.

» New fossil discoveries (e.g. *Homo floresiensis*) challenge existing assumptions about hominin evolution.

Concept questions 11.3b

1 What evidence demonstrates that the cognitive abilities of hominins had advanced with the evolution of *Homo habilis*?

2 What do the postcranial anatomy and global distribution of fossils indicate about bipedalism in *Homo erectus*?

3 What does its dentition suggest about the *Homo erectus* diet? What other evidence may support or refute that assertion?

4 Outline two aspects of *Homo floresiensis* anatomy that argue for contradictory origins.

HOT Challenge

5 What evidence is available to support the idea that the *Homo* species ate different foods from the *Australopithecines*?

11.4 Modern humans and Neanderthals

The first formal record of fossil hominins to be excavated was in 1856 in north-west Germany. The fossils were initially mistaken for the remains of a bear. They would famously come to be known by the name of the valley in which they were exhumed: Neanderthal. The discovery launched the field of palaeoanthropology and ignited public imagination. An icon of pop culture, the Neanderthal was rendered as a 'dull-witted prehistoric brute' (to paraphrase the early 20th century palaeontologist Marcellin Boule). No other ancient hominin has received as much attention or fascination.

There are many questions about the relationship between Neanderthals and modern humans. Why did the Neanderthal disappear while modern humans survived? Did Neanderthals and modern humans coexist peacefully or were they competitors, or adversaries? Did they interbreed? Are modern humans descended from Neanderthals? To answer these questions we will draw on a range of evidence, from over a century and a half of palaeontology to the most recent developments in biotechnology.

Fossil evidence for *Homo neanderthalensis*

The thousands of Neanderthal fossils that have been recovered reveal a hardy, resourceful people, an image that is at odds with the pop culture stereotype.

The distinctive facial appearance of Neanderthals arises from the enlarged brow ridge, sloping forehead and expanded nose. They also had a larger average cranial capacity than modern humans, approximately 1485 cm^3. The enlarged portion of the Neanderthal cranium is at the rear, associated with the visual cortex of the brain. Together with relatively larger eyes, these features suggest Neanderthals were equipped especially for vision in low light, which may have been an advantage in northern European latitudes. Neanderthals were stockier than modern humans, and had a flared rib cage, accommodating an expanded abdomen, and shorter limbs (Figure 11.35). Well-formed muscle attachments indicate strong muscular bodies and a strenuous lifestyle. Neanderthals had to survive episodes of glaciation, so some scientists interpret these features as adaptations for conserving heat in a cold climate. However, others disagree. For example, it was accepted for a long time that the broader nose was associated with a larger

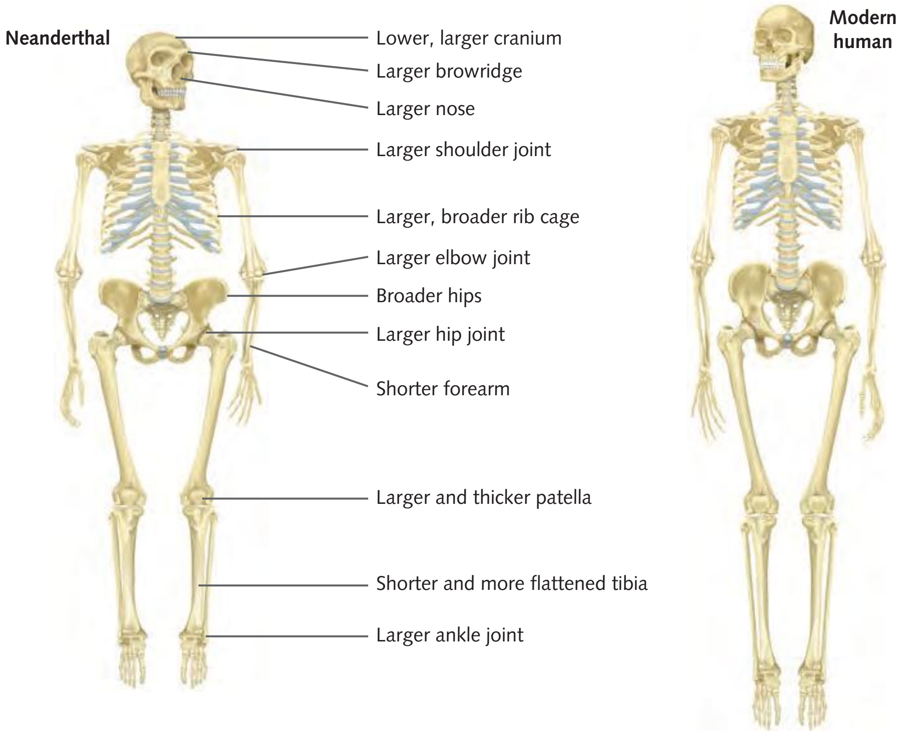

Figure 11.35 A skeletal reconstruction of *Homo neanderthalensis* and modern *Homo sapiens*

sinus cavity, enabling a greater volume of air to be warmed during inhalation. This view was contradicted by 2D X-ray analysis of skulls that revealed Neanderthal sinus cavities are similar in size to those of modern humans.

An alternative hypothesis is that the unique Neanderthal morphology arose by genetic drift in a relatively small, sparsely distributed population.

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INVESTIGATION 11.1

Hominoid skull analysis

Aim

To analyse various hominoid skulls and explore various anatomical adaptations that have emerged in hominids over their evolution

Time requirement

45 minutes

Materials

- Models of skulls from:
  - *Pan troglodytes* (chimpanzee) (modern)
  - *Gorilla* (gorilla) (modern)
  - *Homo sapiens* (human) (modern)
  - *Homo neanderthalensis* (Neanderthal human) (120 000–30 000 years ago)
  - *Homo erectus* (upright human) (2.0 million years ago)
  - *Australopithecus boisei* (2.3–1.2 million years ago)
  - *Australopithecus afarensis* ('Lucy') (4.0 million years ago)
- Tape measure (in millimetres)

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Skulls may have sharp edges.	Handle with care and do not run your fingers over skull teeth.

Method

Examining the brain case

For each skull:

1. Examine the frontal bone (forehead) of each of the skulls and determine whether they appear more vertical or flatter. Ensure that the skull eyes are oriented forward while doing this.
2. Examine above the eye socket and determine whether a supraorbital (brow ridge) is present. If so, see if the brow ridge is continuous or divided in the middle.
3. Measure the width of the brain case at the widest point. Make all measurements in millimetres.
4. Look for evidence of a sagittal crest running lengthwise along the midline of the top of the skull. Identify whether it is prominent, present or absent.
5. Measure the distance between the front teeth and the front ridge of the foramen magnum.
6. Examine behind the ear of the skull and determine if the mastoid process is fairly flat or noticeably protruding.
7. Copy Results table 1 into your logbook. Add extra rows as required. Record the results of your observations.

Examining the facial structure

For each skull:

1. Position the skull so that it is facing you. Examine the nasal bones. Identify whether they are flat or protruding.
2. Measure the maximum breadth (width) of the nasal opening.
3. Measure the maximum height of the nasal opening.
4. Starting at the outside of the back molars, measure the width of the maxilla (the upper jaw).
5. The bizygomatic breadth is the width of the face from the widest part of one cheek bone to the widest part of the other cheek bone. Measure this distance.
6. Copy Results table 2 into your logbook. Add extra rows as required. Record the results of your observations.

Examining the dentition (teeth)

For each skull:

1. Examine the dental arcade (the shape made by the rows of teeth in the upper jaw). Observe the teeth towards the back and identify whether the teeth on each side of the jaw are parallel or diverging.
2. Reposition the skull so that you are viewing it from the side. Examine the incisors and identify whether they are vertical or angled forward.
3. Measure the width of the incisors on the left side of the jaw and then measure the incisors on the right side of the jaw. Add the width of all incisors together to get the combined width.
4. Examine the maxilla (upper jaw) and mandible (lower jaw) together. Identify whether the canine teeth project above or below the chewing surfaces of the other teeth.
5. See if you can identify canine diastema (the gap between the canine and the adjacent teeth).
6. Measure from the back of the last molar to the front of the first premolar on the left side of the jaw. This will give you a measurement of the chewing surface of the teeth.
7. Copy Results table 3 into your logbook. Add extra rows as required. Record the results of your observations.

Results

Results table 1 Examining the brain case

Specimen	Forehead	Browridge present/absent	Browridge continuous/divided	Brain case	Sagittal crest	Foramen magnum	Mastoid

Results table 2 Examining the facial structure

Specimen	Nasal bones	Nasal opening width	Nasal opening height	Maxilla width	Bizygomatic breadth

Results table 3 Examining the dentition (teeth)

Specimen	Dental arcade	Incisors	Incisors width	Canine	Diastema	Chewing surface

Draw a graph of one characteristic (e.g. presence of brow ridge) from each table. Write ‘Specimen’ on the *x*-axis and arrange in order from great apes to modern humans.

Discussion

1. The canine teeth have drastically reduced in size from great apes to modern humans. Explain why this might be.
2. Explain why the face has become progressively flatter over the evolution of hominids.
3. Describe how the position of the foramen magnum relates to body posture and locomotion.
4. Certain areas of the brain case enlarged before others in our evolution. Describe how the areas enlarged throughout our evolution.
5. What traits differentiate modern apes and modern humans?
6. Using your measurements and the facial features you observed as evidence. Are modern humans or modern apes more closely related to extinct hominids?
7. Imagine you found the remains of a skull that only contained the mandible. Is this enough evidence to determine if it belonged to a modern human, an early hominin or an ape? Explain your answer.

Conclusion

Write a statement on the trends in structural changes from great apes to modern humans.

Alamy/The Natural History Museum

Figure 11.36 Mousterian tools associated with Neanderthals

Mousterian technology

There is ample archaeological evidence to show that Neanderthals used relatively advanced stone tools. This Mousterian technology (named from a site in France) dates from about 300 to about 30 kya. Mousterian tools are mostly found throughout Europe but evidence for the technology also occurs in the Middle East and northern Africa. The technology appears to have evolved from Acheulean industry and is characterised by sharp, pointed blades (Figure 11.36) crafted by chipping flint, a type of dark quartz.

Near the end of their history, Neanderthal sites are found with flint-based serrated blades normally associated with modern humans, suggesting Neanderthals were copying or trading the technology.

Neanderthal lifestyle

The Neanderthal diet was mixed, and depended on what was locally available. Chemical analysis of fossil teeth residues and faecal deposits provides direct evidence that Neanderthals consumed starchy tubers, nuts, fruits, grasses and meat. Bone remains indicate Neanderthals effectively hunted and butchered game, particularly reindeer, but also bigger prey such as bison and mammoths. Asymmetric anatomy and frequent broken bones suggest Neanderthals hunted by thrusting spears at large game at close range. Archaeological evidence shows that Neanderthals built hearths and controlled wood-fuelled fires for cooking and for warmth. Neanderthals consistently took refuge in caves and rock shelters, a practice that contributes to the caveman stereotype. Indeed, the rich fossil record for Neanderthals exists because a number of deceased Neanderthals were buried in caves.

There is evidence that Neanderthals buried their dead and occasionally marked their graves. Although disputed, there is no definite evidence that Neanderthal burials were associated with rituals, nor is there any rock art firmly attributed to them. For these reasons, the prevailing if controversial view is that Neanderthals were pragmatic and even altruistic but they displayed little of the symbolic expression that defines the art and ceremonies of *Homo sapiens*.

Migration of modern humans around the world and to Australia

WB
11.4.1
EVOLUTION OF MODERN HUMANS
PAGE 241

The fossil, cultural and molecular evidence suggests that modern humans evolved initially in Africa and then migrated across the world, displacing earlier hominins as they advanced. This model is referred to as the **recent single origin** hypothesis, or more informally as the **Out of Africa** hypothesis. This hypothesis accommodates the possibility that *H. sapiens* interbred with localised populations of ancient humans. However, the migration of modern humans was overwhelming. Evidence suggests that older hominin populations were assimilated into a dominant modern human population, if they were not wiped out altogether.

Considerable nuclear DNA and mtDNA evidence supports the hypothesis that early hominins, migrated out of Africa about 150 kya. DNA evidence supports that modern humans migrated in a later wave into Europe and the Middle East, where they interbred with Neanderthals. DNA evidence suggests that modern hominins reached Australia about 55–50 kya from South-East Asia. Distinct groups from the initial migration, spread rapidly down the western and eastern coasts occupying particular geographic areas. Extinction of the Australian megafauna about 50–40 kya is evidence of this rapid migration across the country. Although evidence from cultural artefacts and DNA varies as to the amount of migration and gene flow between specific populations, there is evidence for prolonged connection over time of specific populations in specific areas of the Australian continent. This is in agreement with the Aboriginal and Torres Strait Islander peoples' cultural beliefs and their central cultural attachment to Country and Place.

Fossil evidence

Anatomically modern humans first appear in the fossil record in Africa. Fossil skull fragments from Morocco dated to about 300 kya have a blend of modern and older hominin features and may represent an early version of modern humans. The earliest unequivocally modern human fossils currently known come from Ethiopia and are dated to about 195 kya. The fossil evidence suggests modern humans evolved first in central Africa. As climatic conditions changed, drought in central Africa pressured modern humans to migrate to the east coast. A coastal existence may have contributed to cognitive evolution by supplementing the diet with seafood rich in omega-3 fatty acids. Modern humans then migrated northwards and southwards along coastal routes.

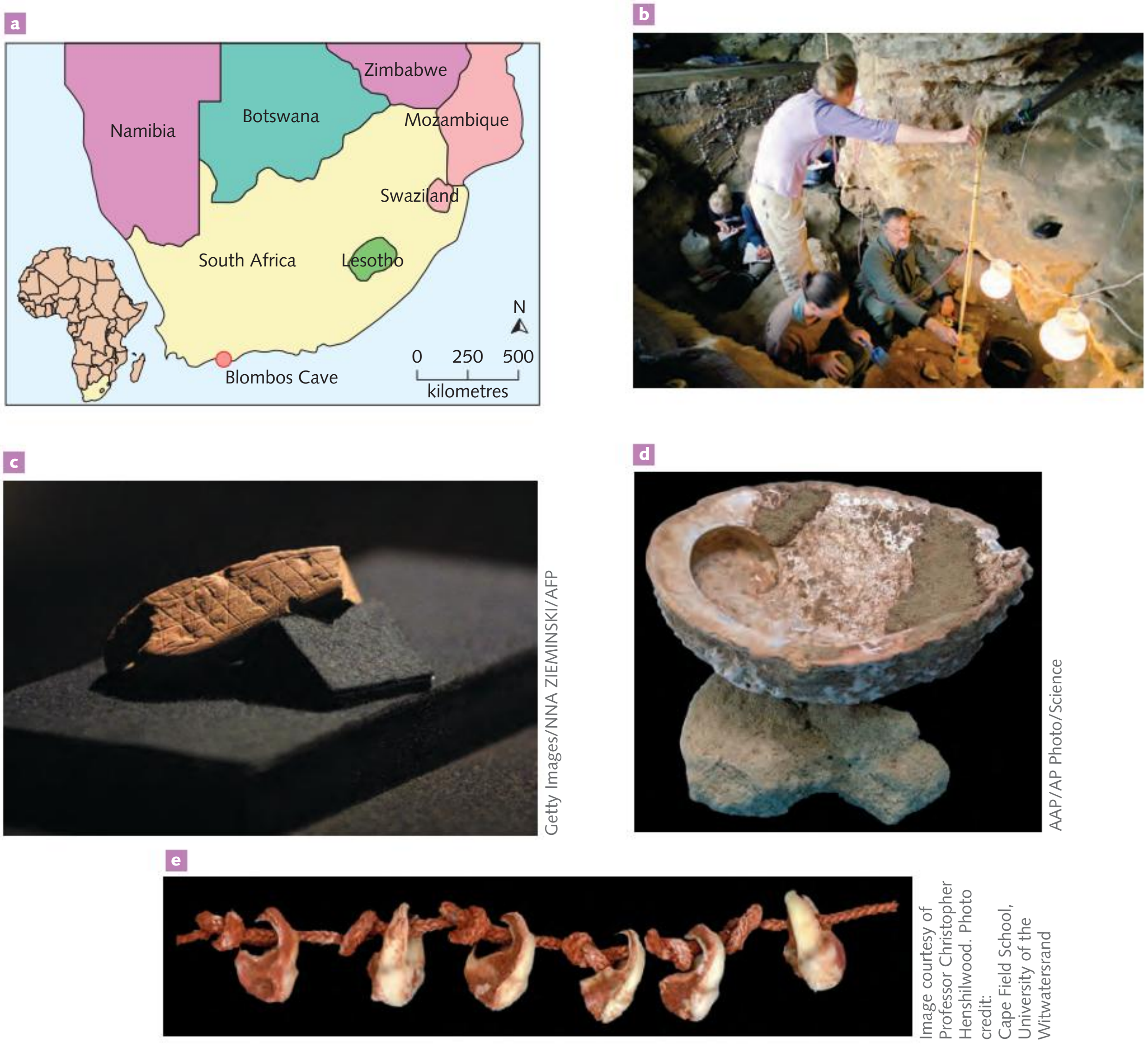
Palaeoanthropologists do not yet agree on the precise timing, the routes or even the number of migration waves of modern humans out of Africa. The current hypothesis is that there were at least two major migration waves north. One of these occurred about 100 kya but ended after reaching the Middle East. A later wave occurred 70–50 kya and pushed into coastal routes along Europe and Asia. The second migration wave ultimately spread the human population worldwide and brought the ancestors of modern Aborigines to Australia.

Skull fragments of an anatomically modern human dated to about 50 kya have been recovered in Niah Cave, Malaysia. This fossil hints at the route modern humans were likely to have taken to enter Australia. Fossil evidence confirms that anatomically modern humans inhabited south-east Australia by 40 kya. The evidence comes from dating of skeletal remains from two burial sites at Lake Mungo on the lands of the Paakantji, Ngyiampaa and Mutthi Mutthi people of southern New South Wales. These remains of a woman and a man are informally referred to as 'Mungo Lady' and 'Mungo Man'.

Modern human fossils in Europe are dated from about 40 kya and are traditionally referred to as Cro-Magnon Man, after the French cave where the first specimens were discovered. The earliest evidence of modern humans currently known on the Asian mainland is dated to about 30 kya. The anatomical proportions of the earliest modern human skeletons in Europe and mainland Asia resemble those of modern Africans, supporting the Out of Africa hypothesis.

Evidence of art and culture

Hominin evolution is associated with the evolution of tools. Evolution of modern humans is also associated with resources being used for artistic, as well as functional, purposes. Ancient art represents the most enduring record of symbolic expression during human evolution. The first convincing signs of art are associated with anatomically modern humans about 120 kya in scattered sites in South Africa. Blombos Cave in South Africa provides evidence of continuous human occupation for more than 100 000 years. It is a significant site for tracking the development of art and culture. Consistent use of particular artistic styles, or 'industries', appears there by about 90 kya. The evidence includes artefacts such as perforated seashell 'beads', engraved ostrich eggs, and patterned engravings in stone tools, bone and ochre (Figure 11.37), as well as evidence for symbolic burial practices. The appearance of industries is significant because it shows that human groups were organising according to shared beliefs, values and behavioural practices. That is, they demonstrate the establishment of culture. Furthermore, some resources at the excavation site, such as abalone shells filled with liquefied ochre, originated from distantly separated locations. This implies the cave inhabitants had the capacity to plan. They were identifying resources, relocating and storing them at their 'workshop', and combining them for later use.



Getty Images/NNA ZIEMINSKI/AFP

AAP/AP Photo/Science

Image courtesy of Professor Christopher Henshilwood. Photo credit: Cape Field School, University of the Witwatersrand

Figure 11.37 a The location of Blombos Cave in South Africa. **b** The interior of Blombos Cave. Artefacts found at the cave include **c** a stone block with patterned carvings and **d** an abalone shell used to prepare ochre. **e** A reconstruction of Blombos Cave beadwork using modern shells.

The quantity, sophistication and geographical distribution of human art expanded abruptly about 40 kya. This is largely represented by cave art in Europe and Australia, including paintings, engravings and carvings (Figure 11.38). The oldest confirmed rock art is from about 45 kya in southern Sulawesi, Indonesia. In Australia, the oldest reliably dated cave art is a charcoal drawing from 28 kya excavated at Nawarla Gabarnmang rock shelter located in Jawoyn country in Arnhem Land. Australia's Aboriginal cave art is considered to be the longest unbroken record of ancient art in the world.

Mungo Lady and Mungo Man, dated to about 40 kya, are the oldest known examples of human cremation and burial in the world. Although the Mungo site skeletons proved to be one of Australia's most significant archaeological discoveries, their removal for scientific study in the 1960s and 1970s caused considerable distress to the local Indigenous communities whose ancestors they represented. The local Indigenous people believe that the spirits of the deceased are restless until their remains are laid to rest on Country. The matter was settled when Mungo Lady and Mungo Man were returned to the Paakantji, Ngyiampaa and Mutthi Mutthi people in 2017.

The removal of ancient artefacts for study and the angst their removal causes Indigenous owners is a cultural and political issue worldwide. The tension underscores a significant lesson about cultural evolution. As modern humans cooperated in larger groups, artwork, ceremony, language and affinity with territory served to bond members and define group identities.

Alamy Stock Photo/Sergi Reboredo

Permission Prof. Maxime Aubert, Griffith University

Photography John Gollings

Figure 11.38 Examples of ancient rock art featuring local fauna from **a** Altamira Cave, northern Spain; **b** Gabarnmang rock shelter, Jawoyn country, northern Australia; and **c** Maros karsts, southern Sulawesi, Indonesia.

Evidence from mitochondrial DNA

From the late 1980s, mitochondrial DNA (mtDNA) was used to explore evolutionary relatedness among modern humans. mtDNA was chosen because its pattern of maternal inheritance provided a relatively uninterrupted lineage of descent from ancestral populations. Global human populations were grouped according to the specific and unique mutations in their mtDNA (their **haplotype**). Members of a group that share a haplotype must be descendants of a common ancestor. These are described as **haplogroups**. Molecular homology was used to produce phylogenetic trees from the mtDNA haplogroups.

It was discovered that, among modern humans, most of the variation in mtDNA sequences occurs in African populations (L haplogroups, Figure 11.39). mtDNA of Europeans, Asians and the Indigenous peoples of Australia, the Americas and Pacific islands represent just a subset of total human mtDNA diversity (M and N haplogroups, Figure 11.39). This provides further evidence for the Out of Africa hypothesis. Molecular clock estimates suggested diverse populations of modern humans evolved more than 200 kya in Africa, with the haplogroups that migrated out of Africa diverging

CONNECT

For more detail on using mitochondrial DNA for evolutionary studies, see Chapter 10.

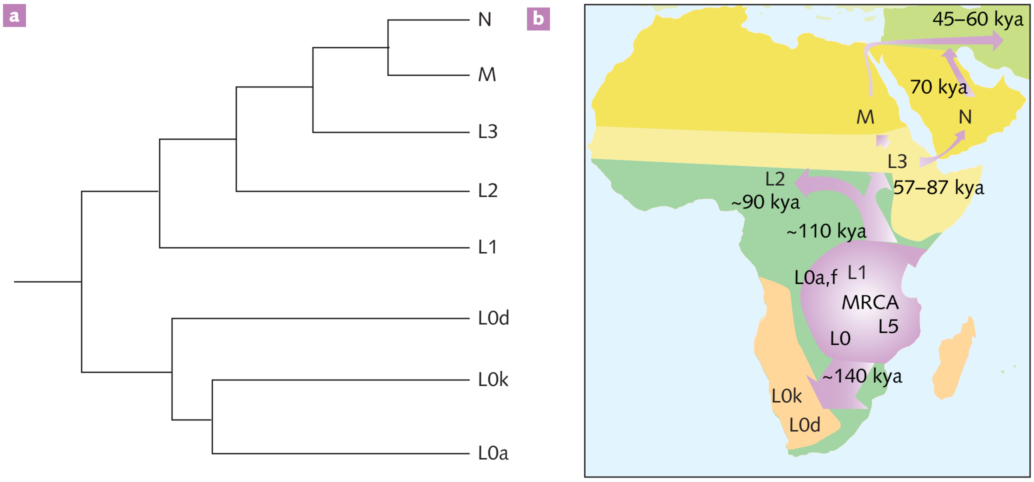


Figure 11.39 a **A phylogenetic tree generated from mtDNA sequences of global human populations. The labels at the tips of the tree represent the haplogroups into which human mtDNA mutations can be classified. b The location of each haplogroup and migration patterns inferred from them taking place thousands of years ago (kya). All haplogroup originate in Africa and the Middle East. Only the M and N haplogroup are found in Indigenous populations throughout the rest of the world. MRCA = most recent common ancestor.**

70–50 kya. The two surviving mtDNA groups (M and N) that colonised the other continents are most closely related to the African L3 group located north-east of Africa and nearest to the Middle East.

Mitochondrial DNA sequences exhibit affinities between northern Indigenous Australian and Papua New Guinean populations, suggesting joint colonisation of the two land masses by related peoples.

Evidence from nuclear DNA

Historically, sequencing nuclear genomes has been far more challenging than sequencing mitochondrial genomes. However, technical advances since the mid-2000s have accelerated the number and rate of whole nuclear genomes sequenced. Nuclear genomic sequences collected from representatives of human populations throughout the world indicate modern humans outside Africa share a common ancestry, consistent with a single major wave of migration dispersing from Africa. Models based on molecular estimates of mutation rates in these sequences suggest that a population bottleneck occurred in the non-African populations about 72 kya, converging with the likely time of migration out of Africa.

CONNECT
Go to Chapter 9 to review bottlenecks.

The models also indicate a likely early split between populations of modern humans. The common ancestors of Australian Aborigines and Papua New Guineans diverged from the ancestors of all other non-Africans about 58 kya. Europeans and east Asians subsequently diverged about 42 kya. This evidence suggests at least two waves of migration across Asia, with the earlier wave establishing the population of modern Indigenous Australian populations, who arrived perhaps about 50 kya. Nuclear DNA sequences indicate native Americans trace back to a common ancestry about 12.6 kya, representing the last major continent-wide migrations by modern humans.

WB
11.4.2 AUSTRALIAN SETTLEMENT PAGE 243

Australian settlement

Migration across the Australian continent was facilitated by lower sea levels that connected New Guinea, the Australian mainland and Tasmania into a single land mass called Sahul until about 8 kya. Sahul was separated from a consolidated southeastern Asian land mass called Sunda. This implies that an approximately 90 km sea crossing was required to settle Sahul.

Dating archaeological artefacts associated with processing food, pigments and stone tools in Madjedbebe rock shelter in Mirarr country 300 km east of Darwin confirms human occupation in northern Australia from at least 65 kya. This early date for human arrival in Australia indicates that models for human migration out of Africa and across the globe still need refinement.

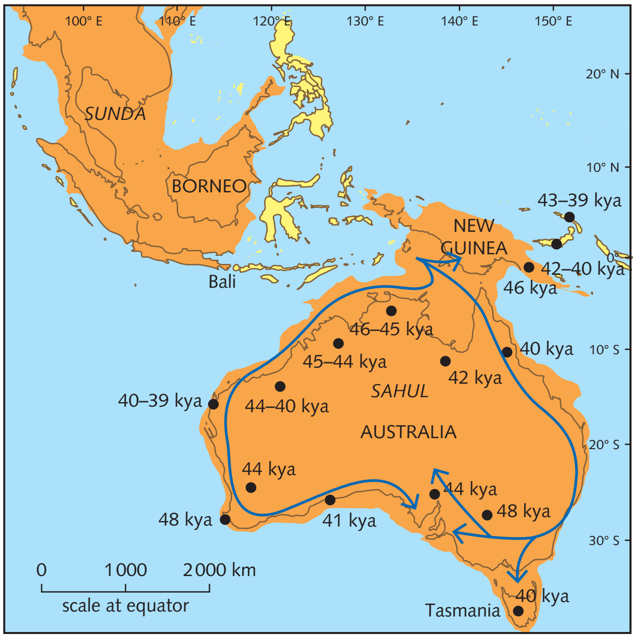


Figure 11.40 Migration routes (blue arrows) taken by Indigenous Australians populating the ancient continent of Sahul inferred from nuclear genome sequences, archaeology, and diversification of language. The dots represent archaeological sites with dates for the oldest artefacts currently known at each site.

Geographically widespread data from archaeological artefacts across Australia confirms extensive human habitation by about 45 kya (Figure 11.40). The evidence includes stone and bone implements, charcoal and burnt bones dated at many Australian sites, including Lake Mungo (about 48 kya), Devil's Lair, Wardandi country in southwestern Western Australia (about 48 kya), Gledswood rock shelter, Waanyi country in northwestern Queensland (about 42 kya) and Warreen Cave on the lands of the Tommeginne in Tasmania (about 40 kya).

Variations in autosomal sequences of Indigenous Australians indicate a genetic divergence between northeastern and southwestern populations with genetic intermediates shared by populations in the interior of the continent. The data informs models for gene flow and shows that the arid interior of the continent acted as a barrier to migration. Migration occurred preferentially in both directions around Australia's coastlines before dispersing inland (Figure 11.40). The combination of nuclear genome data and dating data for human fossils and archaeological artefacts suggests the migration and settlement of Australia was relatively rapid and completed within a few thousand years. Archaeological artefacts indicate ongoing or recurrent human occupation at many Australian sites after they were settled.

Today, Indigenous Australians are represented by hundreds of Indigenous nations and language groups and the clans within them. The principal roots for approximately three-quarters of the Indigenous languages are the Pama (northeastern Australian) and Nyungan (southwestern Australian) languages. The divergence and spread of these languages correspond well with hypothesised migration routes based on nuclear genome sequences (Figure 11.40). However, the timing of the language divergences remains a matter of debate.

Sea levels began rising about 8 kya, eventually cutting off Australia from New Guinea and forming more than 270 islands and 1300 coral reefs in the shallow seas of the Torres Strait (mostly <20 metres deep). Ongoing human occupation in the Torres Strait islands is indicated by archaeological evidence consisting of charcoal and burnt dugong and turtle bones from about 7 kya. The native islanders' connection with the sea is highlighted by archaeological evidence for marine food consumption (Figure 11.41). For example, ancient **middens** from at least 4 kya are rich in discarded bones of small sharks, rays and other fish, indicating subsistence mainly on near-shore fish. Genetic, archaeological and linguistic evidence indicates the local islander populations remained in ongoing contact with the populations of northern Australia and Papua New Guinea.

Figure 3 from M.I. Weisler and I.J McNiven (2016) Journal of Archaeological Science: Reports 7: 764-774 Licensed through Elsevier

Figure 11.41 Artefacts recovered from a fish bone midden in Tigershark Rockshelter on the granite islet of Pulu. Almost 60% of the bones are derived from small sharks and rays (about 1 metre) in this and other middens in western Torres Strait.

KEY CONCEPTS

- Neanderthals were ancient hominins with distinctive anatomical features, who coexisted for a time with modern humans.
- Neanderthals demonstrated cultural and technological evolution but little symbolic expression or art.
- Evidence from fossils, archaeology and mitochondrial and nuclear DNA sequences supports the recent single origin, or Out of Africa, hypothesis.
- Genetic and archaeological evidence provides evidence for patterns of migration of modern humans across the world, including Australia.

Concept questions 11.4a

1. Describe three differences between the skeletal anatomy of Neanderthals and modern humans.
2. Describe fossil evidence that supports the Out of Africa hypothesis.
3. What are haplogroups and how do they support the Out of Africa hypothesis of modern humans?

HOT Challenge

4. Among modern humans, the mtDNA of Europeans, Asians and the Indigenous peoples of Australia, the Americas and Pacific islands represent just a subset of total human mtDNA diversity, through M and N haplogroups. Haplogroup N is derived from the ancestral L3 macrohaplogroup, which represents the migration discussed in the 'Out of Africa' theory. Haplogroup N is the ancestral haplogroup to almost all clades today distributed in Europe and Oceania, as well as many found in Asia and the Americas. Only the M and N haplogroups are found in Indigenous populations throughout the rest of the world.

   How do these findings suggest that modern Aboriginal peoples are the direct descendants of migrants who left Africa up to 75 000 years ago?

WB

11.4.3 RELATIONSHIP BETWEEN MODERN HUMANS AND NEANDERTHALS PAGE 246

Relationship between modern humans and Neanderthals

It is unclear why the second wave of modern human migration was more successful than the first. It may be that modern humans in Africa developed better survival skills in the intervening period. Or perhaps they were cooperating in larger or better organised groups. Whatever the causes, it is certain that, during the second migration wave, anatomically modern humans coexisted with Neanderthals across Europe and Asia. What was the outcome of this encounter?

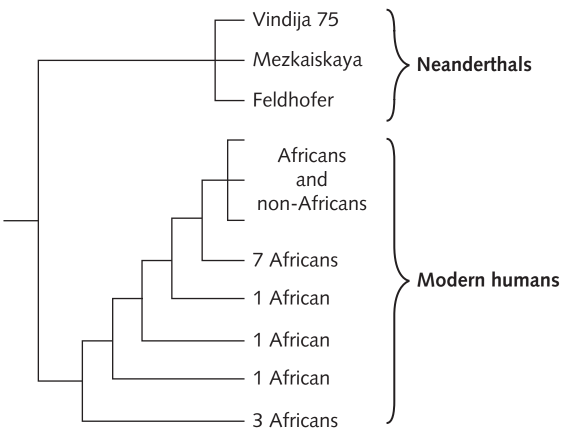


Figure 11.42 A cladogram for mtDNA sequences derived from ancient DNA of fossil Neanderthal bones and DNA from modern humans. The tree shows that the Neanderthal and modern humans samples diverged into separate branches.

A split in the tree

Ongoing speculation about interbreeding between Neanderthals and modern humans was fuelled in part by discoveries of fossils presumed to be anatomical hybrids. At the beginning of the 21st century, modern molecular methods enabled ancient DNA to be isolated and sequenced. This development offered an innovative approach to exploring evolutionary relatedness. Among the first to be studied was the mitochondrial DNA of sufficiently preserved Neanderthal fossils from Asia to western Europe spanning from 70 to 30 kya. These were compared with mtDNA sequences of modern human populations. The data showed that the degree of variation within the mtDNA of both Neanderthals and modern humans was relatively narrow. The mtDNA sequences of modern humans and Neanderthals were very different and had no overlap (Figure 11.42). These early studies offered no proof of interbreeding between Neanderthals and modern humans. Rather, they suggested that Neanderthals and modern humans diverged as two isolated populations.

Branches cross again

In 2010, scientists published a draft nuclear genome sequence from the ancient DNA of Neanderthal bones. This extraordinary technical feat overcame challenges posed by the size of the genome (more than 3 billion nucleotides) and the age of the samples (about 40 kya). The Neanderthal DNA was severely degraded and heavily contaminated by bacterial DNA. The genomes of the scientists working on the project were also sequenced and compared to ensure their DNA had not contaminated the Neanderthal samples.

The nuclear genome of the Neanderthals was compared with nuclear genomes of various modern humans. The comparison revealed that 1–4% of the genomes of modern Europeans and Asians are uniquely identical to those of the Neanderthal. However, these sequences are not shared between genomes of the Neanderthal and sub-Saharan African populations. The simplest interpretation is that 1–4% of the nuclear DNA of modern humans living outside Africa was derived from Neanderthals. This constitutes evidence for a limited amount of interbreeding between Neanderthals and modern humans. Estimates suggest the Neanderthal alleles entered the modern human population 40–80 kya. It is proposed that modern humans encountered and interbred with Neanderthals as they migrated out of Africa and through the Middle East. This is the reason the signature for Neanderthal DNA today is found mainly in descendants of Europeans and Asians but not those of African populations.

As no living human has been found to contain mtDNA of Neanderthals, it is likely that individuals of those lineages have not survived. In 2016, it was reported that chromosome 21 was sequenced from two European Neanderthal specimens dating to about 30 kya. The sequences provided evidence for earlier interbreeding between Neanderthals and modern humans. This was estimated to have occurred about 100 kya, most likely around the Middle East. The evidence indicates multiple interbreeding events between Neanderthals and modern humans.

Another branch, another crossing

In 2010, scientists announced the discovery of bone fragments from a previously unknown ancient hominin in Denisova Cave in Siberia. The bone fragments and the few associated artefacts were dated to about 40 kya. The anatomy of the **Denisovan** hominin remains a mystery but good quality DNA was recovered and sequenced from a single finger bone. The mitochondrial DNA of the Denisovan indicated it was more closely related to the Neanderthals but was sufficiently different to be a distinct branch in the hominin evolutionary tree. Phylogenetic trees show that the split between the Neanderthals and Denisovans occurred after their common lineage diverged from modern humans (Figure 11.43). Molecular clock estimates date the divergence between the Neanderthal/Denisovan lineage and the modern human lineage at about 800 kya. The Neanderthals and Denisovans subsequently diverged about 640 kya.

A fossil jawbone recovered from the Tibetan plateau of northern China was dated at 180 kya. The DNA in the jawbone had degraded but the fossil was later identified as Denisovan based on analysis of teeth proteins. The discovery suggests the Denisovans were an enduring and widespread population, occurring at high and low altitudes in central Asia.

Analysis of nuclear genomes revealed that modern Melanesians share 3–5% of their DNA sequences uniquely with the Denisovans. The Melanesians include Indigenous Australians, native Papuans of New Guinea and the native populations of several western Pacific islands. No other modern humans carry this signature of the Denisovan nuclear DNA. The evidence suggests Denisovans interbred with the ancestors of Indigenous Australian and Papuan populations as they were migrating through southern and southeastern Asia. It is estimated this interbreeding occurred between 50 kya and 44 kya. However, most modern Chinese and southeastern Asian populations lack Denisovan DNA. This supports the view that there were many waves of modern human migration through eastern and southeastern Asia, only some of which resulted in interbreeding with Denisovans. Figure 11.43 summarises the major divergence and interbreeding patterns between modern humans, Neanderthals and Denisovans.

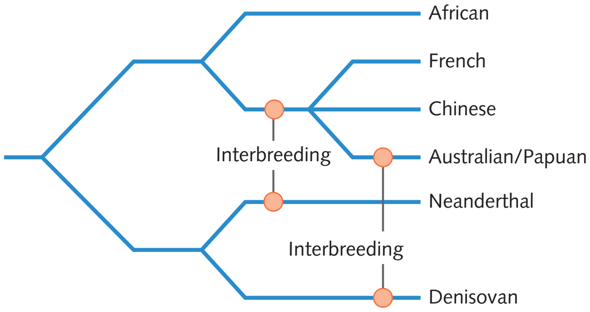


Figure 11.43 Patterns of divergence and subsequent interbreeding during human evolution. The diagram is drawn from evidence revealed by mitochondrial and nuclear DNA sequences.

At a hypothetical boundary between the Neanderthal populations of the west and the Denisovan populations of the east, Denisova Cave continues to be an important focus for the study of human evolution. In 2018, the mitochondrial and nuclear genome sequence of another finger bone found at the cave was reported to belong to a 13-year-old female who was estimated to have lived about 90 kya. Most significantly, her DNA showed she was a first-generation hybrid of a Neanderthal mother and a Denisovan father. The discovery proved that the ancient hominins interbred. The exchange and selection of the fittest genes among hominin populations was surely a key factor contributing to the survival of hominins. Our understanding of gene flow between prehistoric hominins and modern humans will continue to be reappraised whenever the genomes of ancient hominin remains are sequenced.

Denisovans are one of several putative Homo species that have been inferred based on partial fragmentary evidence or genetic evidence. Though it is believed that they exist, where they fit in the evolutionary tree is open to interpretation.

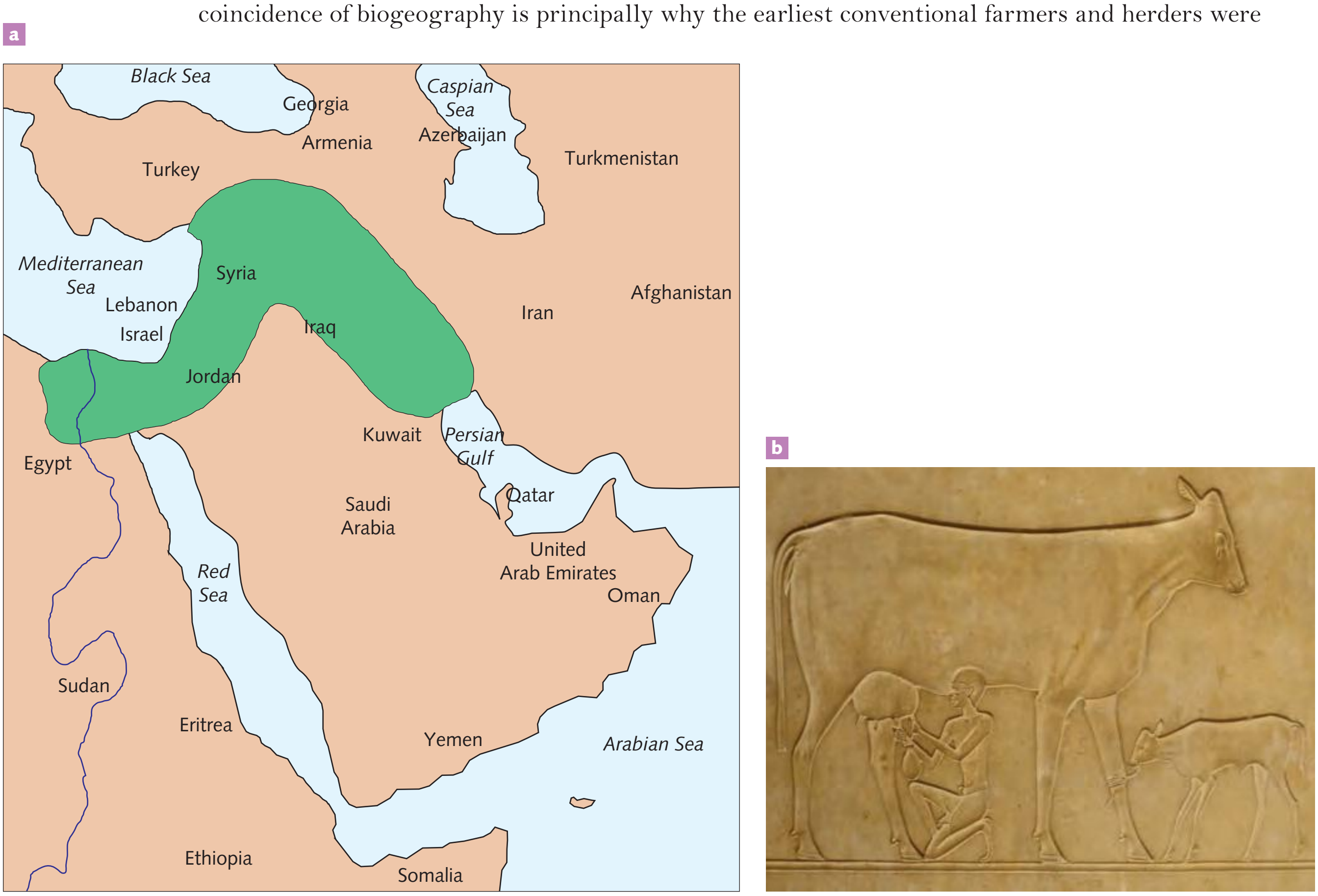
Another putative Homo species is *Homo luzonensis*. Only a few teeth and finger and foot bones were found in the Calleo cave in the Phillipines. In 2010, they were identified as fossils of a modern human, *Homo sapiens*, however in 2019, after discovery of more specimens and based on a wide range of traits, they were placed into this new species. As more evidence is collected from fossils and genetic material, there may be different interpretations and changes to the evolutionary tree of hominin evolution.

Domestication and industrialisation

CONNECT

Artificial selection is discussed on pages 346–348.

In the last 10 000 years, human innovation has been transformed by the practice of domesticating species by artificial selection. The development of agriculture depended upon the availability of domesticable species. The Fertile Crescent in the Middle East was the home range for a variety of suitable species, including wild wheats, barley, peas, cows, sheep, pigs and goats (Figure 11.44). This coincidence of biogeography is principally why the earliest conventional farmers and herders were



Alamy/Images of Africa Photobank

Figure 11.44 a The location of the Fertile Crescent (green); b artwork from Ancient Egypt depicting a cow being milked

located in that region. The plants and animals they domesticated are still some of the most valuable species to humans today.

Domestication also depended on advantageous genes and alleles in humans, as well as in wild plant and animal species. Just as humans selected for favourable traits in other species, the novel sources of nutrition applied selection pressures on modern humans. For example, modern dairying populations have higher allelic frequencies for the gene for lactase persistence. People who produce lactase in adulthood can digest the lactose sugar in milk derived from cattle or goats. Individuals in populations with a long history of agriculture tend to have more copies of the salivary amylase gene. This adapts people to a starch-rich diet of cultivated grains. However, the traits favoured by humans tend to make domesticated species less capable of surviving if re-introduced into the wild. Therefore, domesticated species rely on humans for their dispersal and survival. In essence, humans and their domesticated species have co-evolved and become interdependent.

Indigenous Australians developed holistic agricultural practices to manage the land for a sustainable food supply. Notably among them was Indigenous Australians' relationship with fire. 'Fire-stick farming' was used to stabilise the landscape and control the local food supply of plants and animals. Another example was the unique form of aquaculture practiced by the Gunditjmara people of south-western Victoria. They dug and maintained an extensive system of water channels connecting natural ponds to manage the local eel stocks. Basalt blocks installed in the channels trapped eels at different stages of growth. The transition to an agricultural lifestyle enabled humans to settle and eventually specialise and develop advanced technology.

Domesticated crops selectively bred for yield are grown at higher densities than their counterparts in the wild. The enhanced supply of kilojoules is a key driver of increased human population sizes. The rate of human population growth has accelerated with the pace of technological innovation and industrialisation (Figure 11.45). The tools of modern humans enhance the speed, scale and efficiency of energy production (e.g. power stations), transportation (e.g. motor vehicles and aeroplanes), long-range communication (e.g. television, telephones and Internet), cognitive processing (e.g. computers and software), and the ability to perceive the imperceptible (e.g. microscopes, telescopes and subatomic particle accelerators). Humans have colonised every continent and the human population will soon exceed 8 billion. The scale of the human population now affects every other plant and animal species. The impacts of human activities include depletion of wild species through overharvesting, habitat destruction, widespread pollution, the spread of invasive species and accelerated climate change.

Weblink
A brief history of dogs

Online Worksheet
A brief history of dogs

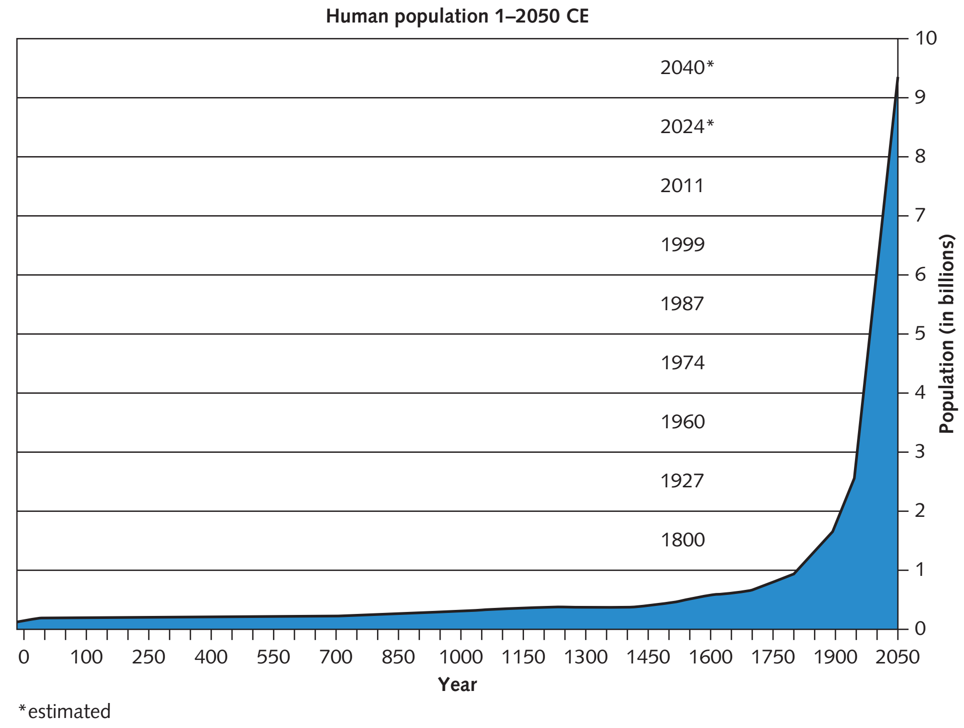


Figure 11.45 Growth in the human population during the past 2000 years

Homo sapiens is an extraordinary outcome of evolution: a conscious, cooperative ape with an unprecedented capability to evaluate, plan and manipulate its environment. It is one species that can, to a considerable extent, shape its own future and that of countless other species. In the course of your lifetime, years from now, how will that future unfold?

KEY CONCEPTS

- Evidence from mitochondrial DNA suggests Neanderthals and modern humans diverged up to 800 kya.
- Complementing fossil evidence, mitochondrial and nuclear genome data indicates that modern humans evolved first in Africa, and then migrated via the Middle East to the rest of the world.
- Evidence from nuclear DNA sequences suggests select groups of modern humans later interbred with Neanderthals and another group of ancient hominins called the Denisovans.
- About 10 kya, the shift from hunter–gatherer to agricultural lifestyles accelerated cultural and technological evolution.

Concept questions 11.4b

1 Explain how mitochondrial DNA shows that the Neanderthal and the modern human lineages diverged about 800 kya.

2 Which modern humans have the signature of Neanderthal DNA in their nuclear genomes today, and which modern humans do not? How do these observations fit with the Out of Africa hypothesis?

3 Who are the Denisovans, and how do they help to explain patterns of migration and colonisation by modern humans?

4 Describe two aspects of the shift to agriculture that made the expansion of the human population possible.

HOT Challenge

5 Recently, Australian Aborigines have been linked genetically to a group of people known as Dravidian Indians from the subcontinent. How might this have happened?

BRANCHING OUT

Is 'Ardi' a hominin?

There are very few fossils of possible hominins dating to before about 4.2 mya and their identity as hominins is frequently debated. The most informative fossil from that time is a nearly half-complete skeleton of *Ardipithecus ramidus* (nicknamed Ardi). It was discovered in Ethiopia in 1994, and the delicate work of excavating and reconstructing Ardi took 15 years.

Scientists debate whether Ardi is a direct ancestor of modern humans, an early side branch of hominin evolution, or a representative of a separate group from the humans and the chimps.

Aim

To examine the characteristics of *Ardipithecus ramidus* and evaluate its classification as a hominin

Observations

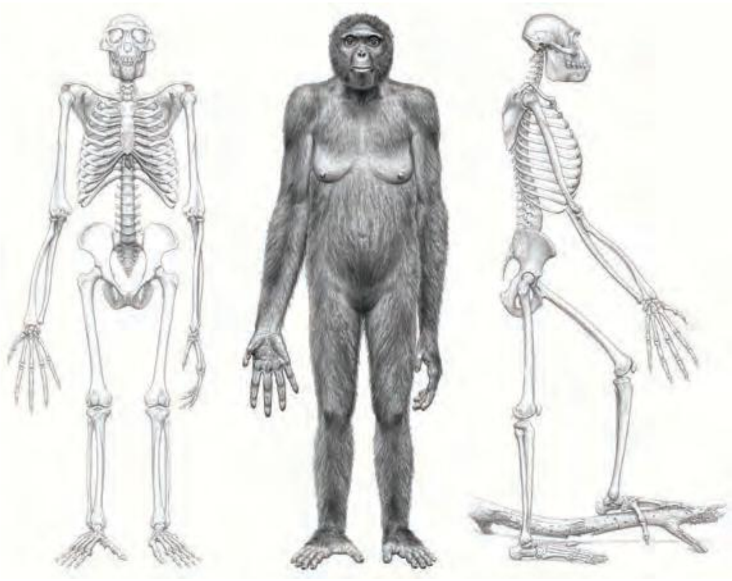
Examine the data for *Ardipithecus ramidus* outlined in Table 11.5 and Figures 11.46 and 11.47. Use your logbook to record your observations for the following features.

- Cranial capacity
- Relative size of the brow ridges
- Relative size of the canines
- Amount of prognathism
- Length of limbs relative to the rest of the body
- Length of the thumb relative to other digits of the hand
- Shape of the spine
- Shape of the pelvis
- Carrying angle
- Length of hallux relative to other toes of the foot

»

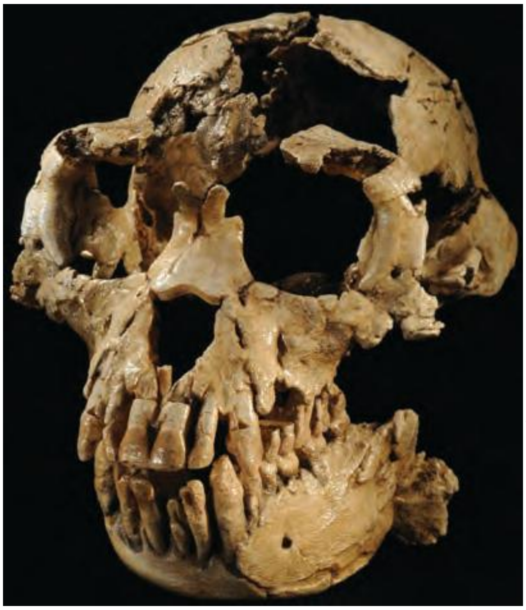
Table 11.5 Characteristics of *Ardipithecus ramidus*

Characteristic	Value
Age	About 4.4 million years
Sex	Female
Height	1.2 m
Weight	50 kg
Cranial capacity	350 cm^3



© J. H. Matternes

Figure 11.46 Sketches of the front view (left) and profile view (right) of the skeleton of *Ardipithecus ramidus*, together with an artist's reconstruction (centre)



Science Source/Bone Clones, Inc.

Figure 11.47 A 4.4 million-year-old reconstructed fossil skull of *Ardipithecus ramidus*, found in the Aramis site, Middle Awash Valley, Afar depression, in north-east Ethiopia. The fossil is housed in the National Museum of Ethiopia.

Discussion

1 What attribute(s) would you consider important in determining whether a hominoid was a hominin? Explain what features you might expect to see in the skeleton of the hominoid if it possessed those attributes.

2 What can you deduce about Ardi's mode of locomotion? Would you say she was quadrupedal, bipedal or both? Did she climb trees? What evidence do you have to support your interpretations?

3 Do the cranial features of Ardi (brow ridges, canines, prognathism) show more similarities with hominins or with other hominoids?

4 What is cranial capacity supposed to indicate about a hominoid? What does Ardi's cranial capacity indicate?

5 Use the evidence and your interpretation to argue the case for including or excluding *Ardipithecus ramidus* in the hominins. Is there anything more you would need to know about the fossil that would help you make your decision?

Conclusion

Write a brief conclusion stating your decision to include or exclude Ardi as a hominin and summarise your reasons.

Online key concepts
Chapter 11: Summary of key concepts

11 Summary of key concepts

11.1 Taxonomy of modern humans

KEY CONCEPTS

p. 425

- Mammals share a range of unique anatomical features, including a body covering of hair or fur, a single jaw with specialised teeth, three bones in the middle ear, specialised exocrine glands, a diaphragm and a four-chambered heart.
- Mammals are divided into monotremes, marsupials and placental mammals.
- Humans are placental mammals.
- Modern humans are classified in the order Primata (primates) within the suborder Catarrhini.
- Key characteristics of primates include opposable digits, stereoscopic colour vision, nails on the digits, and a relatively enlarged brain.
- In contrast to other primates, hominoids lack tails, have eight premolars rather than 12, and molars with five cusps rather four.
- Hominins are uniquely bipedal.
- Chimpanzees are the living species that are most closely related to humans.

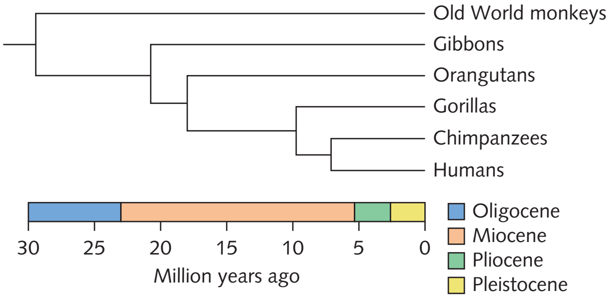


Figure 11.9 A phylogenetic tree depicting molecular clock estimates for divergences between major hominoid lineages

11.2 Adaptations that define humans

KEY CONCEPTS

p. 433

- Many features of the human skeleton, including the skull, spine, pelvis, femurs and feet, have a range of adaptations for bipedalism.
- Humans have a precision grip that enables manual dexterity.
- The anatomy of the brain, including the enlarged prefrontal cortex and increased convolutions, enhances the cognitive capacity of humans.
- Written and spoken language enables humans to convey abstract concepts.
- Cultural evolution is distinguished from biological evolution by the speed, the means and the choice exercised in its dispersal.

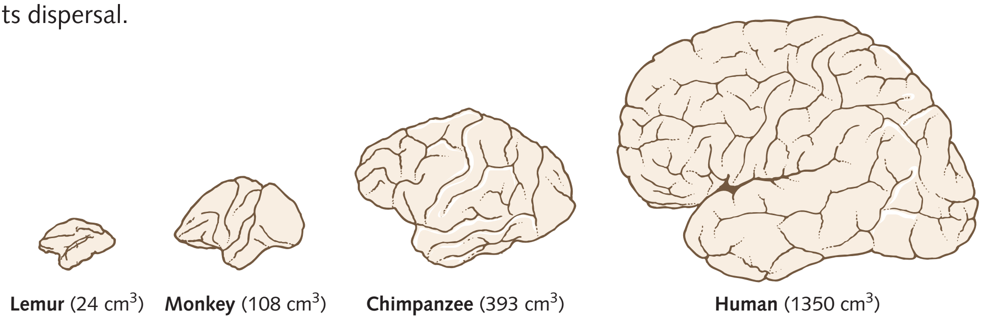


Figure 11.17 The brain sizes of various primates (drawn to scale)

11.3 Meet the ancestors

KEY CONCEPTS

p. 442

- Hominin evolution is represented by a ‘bushy’ evolutionary tree. Interpretations about the fossil record are subject to ongoing debate and refinement as new discoveries are made.
- Australopithecines were relatively small, bipedal apes.
- Australopithecine species are grouped into the genera *Australopithecus* (gracile) and *Paranthropus* (robust).
- Evolution of the genus *Homo* is associated with expansion of the cranium, as well as enhancements in bipedal evolution.
- New fossil discoveries (e.g. *Homo floresiensis*) challenge existing assumptions about hominin evolution.

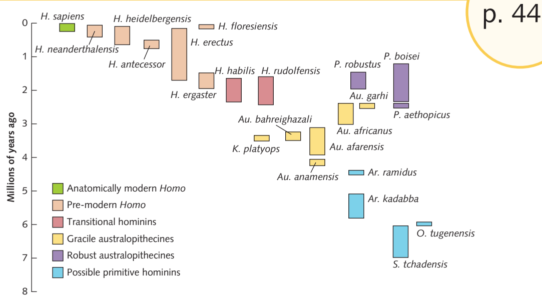


Figure 11.22 Species recognised in the hominin fossil record under the genera *Homo* (H.), *Ardipithecus* (Ar.), *Australopithecus* (Au.), *Kenyanthropus* (K.), *Orrorin* (O.), *Paranthropus* (P.) and *Sahelanthropus* (S.). The rectangles represent an estimate of the periods in the geological record during which the species are presumed to have lived, based on fossil evidence.

11.4 Modern humans and Neanderthals

KEY CONCEPTS

p. 451

- Neanderthals were ancient hominins with distinctive anatomical features, who coexisted for a time with modern humans.
- Neanderthals demonstrated cultural and technological evolution but little symbolic expression or art.
- Evidence from fossils, archaeology and mitochondrial and nuclear DNA sequences supports the recent single origin, or Out of Africa, hypothesis.
- Genetic and archeological evidence provides evidence for patterns of migration of modern humans across the world, including Australia.
- Evidence from mitochondrial DNA suggests Neanderthals and modern humans diverged up to 800 kya.
- Complementing fossil evidence, mitochondrial and nuclear genome data indicates that modern humans evolved first in Africa, then migrated via the Middle East to the rest of the world.
- Evidence from nuclear DNA sequences suggests select groups of modern humans later interbred with Neanderthals and another group of ancient hominins called the Denisovans.
- About 10 kya, the shift from hunter–gatherer to agricultural lifestyles accelerated cultural and technological evolution.

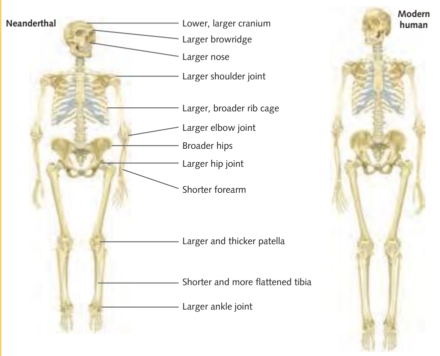


Figure 11.35 A skeletal reconstruction of *Homo neanderthalensis* and modern *Homo sapiens*

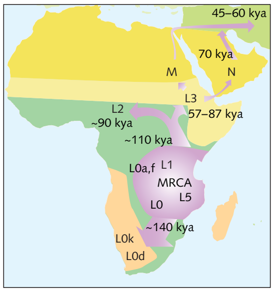


Figure 11.39 b The location of each haplogroup and migration patterns inferred from them taking place thousands of years ago (kya). All haplogroup originate in Africa and the Middle East. Only the M and N haplogroup are found in Indigenous populations throughout the rest of the world. MRCA = most recent common ancestor.

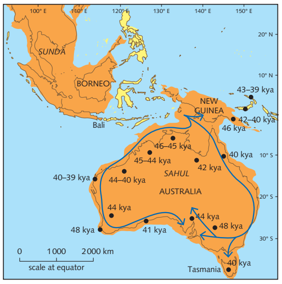


Figure 11.40 Migration routes (blue arrows) taken by Indigenous Australians populating the ancient continent of Sahul inferred from nuclear genome sequences, archaeology, and diversification of language. The dots represent archaeological sites with dates for the oldest artefacts currently known at each site.

WB
11.5.1
KEY TERMS
PAGE 247

11 Chapter glossary

arboreal related to, or living in, trees

australopithecine a term for bipedal apes of the fossil genera *Australopithecus* and *Paranthropus* that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

bipedalism a type of locomotion in which an organism walks on two hind limbs

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

brow ridge a bony ridge above the eye sockets

carrying angle the angle at which the femur is tilted in towards the knee

cerebral cortex the outermost layer of the brain

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions and apply new knowledge and skills

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

cultural evolution the way beliefs, social practices, skills and technology change over time

Denisovan a distinct, but undescribed, ancient hominin known primarily from bone fragments found in Denisova Cave in Siberia

dentition arrangement of the teeth

exocrine gland a gland that secretes a substance through a duct to the outer surface of the body

foramen magnum the hole in the base of the skull through which the spinal cord passes

gracile of slender build

hallux the big toe, or innermost toe of the foot

haplogroup a group of organisms that have the same genetic mutations in a single chromosome and are descendants of a common ancestor through either the maternal (mtDNA) or the paternal (Y chromosome) line of inheritance

haplotype the unique combination of genetic mutations in the DNA sequences of a single chromosome (e.g. mtDNA or Y chromosome) that are shared by different organisms and indicate common ancestry

hominin a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a member of the superfamily Hominoidea; an ape, or tail-less primate

kya thousands of years ago

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

mammal a warm-blooded vertebrate animal that has hair or fur, and the females secrete milk to nourish their offspring

mammary gland a gland in female mammals that produces milk

mandible the lower jawbone of the skull

midden a pile of discarded waste left by humans in the past

Out of Africa hypothesis *see* recent single origin

oviparous an animal that lays eggs that are expelled from the body and from which juveniles hatch

palaeoanthropology the field of study concerned with fossil hominins

placental describes an animal in which the foetus develops inside the mother while attached to an organ that provides nutrients and oxygen and removes wastes

postcranial all of the skeleton, except the skull

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manipulate an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of curling and grasping

primate a member of the order Primata; includes lemurs, lorises, tarsiers, monkeys, apes and modern humans

prognathism a condition in which the jaws protrude from the plane of the face

quadrupedalism a type of locomotion in which an organism walks on four limbs

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents; also known as Out of Africa hypothesis

robust of sturdy build

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

sebaceous gland a gland in the skin that secretes oils that lubricate and protect patches of skin and hair

sexual dimorphism where males and females of a species have different morphologies, often in shape or size

stereoscopic describes vision that has a sense of depth

superfamily a taxonomic rank immediately superior to the traditional rank of family; a superfamily may contain multiple taxonomic families

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

sweat gland a gland in the skin that secretes sweat

tribe a taxonomic rank inserted between family and genus

viviparous an animal that gives birth to live young

WB
11.5.2
EXAM PRACTICE
PAGE 249

11 Chapter review

Remembering

1 Sketch a diagram of a modern human and label the features that show the human is a mammal.

2 Describe how the fingers and toes of modern humans differ from the digits of other great apes. What advantages do these adaptations confer to modern humans?

3 Outline the ways that *Homo floresiensis* contrasts with other ancient *Homo* species.

Understanding

4 Summarise features of the postcranial anatomy of modern humans that adapt them for bipedal locomotion.

5 Describe at least five changes that have occurred to the hominin skull during evolution and indicate likely reasons for why they came about.

6 Describe the similarities and differences between the ways a monotreme, marsupial and placental mammal nourish their unborn and newborn young.

7 Sketch a cladogram that traces the evolutionary relationships among hominoids. On the cladogram, include the genera *Pongo*, *Gorilla*, *Pan*, *Australopithecus*, *Paranthropus* and *Homo*. Justify your positioning of each of the genera in the cladogram. Explain whether or not the hominins in your cladogram are a monophyletic group.

8 Explain how three anatomical and/or behavioural features of *Homo sapiens* have contributed to cultural evolution.

9 Is cranial capacity enough to explain the cognitive capacity of modern humans? Explain your point of view and provide evidence, wherever available, to support it.

Applying

10 Discuss the advantages conferred on primates by at least three adaptations that enabled them to live in trees.

11 Draw an annotated timeline from 2.5 mya to 100 kya showing:
  a the appearance of stone technologies and other cultural artefacts
  b which hominins the technologies were associated with
  c what these artefacts say about hominin cultural and technological evolution.

12 Draw a map outlining the major migration pathways of modern humans out of Africa and their timing based on nuclear genome sequences.

13 Describe key observations from mitochondrial and nuclear DNA of modern and ancient hominins that provide evidence for patterns of migration and interbreeding.

Analysing

14 Alleles for lactase persistence occur in many human populations around the world. The alleles are different, for example, between North African, European and Indian populations, but the phenotype is the same: lactase production persists beyond infancy and adults can digest lactose in milk. Studies of ancient DNA show the alleles were present at low proportions in early dairying populations 5–10 kya. Explain what has happened to result in higher frequencies of lactase persistence alleles in modern human populations. Your explanation should discuss:
  a why the alleles differ in different populations
  b which events represent natural selection
  c which events represent artificial selection
  d which events represent cultural evolution.

15 Social cooperation is observed in insect species such as termites and honey bees. Would you argue that this social cooperation is similar to or different from that observed in humans? Outline the evidence in support of your argument.

16 Distinctive pottery of Lapita culture is dated to 3.5 kya on islands off northern New Guinea, 2.9 kya in eastern mainland New Guinea and 2.5 kya in the Torres Strait islands. Pottery is not a feature of Torres Strait islander culture.

a What might explain how the pottery arrived in the Torres Strait islands?

b What evidence would you seek to support or disprove the hypothesis that Lapita people were absorbed into the Torres Strait islander population?

Evaluating

17 Consider the representative species of genus *Homo* discussed in this chapter. How satisfied are you with their designation as a distinct species? Give reasons to support your response.

18 Humans exercise great control over their environment and often adapt the environment to their needs. So have humans ceased to evolve? Provide evidence to justify your response.

Creating

19 Write an account of the changes in diet during hominin evolution from australopithecines to modern humans. Support your account with evidence from a range of sources, including the fossil record, comparative anatomy, biogeography and archaeological artefacts.

20 Sketch and annotate a drawing of what you predict the common ancestor of the chimpanzee and modern human might have looked like. Summarise the features you expect to see in the ancestor and list dot points to explain your reasoning.

Unit 4 Area of Study 2 review

Multiple choice

Question 1 ©VCAA 2012 EXAM 2 Q25 ADAPTED EASY

Cultural evolution

A includes physical responses to life events and the behaviour of individuals in a population.

B occurs faster than biological evolution.

C describes change in a population over many generations through the inheritance of traits.

D is defined as simply change over time.

Question 2 ©VCAA 2017 Q30 ADAPTED EASY

Farming practices adopted by Mongol farmers involved separating horses with docile temperaments and ease of riding from the native stock to interbreed within themselves for future horse herds. This is an example of

A genetic drift.

B natural selection.

C allopatric speciation.

D selective breeding.

Question 3 ©VCAA 2010 EXAM 2 Q18 ADAPTED MEDIUM

Index fossils are useful because they

A represent the oldest rocks in a series of strata.

B show the absolute age of any sedimentary rock stratum.

C are easily recognisable and have a wide geographic distribution.

D date the age of rocks from the Triassic period only.

Question 4 ©VCAA 2006 EXAM 2 Q16 ADAPTED HARD

A type of genetic drift is

A speciation.

B founder effect.

C gene flow.

D selection pressures.

Question 5 ©VCAA 2019 Q28 ADAPTED MEDIUM

Shutterstock.com/MillaF

The image shows a marine ammonite discovered by scientists. This type of fossil is best described as

A preserved remains.

B a petrified fossil.

C a cast.

D a trace fossil.

Question 6 ©VCAA 2012 EXAM 2 Q23 ADAPTED EASY

When scientists discover a new species of any organism, their claims need to be supported by evidence that shows that the new species

A looks similar to other species.

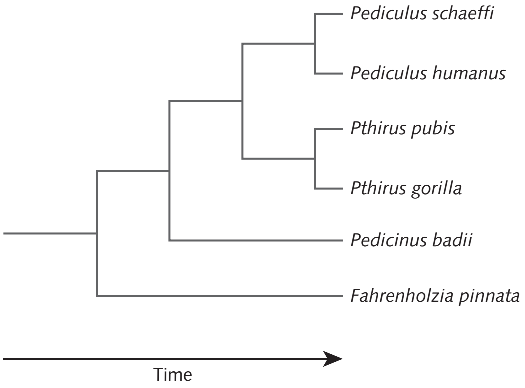
B cannot produce fertile offspring with members of known species.

C exhibits different behaviours to other known species.

D has DNA sequences that are similar to known species.

Question 7 ©VCAA 2016 Q29 ADAPTED MEDIUM

The following diagram is a phylogenetic tree for six different species of lice. The tree has been constructed from molecular and morphological data.



This information suggests that

A *Pediculus humanus* is the ancestor of *Pediculus schaeffi*.

B *Pediculus humanus* is more closely related to *Pedicinus badii* than it is to *Pthirus gorillae*.

C *Pedicinus schaeffi* shares a more recent common ancestor with *Pthirus gorillae* than it does with *Pediculus humanus*.

D *Pedicinus badii* shares a more recent common ancestor with *Pthirus pubis* than it does with with *Fahrenholzia pinnata*.

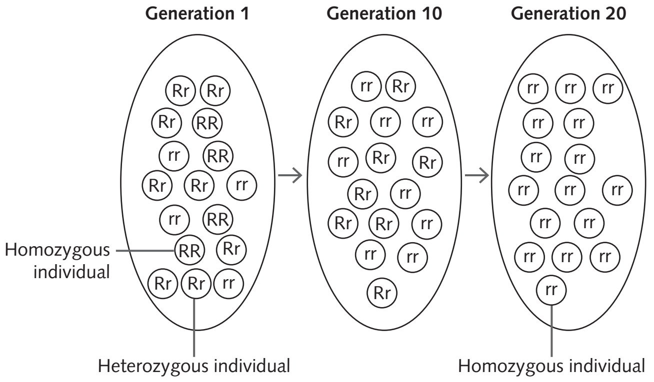
Question 8 ©VCAA 2016 Q30 ADAPTED EASY

Height is an example of a human trait that displays continuous variation. A trait that displays continuous variation

A may be influenced by many genes and the environment.
B is controlled by a single gene with very few alleles.
C is human ABO blood groupings.
D would have equal numbers of individuals in the population carrying each of the different genotypes.

Question 9 ©VCAA 2019 Q26 ADAPTED HARD

Consider the following diagram, which shows the gene pool of a population over 20 generations.



It would be correct to conclude that, over the 20 generations

A new advantageous alleles for this gene were introduced via individuals joining the population.
B individuals with the genotype rr had a selective disadvantage in this population.
C the frequency of each allele is equal in Generation 10 but not in other generations.
D genetic diversity is decreasing in this population.

Question 10 ©VCAA 2013 Q31 MEDIUM

The thylacine (*Thylacinus cynocephalus*) was a large, dog-like marsupial whose last known specimen died in 1935. A mummified carcass of a thylacine, found in a cave on the Nullarbor Plain, was dated at about 5000 years old. Dating a mummified carcass this old is most successfully done by

A carbon dating of the mummified remains.
B uranium–thorium dating of the mummified remains.
C relative dating of the layers of rocks within the cave.
D comparing the teeth in the mummified carcass to the teeth of other fossilised dogs.

Question 11 ©VCAA 2019 Q33 ADAPTED MEDIUM

Significant trends in hominin evolution occurred in the transition from the *Australopithecus* species to the *Homo* species. Which of the following shows the group of characteristics that best reflects these trends?

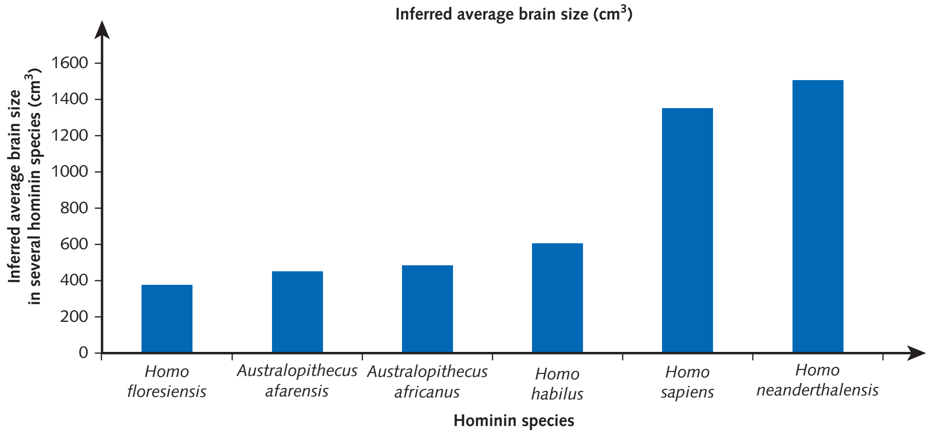
A Increasing size of brow ridges, decreasing tooth size, increasing size of zygomatic arch, increasingly bowl-shaped pelvis
B Increasing cranial capacity, decreasing size of canines, increasingly bowl-shaped pelvis, increasing arch of feet
C Decreasing cranial capacity, increasing jaw size, increasing arch of feet, decreasing tooth size
D Decreasing tooth size, decreasing arch of feet, decreasing size of brow ridges, more-opposable big toe

Question 12 ©VCAA 2019 Q32 HARD

The inferred average brain size of a number of hominin species is represented in the following bar graph.

Using the information in the graph and your knowledge of human evolution, which one of the following species could be the transitional form between *Australopithecus* and *Homo* species?

A *H. habilis*
B *H. sapiens*
C *H. floresiensis*
D *A. afarensis*



Source: Australian Museum, https://australianmuseum.net.au

Question 13 ©VCAA 2007 EXAM 2 Q7 ADAPTED **EASY**

Comparison of sequences in mitochondrial DNA is often used to establish the degree of relatedness between organisms, and thus to suggest evolutionary relationships, particularly in complex, higher-level organisms. Mitochondrial DNA is used because it

A is only inherited through the female line.
B has a higher rate of crossing over than nuclear DNA.
C has more genes than nuclear DNA.
D contains different nitrogen bases from those found in nuclear DNA.

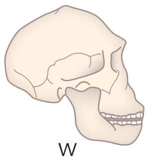
Question 14 ©VCAA 2017 Q35 ADAPTED **MEDIUM**

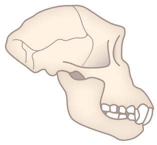
The genome of modern-day African *Homo sapiens* does not contain Neanderthal DNA. Modern non-African *H. sapiens* contain a small percentage of Neanderthal DNA due to interbreeding between *H. sapiens* and Neanderthals. This interbreeding is thought to have happened between 65 000 and 47 000 years ago. A recent study has found that the genomes of 100 000-year-old Neanderthal remains contain *H. sapiens* DNA. From this new discovery, it would be reasonable to conclude that

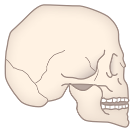
A modern Africans are descended from Neanderthals.
B between 65 000 and 47 00 years ago, the ancestors of modern Africans migrated from Europe to Africa.
C *H. sapiens* migrated out of Africa before 100 000 years ago.
D approximately 100 000 years ago, *H. sapiens* bred with Neanderthals in Africa before the Neanderthals migrated out of Africa to the rest of the world.

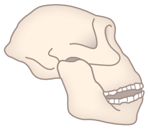
Question 15 ©VCAA 2006 EXAM 2 Q 22 **HARD**

Consider the following diagrams of skulls.


W


X


Y


Z

The skull most likely to be that of a Neanderthal is:

A W.
B X.
C Y.
D Z.

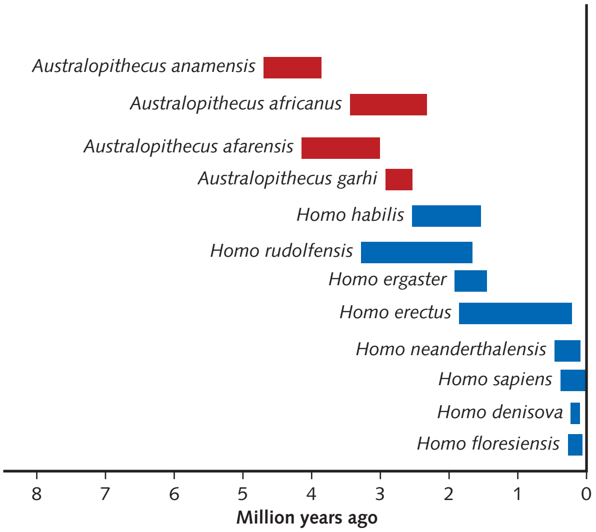
Short answer

Question 1 ©VCAA 2017 SECTION B Q7 ADAPTED

In 2013, about 1500 fossil bones of a hominin species were found in a cave in South Africa. From these bones, scientists have managed to construct an almost complete skeleton. The fossil bones have some features in common with those of the genus *Australopithecus*; however, they have enough similarities to the genus *Homo* that scientists have classified the fossil skeleton as belonging to a new species, *Homo naledi*.

a What two features would the fossil skeleton of *H. naledi* have shown to cause the scientists to classify it into a different genus from *Australopithecus*? 2 marks

Determining the age of these *H. naledi* fossils has been both difficult and controversial. A group of scientists claims that the age of the fossils is more than 2 million years and suggests that *H. naledi* might be a 'link' between *Australopithecus* and *Homo*. A second group of scientists has calculated the age of the *H. naledi* fossils to be only about 900 000 years and claims that *H. naledi* cannot be the 'link' between *Australopithecus* and *Homo*. The following diagram indicates the time periods for different *Australopithecus* and *Homo* species.



b Draw a horizontal arrow on the diagram to show where *H. naledi* would have occurred if the first group of scientists was correct.

1 mark

Question 2 ©VCAA 2009 EXAM 2 SECTION B Q4 ADAPTED

A slab of rock contained a fossil of an ancient fish form.

Alamy Stock Photo/The Natural History Museum

a What type of rock is this most likely to be?

1 mark

b List the sequence of events that led to the formation of this fossil.

2 marks

c Scientists discovered more fossils in layers below this fish fossil. They declared that these fossils were older than the fish fossil. Were they right or wrong? Explain your choice.

2 marks

Question 3 ©VCAA 2003 EXAM 2 SECTION B Q6 ADAPTED

The following table shows the number of nucleotide differences between a region of mitochondrial DNA in humans, chimpanzees and a Neanderthal.

a Based on the data in the table, list the other organisms in order of relatedness to Human 1.

2 marks

b The Neanderthal DNA was extracted from a fossil approximately 25 000 years old.

- **i** What other type of information obtained from the fossil could be used to assist in determining the evolutionary relationship of Neanderthals with humans and chimpanzees?
- **ii** One scientist suggested using potassium–argon dating to determine the absolute age of the fossil. The other scientists disagreed. Explain why they disagreed and what they would have suggested instead.
- **iii** Would dating this fossil using relative dating techniques provide a more accurate estimate of age than using absolute dating techniques? Explain your answer.

1 + 2 + 2 = 5 marks

	Human 2	Chimpanzee 1	Chimpanzee 2	Neanderthal
Human 1	15	77	76	20
Human 2		79	80	27
Chimpanzee 1			23	72
Chimpanzee 2				71
Neanderthal				

Glossary

A

α-helix a type of secondary protein structure in which the polypeptide chain folds into a tight coil

absolute dating the process of determining the age in years of rocks and the fossils they contain on the basis of the physical or chemical properties of materials in the rock

accurate without any mistakes

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

activation energy the energy required to initiate a reaction

active immunity when, after vaccination, memory cells are created that provide immunity against further exposure to antigens

active site the place on the surface of an enzyme molecule where substrate molecules attach

adaptation an anatomical, behavioural and physiological characteristic that allows an organism to exploit a specific ecological role

adaptive immune response an immune response directed against a specific antigen; it retains memory of that antigen so that, on subsequent exposure to the same antigen, it responds with a secondary response

adaptive radiation when a single species diversifies relatively rapidly into many new species because of the availability of many different ecological niches

adenosine diphosphate (ADP) a low-energy compound made of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound made of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

aerobe an organism that requires oxygen to survive and reproduce

aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell

agarose gel a gel matrix used for electrophoresis

agglutination when antigens or pathogens become stuck together because of antibody binding

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

allele a different version of the same gene (at the same locus) determined by small differences in the DNA sequence of the gene

allergen an antigen that is normally innocuous but can sometimes cause an over-reaction from the immune system known as an allergy

allergy an immune response characterised by IgE production to an innocuous substance

allopatric speciation speciation that occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species

allosteric site a binding site on a protein (usually an enzyme) that is not part of the active site; binding of a specific molecule at this site results in a change in activity of the protein

alternative splicing a process in which one or more exons are removed with the introns to produce mRNA molecules of different length and sequence

amino acid a nitrogen-containing compound that is the monomer from which proteins are built

amino acid sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide, sometimes referred to as 'polypeptide sequence'

anabolic reaction a reaction in which atoms and small molecules are joined together to make larger molecules

anaerobe an organism that does not require oxygen to survive and reproduce

anaerobic cellular respiration cellular respiration in the absence of oxygen

analogous structure an anatomical or morphological feature in different organisms that has the same function but not the same basic underlying structure

anaphylactic shock a severe allergic reaction that causes widespread swelling, including of the face and neck, which can lead to difficulty breathing and a life-threatening reaction

annealing a process used in the polymerase chain reaction to join separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic a naturally or synthetically produced compound that is toxic to bacteria

antibiotic resistance the capacity for a microbe to withstand the lethal effects of an antibiotic to which it was once susceptible

antibiotic resistance gene a gene that codes for an antibiotic resistant phenotype

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow

antibody a Y-shaped protein that binds to foreign substances that invade the body; also called immunoglobulin

anticodon the three nucleotides in tRNA that bind to the complementary codon in mRNA according to base-pairing rules, resulting in the addition of a specific amino acid to the polypeptide chain

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antigen-presenting cell (APC) a cell that displays peptides derived from processed antigens on major histocompatibility complex class II molecules for presentation to T_H cells; can be B cells, macrophages and dendritic cells

antigenic drift a change in the antigen of a virus that would otherwise be recognisable by the adaptive immune system, resulting from the gradual accumulation of mutations in the virus

antigenic shift a sudden change in the antigen of a virus resulting from the rearrangement of genetic material from two or more strains or subtypes of the virus

antiparallel parallel but orientated in the opposite direction

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cells by various enzymes, including caspases

arboreal related to, or living in, trees

artificial selection breeding of plants and animals over successive generations to produce traits that are desirable to humans; also known as 'selective breeding'

ATP synthase an enzyme that provides energy for the cell through synthesis of ATP

australopithecine a term for bipedal apes of the fossil genera *Australopithecus* and *Paranthropus* that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

authentication confirming that the submitted assessment has been completed by the student

autoantibody an antibody produced by a person's immune system that is directed against the person's own proteins

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

autotroph an organism that makes its own food from inorganic substances, using light (through photosynthesis) or chemical energy (through chemosynthesis); includes green plants, algae and certain bacteria

B

β-pleated sheet a type of secondary protein structure in which segments of the polypeptide chain bond side by side into a flattened assembly

B cell a class of lymphocyte that, once activated, produces antibodies; also called a B lymphocyte

B cell receptor a surface-bound antibody that serves as a receptor so that B cells can detect antigens

B plasma cell a cell that originates in the bone marrow and produces large quantities of antibodies

bacteria unicellular prokaryotes that can be pathogenic and therefore carry disease

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

bacteriophage a virus that can infect bacteria and replicate

base pair two complementary nitrogen bases linked by hydrogen bonding

beneficence an ethical concept that involves taking positive action that maximises the benefit or 'good', and minimises the risks and potential harms

beneficial mutation a mutation that increases the organism's chances of survival and reproduction

bias an error that occurs when an investigation is not randomised, particularly if the investigator is affected by their expectations of the outcome

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to a final product

bioethics the study of ethical issues emerging from advancements in biology

biofuel a fuel that has used biomass as its original source

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

biological functionality the function of a protein

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

biomass the total dry weight of organic material

biotechnology the use of living organisms and biological systems and processes for human benefit

bipedalism a type of locomotion in which an organism walks on two hind limbs

block mutation a mutation involving rearrangements of chromosomal segments

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

bone marrow soft tissue found inside some bones that contains stem cells that produce cells of the immune system

bottleneck effect when a catastrophic event or period of adverse conditions drastically reduces the size of a population and its genetic diversity

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

broad spectrum describes an antibiotic (or insecticide) that is effective against a variety of organisms

brow ridge a bony ridge above the eye sockets

C

C_3 plant a plant that directly uses CO_2 as an input for photosynthesis

Calvin–Benson cycle a biochemical pathway in which sugar molecules are produced using carbon dioxide

CAM (crassulacean acid metabolism) plant a plant that shuts its stomata during the day and fixes carbon during the night when its stomata are open; an adaptation to hot dry environments

cancer uncontrolled abnormal division of cells that are not kept in check by the immune system and invade other areas of the body

carbon fixation the conversion of atmospheric carbon dioxide into carbohydrates in the stroma of chloroplasts in eukaryotic cells

carrier a person who does not show symptoms of a disease but can transmit the infection to others

carrying angle the angle at which the femur is tilted in towards the knee

Cas9 protein an endonuclease that cuts double-stranded DNA at a target location in the genome

catabolic reaction a reaction in which larger molecules are broken down into smaller molecules

catalyse to speed up a biochemical reaction by using an enzyme

catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change

cell-mediated immunity an immune response initiated by cells, which does not involve antibodies

cellular metabolism the sum of metabolic reactions in a cell

cellular pathogen a disease-causing pathogen that is made up of one or more living cells such as bacteria or fungi

cellular respiration a process occurring in all living cells where large molecules are broken down to release energy

cerebral cortex the outermost layer of the brain

chemoautotroph an organism that makes its own food from inorganic substances, using chemicals as the primary energy source

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or a cell along a chemical concentration gradient either towards or away from a chemokine

chlorophyll the green pigment in plant chloroplasts; it absorbs light energy, making it available for photosynthesis

chloroplast a membrane-bound organelle containing chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates

chromosome a thread-like structure made of nucleic acids and proteins that encode genetic information

cilia slender hair-like structures projecting from a cell surface that beat against fluid

cisterna a flattened membrane disc that makes up the Golgi apparatus and endoplasmic reticulum

clade a branch of a cladogram that represents a common ancestor and all of its descendants

cladogram a phylogenetic tree that depicts a hypothesis about the evolution of a group of organisms from a common ancestor

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

codon a group of three nucleotides in mRNA that specifies an amino acid

coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction

cofactor a molecule that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions and apply new knowledge and skills

companion plant a plant that is grown with another plant because one species improves the growth of the other

comparative dating *see* relative dating

competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T and C pairs with G

condensation polymerisation a reaction in which monomers are linked together into a polymer with the release of a small molecule, such as water, as a by-product

conformation the proper or functional shape of a protein

conserved amino acids of polypeptide sequences or nucleotides of DNA sequences that remain consistent across species

control group a group in an investigation that receives no treatment (independent variable) so a baseline value can be established

controlled variable the variable that is kept constant during an investigation in order to determine the relationship between the independent and dependent variables

convergent evolution when organisms that are not closely related independently evolve similar traits as a result of having to adapt to similar environments or ecological niches

correlation the inference that rock layers located in distant sites must be of the same age if they have identical mineral and fossil composition

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

CRISPR-Cas9 a bacterial immune defence mechanism in which short RNAs target complementary sequences in viral genomes to guide Cas9 proteins to destroy an invading virus

cristae the folding of the inner mitochondrial membrane into the matrix, thus increasing the total surface area of the inner membrane

crRNA (CRISPR RNA) RNA transcribed from the CRISPR locus; guides Cas9 proteins to their complementary sequence in the invading viral DNA genome, targeting it for destruction by Cas9

cultural evolution the way beliefs, social practices, skills and technology change over time

cytochrome a family of membrane-bound proteins that carry out electron transport; located in the mitochondrial inner membrane and in chloroplast thylakoid membrane

cytokine a signalling molecule that coordinates inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

cytotoxic T cell (T_C cell) a class of lymphocyte that destroys virally infected or cancerous cells by secreting proteins that result in the extrinsic pathway of apoptosis; also called a cytotoxic T lymphocyte

D

defensin a type of small antimicrobial peptide secreted by nearly all plants and animals

degenerate a property of the genetic code in which most amino acids are encoded by two or more codons

degranulation a cellular process in which the granules of neutrophils, mast cells, basophils or eosinophils are emptied into extracellular surroundings

deleterious mutation a mutation that decreases the organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

denature to permanently change the molecular structure of a protein or DNA

dendritic cell a phagocyte with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system

Denisovan a distinct, but undescribed, ancient hominin known primarily from bone fragments found in Denisova Cave in Siberia

dentition arrangement of the teeth

deoxyribonucleic acid (DNA) the information molecule that is the basis of an organism's genetic material

dependent variable the variable that is measured and whose value depends on the independent variable, i.e. it responds to the independent variable

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

disease any condition that interferes with how an organism, or any part of it, functions

divergent evolution when members of a population develop adaptations to the different selection pressures over many successive generations and eventually become new species

DNA ligase an enzyme that catalyses the formation of a phosphodiester bond between two pieces of DNA

DNA polymerase the enzyme that catalyses the bonding of nucleotides to form new strands of DNA

DNA profiling comparison of individuals based on patterns of non-coding base sequences in the genome

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

double-strand break a mutation involving breaks in the sugar-phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

duplication a mutation that occurs when one or more extra copies are made of a section of chromosome

E

electron spin resonance a method for determining the age of a rock or fossil based on the properties of electrons trapped inside the crystals of minerals

electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the addition of phosphate of ADP to yield ATP

endemic restricted or native to a certain locality

endergonic reaction a chemical reaction that requires the input of energy for it to proceed

endoplasmic reticulum an organelle made up of a network of membranous tubules involved in protein synthesis and folding for secretion

endothermic reaction a reaction that absorbs energy from its surroundings

enzyme a specific protein catalyst that increases the rate of a biochemical reaction within the cell by lowering the amount of energy required for the reaction to proceed

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular organisms

epidemic the rapid spread of a disease across a number of countries

epitope a small part of a larger molecule that binds to a receptor site such as B cell receptors and T cell receptors

ethics a system of moral principles that considers what is good and bad for society

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

exergonic reaction a spontaneous reaction that releases energy

exocrine gland a gland that secretes a substance through a duct to the outer surface of the body

exon a segment of DNA or RNA containing information that codes for a polypeptide or part of a polypeptide

exothermic reaction a chemical reaction that releases energy, usually in the form of heat or light

extinct when all the members of a population or species have died out

extraneous variable a variable, other than the independent variable, that can influence the dependent variable

F

$FADH_2$ the loaded form of flavin adenine dinucleotide, a coenzyme that acts in both cellular respiration and photosynthesis

feedback inhibition a control mechanism used by cells in which an enzyme's activity is stopped or reduced by the product

first line of defence physical and chemical barriers that keep pathogens from entering the body of a living thing

fitness the capacity of an individual to survive and produce viable offspring

fixed describes an allele when it is the only variant available for a particular gene in the gene pool of a population

flagellum a helical filament that rotates to give bacteria locomotion

foramen magnum the hole in the base of the skull through which the spinal cord passes

fossil the preserved remains or traces of an organism

fossil record the worldwide collection of fossils as they occur in the surface layers of Earth

fossil succession when fossils appear in a consistent order in the fossil record from older rock layers to younger overlying rock layers; the same order is found worldwide

founder effect the type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

frameshift mutation a mutation in DNA caused by the addition or deletion of a nucleotide or nucleotides resulting in a change in the amino acid sequence and protein being made

functional proteomics the study of how proteins work together in different cells or tissues, or under different circumstances

fungus a heterotropic organism made up of one or many cells; has cell walls but is not a plant

G

gel electrophoresis a technique that separates DNA fragments according to their size and charge

gene a segment of DNA in a chromosome that codes for a polypeptide; comprises the promoter, exons and introns

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

gene duplication generating an extra copy of a gene within a genome as a result of duplication of a chromosomal segment

gene expression the process by which the information in a gene is turned into a polypeptide

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

gene regulation the process by which gene expression is switched on or off

gene sequence the sequence of nucleotides in a gene

genetic code the complete set of mRNA codons and the corresponding amino acids they specify

genetic drift the change in the gene pool of a population as a result of chance; usually occurs in small populations

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organism (GMO) an organism whose genome has been genetically engineered

genome the complete sequence of DNA in a single (haploid) set of an organism's chromosomes, including nuclear, mitochondrial and chloroplast DNA

genotype a specific combination of alleles for a particular gene locus belonging to an individual

germline a cell line in eukaryotic organisms from which the gametes are derived

glycogen an energy-storage polysaccharide in animals that is made of many connected glucose molecules

glycolysis an energy-yielding process occurring in the cytosol of cells in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

Golgi apparatus a collection of membranes that package and store substances into vesicles in preparation for their release from the cell

gracile of slender build

gradient the slope of a graph

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

granulocyte a white blood cell that has granules in the cytoplasm

guide RNA RNA that guides the Cas9 protein to the target sequence in a genome for gene editing

H

hallux the big toe, or innermost toe of the foot

haplogroup a group of organisms that have the same genetic mutations in a single chromosome and are descendants of a common ancestor through either the maternal (mtDNA) or the paternal (Y chromosome) line of inheritance

haplotype the unique combination of genetic mutations in the DNA sequences of a single chromosome (e.g. mtDNA or Y chromosome) that are shared by different organisms and indicate common ancestry

helper T cell (T_H cell) a lymphocyte that assists cytotoxic T cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling; also called a helper T lymphocyte

herd immunity when unvaccinated individuals are protected against a disease because a large number of people between 60–95% depending on the disease have been vaccinated, thereby making it unlikely that unvaccinated people will come into contact with anyone suffering from the disease

heritable capable of being passed on to the next generation

heterotroph an organism that cannot make its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

histone a protein that binds and packages DNA in eukaryotic chromosomes

hominin a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a member of the superfamily Hominoidea; an ape, or tail-less primate

homologous refers to genes or polypeptides that have similar sequences and indicate a shared evolutionary ancestry

homologous structure an anatomical feature in different organisms that has the same basic underlying structure but different functions

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

host the organism in which a parasite lives

humoral immune response an adaptive immune response mediated by antibodies

hybridoma a cell involved in the production of large amounts of monoclonal antibodies

hydrogen bond a weak chemical bond between a hydrogen atom on one molecule and a more electronegative element, usually an oxygen or nitrogen atom, on another molecule

hydrophilic describes substances such as polar molecules and ionic compounds that dissolve readily in water

hydrophobic describes substances such as non-polar molecules that are insoluble in water

hypothesis a tentative prediction, or explanation of an observation, based on an existing model or theory

I

immune having resistance to infection by a specific pathogen

immune system a complex network of cells, tissues and organs in the body that detect differences between self-molecules and foreign (non-self) organisms, and mounts an immune response that results in the formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

immunisation the process of making a person immune to a disease through vaccination

immunoglobulin (Ig) a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called antibody

immunotherapy boosting the ability of a person's own immune system to fight cancer

independent variable the variable changed or manipulated by the scientist and assumed to have an effect on the dependent variable

index fossil a fossil that is representative of a specific geological time

induced-fit model a model of enzyme action that explains that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

inducer a signalling molecule that switches on expression of a gene

infectious disease a disorder caused by bacteria, viruses, fungi and other organisms, that can often be transmitted to other members of a population

infectivity the ability of a pathogen to spread from one host to another host

inflammation an innate response to infection or damage that causes pain, swelling, heat and redness

innate immune response a response to a pathogen that is not specific to the antigen, only that it has been identified as being non-self; the response does not generate antibodies or memory lymphocytes

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

integrity an ethical concept that means being honest about one's actions; in science it means fully reporting data (even if it doesn't fit your hypothesis) and acknowledging all sources of information

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

interleukin a subset of cytokines that assists with the coordination of cells involved in the immune response

interstitial fluid a fluid that lies between cells; also known as tissue fluid or extracellular fluid

intron a segment of DNA within a gene or pre-mRNA that does not code for a polypeptide and interrupts the sequence of a gene

inversion mutation a mutation resulting in the normal sequence of genes being reversed in a chromosome

isotope one of two or more atoms of the same element with the same atomic number and number of protons, but different numbers of neutrons and therefore different relative atomic masses

J

justice a moral obligation to give fair consideration to competing claims, not place unfair burden on a particular group, and ensure fair access and distribution of benefits of an action

K

keratin the tough, fibrous protein of the outer epidermis layer

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

Koch's postulates a set of criteria to determine the causative agent of a disease

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl CoA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

kya thousands of years ago

L

lactic acid a product of anaerobic cellular respiration in animals

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

latent not active

leukocyte the general term for a white blood cell

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll to split water molecules to produce oxygen, hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions, carbon dioxide, hydrogen ions and ATP produce carbohydrate

limiting factor the factor that limits the rate of a reaction

lineage in evolution, a population that represents a separate line of descent from a common ancestor to modern species

loaded describes coenzymes that are attached to the specific group of atoms they transfer

lock-and-key model a model of enzyme action that suggests that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

logbook a record of experimental investigation kept by scientists performing the investigation; it is a legal record of the investigations and their results

luminescence in absolute dating, a method for determining the age of a mineral crystal based on measuring the emission of light by electrons as they are stimulated to escape from the crystal

lymph a colourless fluid that originates from tissue fluid

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes to present to lymphocytes and initiate an adaptive immune response

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and removing foreign matter

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

M

macrophage a large white blood cell that phagocytoses pathogens; originates as monocytes in circulation

major histocompatibility complex (MHC) protein markers found on cell surfaces that are important in distinguishing self from non-self; MHC class I is found on all cells and MHC class II is found only on antigen-presenting cells

mammal a warm-blooded vertebrate animal that has hair or fur, and the females secrete milk to nourish their offspring

mammary gland a gland in female mammals that produces milk

mandible the lower jawbone of the skull

mass extinction the extinction of many species over a relatively short (geological) period

mast cell a cell that is located in the tissues and releases granules containing histamines when activated

maternally inherited describes a genotype that is transmitted entirely from the female parent to the offspring

matrix a gel-like fluid in mitochondria, where the Krebs cycle (citric acid cycle) of cellular respiration takes place

memory cell a long-lived lymphocyte capable of responding to a particular antigen when it is reintroduced; made from B cells and T cells

messenger RNA (mRNA) RNA copied from DNA that conveys the instructions needed for polypeptide synthesis from the nucleus to the cytoplasm

method the steps taken to carry out a scientific investigation

methodology the broader framework of approach taken in the investigation to test your research question

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

microbiome the bacteria, viruses and fungi that live in the gut plus their released metabolites and nucleic acids that exist in a specific environment

microbiota a community of micro-organisms, including fungi and bacteria, that live in or on another organism

microflora *see* microbiota

midden a pile of discarded waste left by humans in the past

mineralisation the process by which minerals from sediments have replaced the biological matter in a deceased organism, making it prone to become a fossil

missense mutation a mutation that results in one amino acid being replaced by another amino acid in the encoded protein

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

model a representation of a system or phenomenon that explains the system or phenomenon; a model may be mathematical equations, a computer simulation, a physical object, words or some other form

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

monoclonal antibody a laboratory-produced molecule that serves as a substitute antibody to fight cancerous cells

monoculture the practice of cultivating a single genetically uniform breed of plant or livestock

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

morphological species concept usually applied to fossils, defines a species by measurable anatomical criteria and characteristics

mucosal-associated lymphoid tissue (MALT) an extensive system of lymphoid tissue deposited all over the body; initiates immune responses along mucosal areas such as gastrointestinal tract, eyes and lungs

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

multidrug resistance when a bacterium becomes resistant to two or more antibiotics

mutagen an agent capable of inducing mutations

mutation when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

mya millions of years ago

N

NAD$^+$ the unloaded form of the nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

NADH the loaded form of nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

NADP$^+$ the unloaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

NADPH the loaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

natural killer cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

natural selection the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals

necrosis cell death that results from tissue damage or infection when the plasma membrane is breached; results in inflammation

neutral mutation a mutation that has no effect on the organism's chances of survival and reproduction

neutralisation the process by which antibodies prevent toxins from acting by binding to them and blocking them from binding to their targets

neutrophil a phagocytic leukocyte found in the blood and tissues

niche an organism's ecological role; the way the organism lives and functions in its environment

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

non-cellular pathogen a disease-causing pathogen that is not made of living cells; for example, viruses and prions

non-coding region DNA that does not encode a protein sequence

non-template strand the DNA strand that has the same sequence of nucleotides as the mRNA (except it has T instead of U)

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

non-maleficence an ethical concept that involves avoiding harm or ensuring that harm caused by action is proportionate to the benefit gained from the action

non-self antigen a molecule that is not recognised by the immune system as being part of the organism itself

non-specific response a response that is the same regardless of the type of antigen

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

nucleic acid a large, linear polymer built from nucleotide monomers bonded together; includes DNA and RNA

nucleosome a histone with a length of DNA coiled around it

nucleotide the monomer, or building block, of DNA and RNA, consisting of sugar, phosphate and a nitrogen base

O

obligate parasite a parasite that cannot complete its life cycle without a suitable host; without a host, the parasite cannot reproduce

observation acquisition of information through your senses

operator a segment of DNA to which a protein binds, usually to switch off gene expression

operon a group of genes that are expressed as a single unit

opsonisation the process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

optically stimulated luminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is exposed to coloured light

optimum pH the pH at which an enzyme works fastest

optimum temperature the temperature at which an enzyme works fastest

Out of Africa hypothesis *see* recent single origin

outlier a data point that does not fit the pattern shown by the other measured data points

oviparous an animal that lays eggs that are expelled from the body and from which juveniles hatch

P

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

palaeoanthropology the field of study concerned with fossil hominins

pandemic the spread of a disease across the world

passive immunity immunity characterised by the transfer of antibodies from one individual to another; does not generate immunological memory

pathogen an organism foreign to the body and capable of causing disease

peptide bond a chemical bond that links two amino acids in a chain

personal error a mistake or miscalculation due to human error

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis a process by which phagocytes engulf a particle or cell

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

phosphodiester bond a chemical bond that links two nucleotides in a growing chain

photoautotroph an organism that makes its own food from inorganic substances, using light as its primary energy source

photorespiration the process in which plants take up oxygen and release carbon dioxide

photosynthesis the anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny the evolutionary relationships that exist between species, often expressed as a tree-like diagram or represented by taxonomic classification

phylogram a type of phylogenetic tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

pigment a molecule that absorbs certain wavelengths of light and reflects all others

placental describes an animal in which the foetus develops inside the mother while attached to an organ that provides nutrients and oxygen and removes wastes

plasmid a small, circular DNA structure independent of the chromosome in prokaryotic cells

platelet a cell fragment found in the blood involved in blood clotting

point mutation a mutation that affects a single base-pair position within a gene

poly-A tail a chain of 100–200 adenine nucleotides added at the 3′ end of an mRNA strand

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

polymorphism a variation in DNA sequences among individuals

polypeptide a linear polymer built from amino acid monomers

polyribosome a chain of ribosomes formed by attaching to and translating from a single mRNA strand

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time

postcranial all of the skeleton, except the skull

pre-mRNA an unprocessed RNA strand that is transcribed directly from the DNA

precise how closely together measurements are to one another

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manipulate an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of curling and grasping

primary data data that you have measured or collected yourself

primary host an organism in which a pathogen reproduces

primary lymphoid organ the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

primary source an original source of information, created by the author and usually including primary data

primary structure the linear sequence of amino acids that makes up a polypeptide chain

primate a member of the order Primata; includes lemurs, lorises, tarsiers, monkeys, apes and modern humans

primer a single-stranded DNA molecule that acts as the start of the amplification process

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

product the outputs of a chemical reaction that are formed from the reactants or inputs

prognathism a condition in which the jaws protrude from the plane of the face

promoter region a segment of DNA to which RNA polymerase binds to begin transcription

protein a polymer made up of amino acid monomers; may consist of a single polymer chain or many polymers bonded together into a functional molecule

protein secretory pathway the pathway through which cells package proteins into vesicles for release into the extracellular environment

proteome the complete set of proteins produced by a cell, a tissue, or an organism

proteomics the study of proteomes

protist a unicellular eukaryotic organism

pyruvate a three-carbon molecule that is the end product of glycolysis

Q

quadrupedalism a type of locomotion in which an organism walks on four limbs

qualitative data a measurement with descriptive or nonnumerical results

quantitative data a measurement with numerical values

quarantine restricting the mobility of person or persons to a certain area so they reduce contact with other people in order to stop the spread of a pathogen

quaternary structure the structure formed when two or more polypeptides associate into a mature protein

R

radioactive decay a process by which the nucleus of an unstable isotope splits and emits energy in the form of radioactivity

radiometric dating a method for determining the age of a rock or fossil based on the predictable rates of decay of naturally occurring radioactive isotopes present

random coil a secondary protein structure in which the polypeptide chain does not fold into a specified arrangement

random error an unpredictable variation in measurement; can be improved by taking multiple measurements and calculating an average

reactant the inputs of a chemical reaction that are required to form products or outputs

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents; also known as Out of Africa hypothesis

recombinant DNA technology the process of transferring a gene from a cell of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

references a list of all the sources that have been used in the write-up of a scientific investigation

regulatory gene a gene whose product switches on or switches off expression of one or more other genes

regulatory T cell (T_{reg} cell) a class of lymphocyte that helps to negatively regulate the immune response; also called a regulatory T lymphocyte

relative dating the process of determining the age of rocks and the fossils they contain relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

reliable highly likely to be a trustworthy source of information or reproducible data

repeatable an investigation that can be conducted again by the same investigator under the same conditions to generate similar results

replicates independent samples that allow you to take multiple measurements, increasing the reliability of your data

reporter gene a gene that enables visualisation or quantification of gene expression

repressor protein a protein that binds DNA to prevent RNA polymerase attaching or transcribing; essentially shuts off gene expression

reproducible giving the same result within uncertainty limits; when repeated measurements are made by a different investigator

reproductively isolated when sexual reproduction can no longer occur freely among any adult members of the population

research question a specific question that a particular investigation is attempting to answer

respect an ethical concept that considers the rights of an individual or a group; e.g. respect for animals considers their welfare

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

ribonucleic acid (RNA) a type of nucleic acid consisting of a single strand of nucleotides; has essential roles in protein synthesis

ribosomal RNA (rRNA) an RNA strand that serves as a structural component of a ribosome

ribosome a small structure consisting of RNA and proteins where amino acids are joined to form polypeptides

risk assessment a process of evaluating potential risks of an investigation

RNA polymerase the enzyme that catalyses the synthesis of RNA

robust of sturdy build

rough endoplasmic reticulum endoplasmic reticulum with ribosomes attached

S

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

sebaceous gland a gland in the skin that secretes oils that lubricate and protect patches of skin and hair

second line of defence non-specific immune responses including fever and inflammation

secondary data data that has been measured and collected by someone other than you

secondary host an organism in which the immature pathogen becomes mature

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and mucosal-associated lymphoid tissue

secondary metabolite an organic compound produced by bacteria, fungi or plants; its role is to sustain functional and homeostatic health of cells within organs by assisting cells to excrete wastes and toxic substances

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

secondary source a source of information that has been obtained from another source and/or summarised, e.g. a popular science magazine

secondary structure the localised folding of a polypeptide chain when neighbouring amino acids bond to each other to form α-helices, β-pleated sheets or random loops

selection pressure a factor that favours the survival of some individuals over others within a population

selective breeding *see* artificial selection

self-antigen an antigen or a molecule that is a normal body component

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self-antigens to prevent an immune response to these antigens

semi-conservative replication the replication of DNA in which the product contains one original and one newly made strand

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

sexual dimorphism where males and females of a species

short tandem repeat (STR) a short non-coding region of DNA of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be used in DNA profiling

silent mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a synonymous mutation

somatic describes a body cell that will not pass its genes on to the next generation

speciation the evolution of one or more new species from an ancestral species

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same; also describes the lowest formal taxonomic rank and forms the second part of an organism's scientific name

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

spleen an abdominal organ that stores white blood cells

sporadic seen infrequently in a small number of people

stereoscopic describes vision that has a sense of depth

sterile inflammation inflammation resulting from the detection of damage- or danger-associated molecular patterns released during tissue injury in the absence of infection

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

strata (singular: stratum) successive layers of sedimentary rocks; each layer represents a unique age range and contains a unique collection of fossils

stroma the jelly-like, semifluid interior of a chloroplast

structural gene a gene that codes for tRNA, rRNA or a polypeptide other than a regulatory molecule

structural morphology the study of the physical structure and form of organisms

subspecies the level of classification below species, referring to races of a species that are geographically isolated from each other

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

substrate the substance that an enzyme acts on

subunit a distinct component of a biological particle; in proteins, it refers to each polypeptide that contributes to the quaternary structure

superfamily a taxonomic rank immediately superior the traditional rank of family; a superfamily may contain multiple taxonomic families

superposition the principle that strata are deposited in a time sequence, with the oldest at the bottom and the youngest at the top

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

sweat gland a gland in the skin that secretes sweat

sympatric speciation when two species evolve from an ancestral population while still inhabiting the same geographical area

synonymous mutation *see* silent mutation

systematic error a predictable deviation in data, e.g. as a result of the equipment used

T

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens presented on major histocompatibility complex proteins

T-cell transfer therapy a therapy that boosts the ability of T cells to fight cancer cells

taxonomy a system of scientific conventions for naming and classifying organisms

template a pattern that can be used for making many more copies

template strand a strand of DNA that is copied during DNA or RNA synthesis

tertiary structure the overall three-dimensional shape of a completely folded polypeptide

tetrapod a 'four-footed' vertebrate animal; includes amphibians, reptiles and mammals

theory a collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

thermoluminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is heated

thylakoid membrane the interconnected, folded membrane within a chloroplast

thymus the gland in the upper chest where T cells mature

trace fossil a fossil produced by the organism's activities, such as fossil footprints or burrows

transcribe to copy DNA into mRNA

transcription the process by which DNA is copied into mRNA

transcription factor a protein that binds to DNA to control the rate of transcription from a gene

transfer RNA (tRNA) an RNA molecule that transports an amino acid to the ribosome for assembly into a polypeptide

transformation the process by which the genetic material of an organism is changed by the addition of new genetic material.

transgenic organism an organism that has been modified by incorporating a piece of foreign DNA into its genome

transitional fossil a fossil that bears features of both an older ancestral life form and a younger descendant

translation the process of turning the nucleotide sequence of mRNA into the amino acid sequence of a polypeptide

translocation a mutation occurring when a section of one chromosome breaks off and reattaches to another chromosome

transmitted when an infection is passed from one person or organism to another

transport vesicle a small membrane-bound sac containing protein that is transported from the Golgi apparatus to the plasma membrane for release into the extracellular environment

tribe a taxonomic rank inserted between family and genus

triplet a set of three nucleotide codes

true value a value obtained in an ideal measurement

tumour a mass of abnormally growing cells

U

uncertainty a range of values that the true value falls within

unloaded can accept protons, electrons or chemical groups that are released from catabolic reactions

V

vaccination the administration of a vaccine to protect someone from a disease

valid describes results that are affected by only a single independent variable and hence are reproducible

variable something that can change or be changed, as distinct from a constant, which does not change

vasodilation widening of blood vessels, particularly arterioles

vector a vehicle used to transfer DNA sequences from one organism to another

vestigial structure a structure found in organisms that has lost most, if not all, of its original function in the course of evolution; in ancestral organisms, the structures served a purpose, but in their descendants, the structures become atrophied or rudimentary

virulence the capacity of a pathogen to cause severe disease within its host

virus an obligate intracellular pathogen that can use the host cell's machinery to replicate itself; usually consists of a nucleic acid surrounded by a protein coat

viviparous an animal that gives birth to live young

W

wild type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

Z

zoonotic describes a disease that can be transmitted from animals to humans

Answers

Unit 3 Area of Study 1 review

Multiple choice

1 A
2 A
3 D
4 D
5 C
6 A
7 A
8 B
9 A
10 D
11 A
12 A
13 D
14 B
15 A

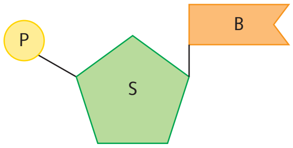
Short answer

1 **a** RNA polymerase
  b To carry a copy of the instruction for polypeptide/protein production from the DNA in the nucleus to the ribosome in the cytosol.
  c Processing (any two of): intron removal or exons joined; addition of a methyl cap/guanine cap; addition of poly-A tail; add 5' cap.
  d Different factors expressed by regulator genes could lead to production of the different proteins OR Alternate splicing of the pre mRNA means that exons are joined in a different order, creating a different base sequence that would lead to the translation of a different protein.
  e The genetic code being universal

2 **a** The ribosome binds to or reads the mRNA; tRNA brings in specific amino acids; or the tRNA anticodon is complementary to the mRNA codon. The amino acids are joined by condensation polymerisation.
  b Any three of the following:

Organelle	Role
Rough endoplasmic reticulum	Transports trypsin within cell, including to the Golgi apparatus
Golgi apparatus	Packages trypsin into vesicles for export from the cell
Vesicles	Carries trypsin to plasma membrane where it fuses and releases the trypsin from the cell
Plasma membrane	Vesicles fuse with it and are released by exocytosis
Mitochondria	Provides the energy required e.g. for packaging

3 **a** mRNA: C A C U G U A A U G A G
  b 30%
  c **i** ribosomes
    ii rRNA (ribosomal RNA) associates with proteins to from ribosomes
  d Histidine, cysteine, asparagine, glutamic acid
  e



Unit 3 Area of Study 2 review

Multiple choice

1 C
2 B
3 C
4 A
5 D
6 B
7 B
8 D
9 D

10 B
11 D
12 B
13 A
14 B
15 D

Short answer

1 a Oxygen levels decreased as the yeast cells were respiring aerobically
  b Anaerobic respiration.
  c Oxygen levels would stay the same as yeast cells would switch over to anaerobic respiration. Ethanol levels would rise as ethanol is a by-product of anaerobic respiration.

2 a Light-independent stage or Calvin (Benson) cycle
  b Light-dependent stage; Input: light, water
    Role: to provide energy to generate energy molecules such as ATP from ADP + Pi
    Input: ADP or NADP$^+$; provides H+ needed to produce NADPH
    Role: to carry energy from the light-dependent reaction to the light independent reaction

3 a Rubisco
  b To carry energy needed for the reactions in the light independent reactions to occur. NADPH also provides the hydrogen ions needed to make glucose
  c Glucose

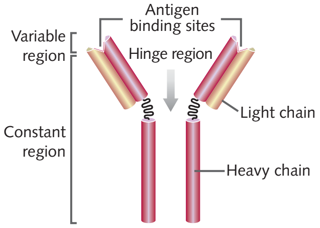
Unit 4 Area of Study 1 review

Multiple choice

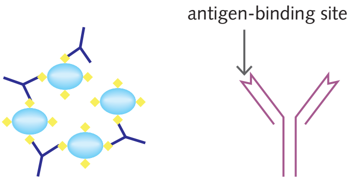
1 C
2 B
3 C
4 D
5 B
6 B
7 C
8 C
9 A
10 A
11 C
12 B
13 B
14 C
15 D

Short answer

1 a A pathogen is an contagious/infectious disease causing agent 1 mark.
  b 2 marks



  c i 2 marks



    ii The antigen–antibody complex either immobilises the pathogen, agglutinates the pathogen or allows phagocytes to engulf the pathogen.

2 a Thick waxy cuticle on leaves and stems or thick bark on trees that act as a barrier to the entry of pathogens. Thorns or hairs to deter insects (2 × 1 mark)
  b Two of the following: Intact skin; nose lined with thick, sticky mucous; fine nasal hairs to trap pathogens (2 × 1 mark)
  c Macrophages engulf pathogens; dendritic cells engulf pathogens, process them and present them to other cells of the immune system. (2 × 1 mark)

3 **a** Lymph or lymphatic fluid (1 mark)

b Filter the lymph for foreign particles and invading pathogens; activation of white blood cells causing the influx of more white blood cells; and enlargement of lymph node (2 × 1 mark)

c B cells produce antibodies; rough endoplasmic reticulum produces proteins that make up antibodies. (2 × 1 mark)

Unit 4 Area of Study 2 review

Multiple choice

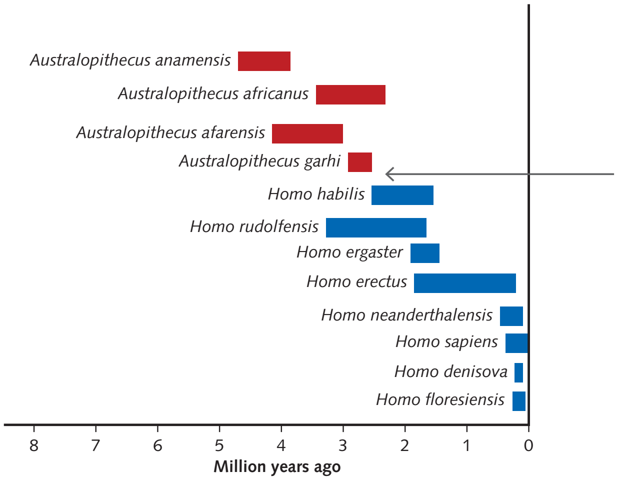
1 B
2 D
3 C
4 B
5 C
6 B
7 D
8 A
9 D
10 A
11 B
12 A
13 A
14 C
15 A

Short answer

1 **a** Two of the following suggested answers.

The skull shows: a flatter face, a more parabolic jaw, a relatively larger brain case or teeth of a more uniform shape. The skeleton shows upper limbs/forelimbs shorter and hind limbs/legs longer. The features needed to be comparative.

b



2 **a** Sedimentary rock

b Organism must have hard body parts; is covered quickly; high pressure to promote mineralisation; low oxygen conditions to stop decay (1/2 mark each)

c Correct. Layers of sediment build up over time; the oldest fossils are found in the lowest stratum; the youngest at the highest stratum

3 **a** Human 1, Human 2, Neanderthal, Chimpanzee 2, Chimpanzee 1

b **i** Skeletal structure/morphology may be used to assist in determining the evolutionary relationship of Neanderthals with humans and chimpanzees.

ii The fossil is only 25,000 years old, it would be more accurate to use carbon dating to date the fossil. Carbon dating compares the ratio of carbon 14 to carbon 12 and the known rate of carbon 14 decay can be used to find the age of the fossil.

iii Absolute dating is more accurate than relative dating. Absolute dating uses know decay rates of parent isotopes to daughter isotopes to find the absolute age of a fossil. Relative dating uses rock layers to determine if a fossil is older or younger than other fossils or the presence of index fossils to give an age range only.

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