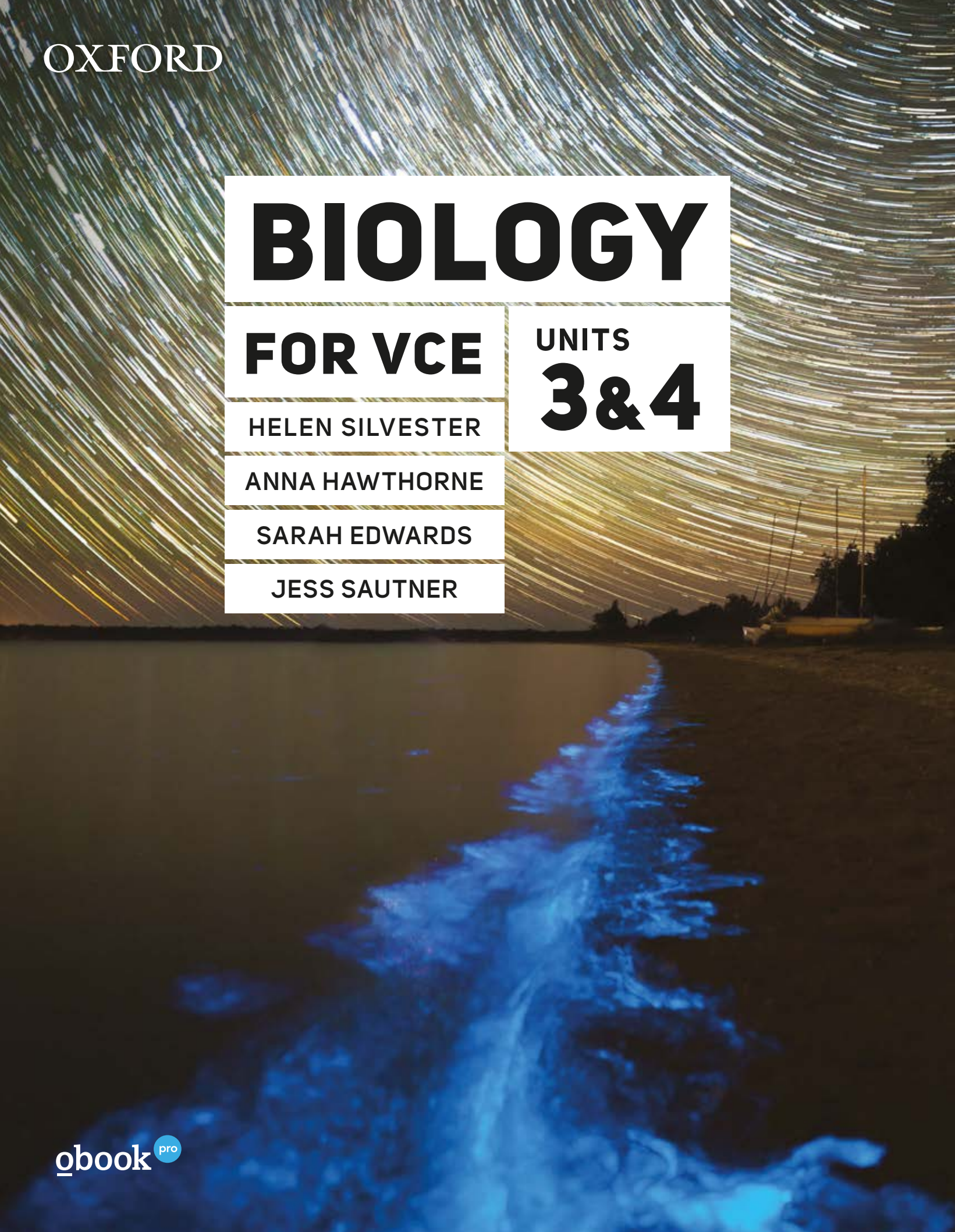

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CONTENTS

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Helen Silvester has been an educator for over 20 years, is extremely passionate about writing and reviewing, and has held Head of Science positions in a number of schools. She was shortlisted for the 2014 and 2017 Prime Minister's Secondary Science Prize for Excellence in Science Teaching in Secondary Schools and was Victoria's representative for the BHP Billiton Science Teachers Awards. Helen has also worked as a researcher at Walter and Eliza Hall Institute and the Royal Children's Hospital. Currently, Helen is Director at STEAM-focused Casey Tech School and a reviewer and moderator of VCE Biology exams.

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Dr Gary Simpson

Dr Gary Simpson has a wealth of experience in teaching VCE Biology, Environmental Science and Geography. He currently teaches Junior Science 9–10 at Woodleigh High School and was previously Head of Science. Gary is an exam assessor for Biology and Geography, a Panel Chair for Environmental Science, and a co-author of the VCE Biology Study Designs 'Units 1 & 2 2016–2020' and 'Units 3 & 4 2017–2021'. Gary received ACEL's inaugural Teacher Leader Award in 2007 and was inducted into the Zoos Victoria Hall of Fame in 2015.

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Dr Kyi Muller is a highly trained biology researcher and educator with over 8 years teaching experience. She has a doctorate in marine biology and has worked at Haileybury for 4 years as Head of Science and the Director of Innovation and Entrepreneurship. Kyi now works as a learning designer at Monash University. She is extremely passionate about science education and was previously the Program Director for Curious Minds – an Australian Government initiative to increase access for girls to STEM education. Kyi was also awarded the 2019 Graeme Clarke Science Innovation in Schools Award.

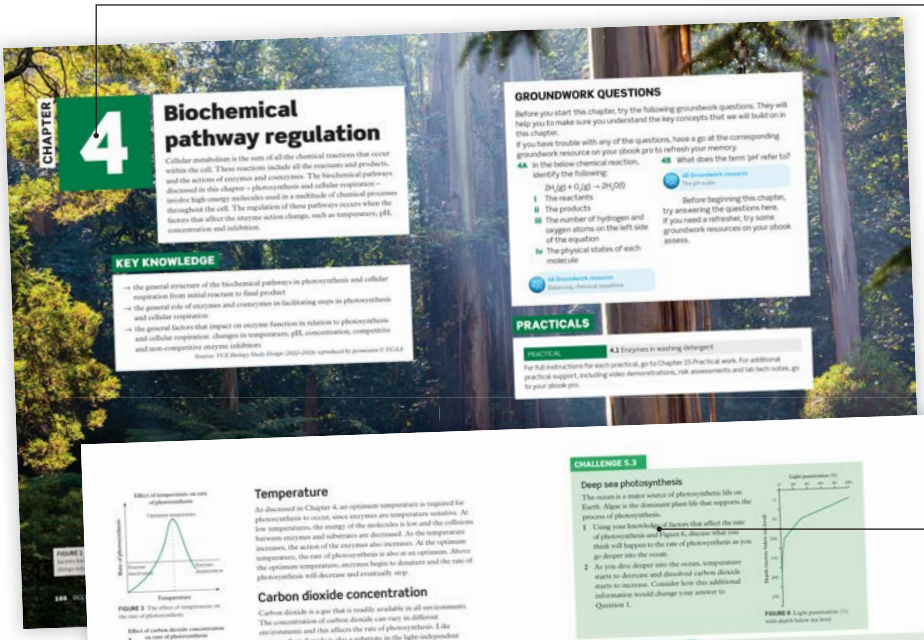
Dr Ngaree Blow

Dr Ngaree Blow is a Noonuccal, Goreng-Goreng, Yorta-Yorta woman living on Wurundjeri country. She is currently working as the Director of First Nations Health for medical education at the University of Melbourne, as well as completing research in the Intergenerational Health team at the Murdoch Children's Research Institute (MCRI). Ngaree completed both her Doctor of Medicine and Masters of Public Health (MD/MPH) degrees concurrently and has a keen interest in public health and paediatric medicine. Ngaree is also a board member of the Australian Indigenous Doctors Association (AIDA) and has been involved in many First Nations health, research and education roles.

Biology for VCE Units 3 & 4 has been developed for the VCE Biology Study Design for 2022–26. This new series offers a suite of resources including Student Books, Workbooks and digital resources that offer teachers and students a clear pathway to VCE Biology success.

Student Books

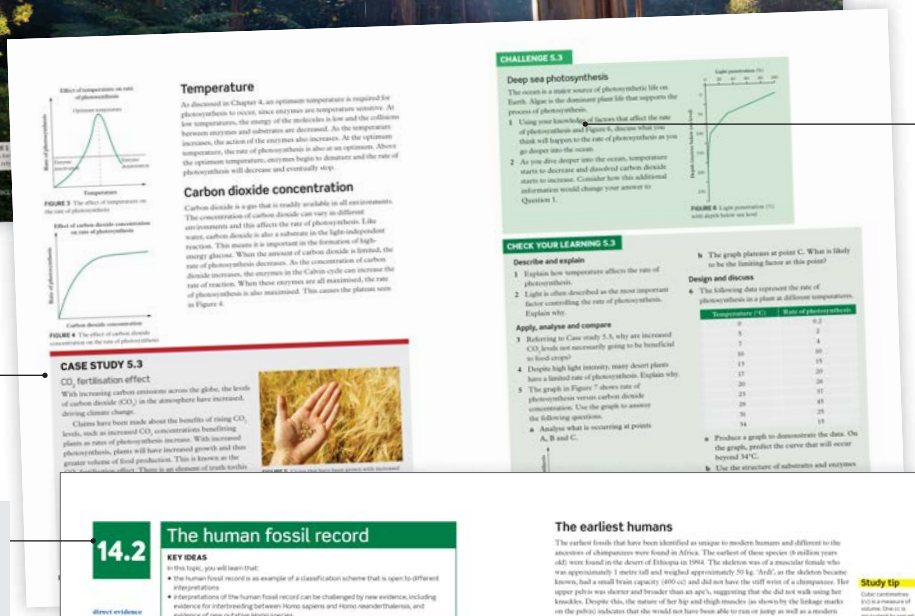
The Biology for VCE Student books feature a clear and engaging design, with targeted on-page features to support student understanding and preparation for VCE success.



Chapter opener

Each chapter begins with a chapter opener that includes:

- Key Knowledge from the Study Design
- Groundwork questions to test and support students' assumed knowledge
- a list of no-tech, standard and high-tech practicals to support key concepts



Challenge questions

Extension questions and scenarios encourage critical thinking.

Worked examples

Detailed worked examples take students through how to solve different problems.

Case studies

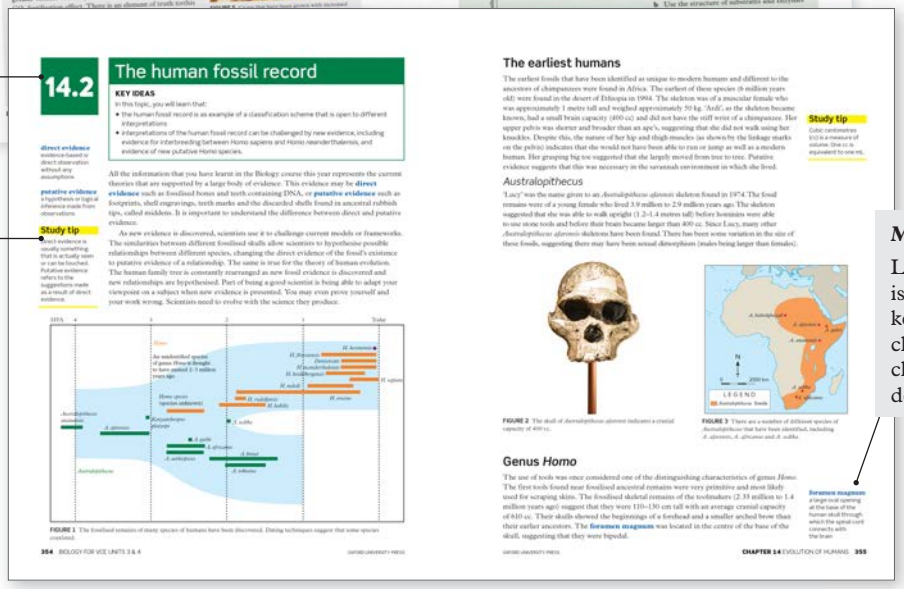
Real-life examples provide opportunities to apply key knowledge.

Topic-based approach

Content is structured in clear topics with key ideas signposted at the beginning

Study tips

Practical tips support student success in SACs and exams.

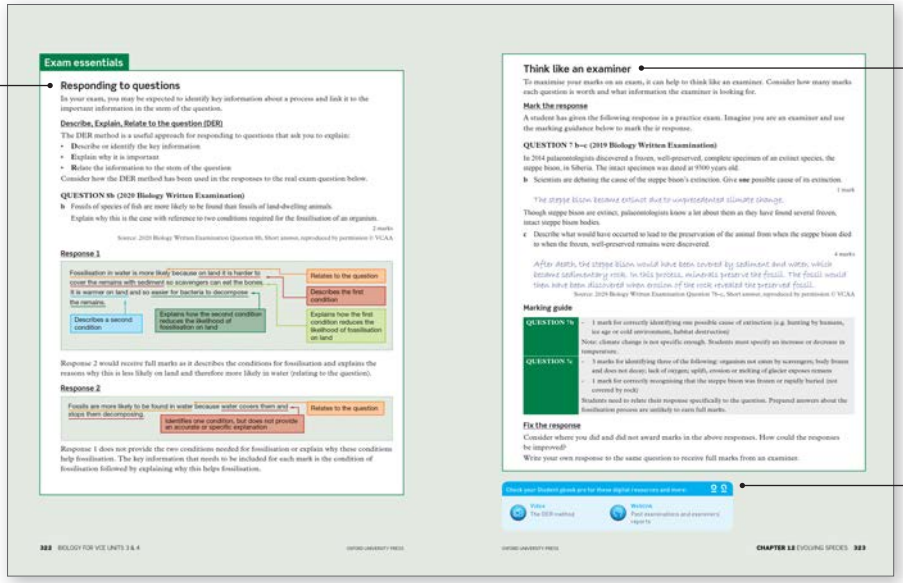


Margin glossary

Literacy support is provided for key terms in the chapter with clear and concise definitions.

Responding to questions

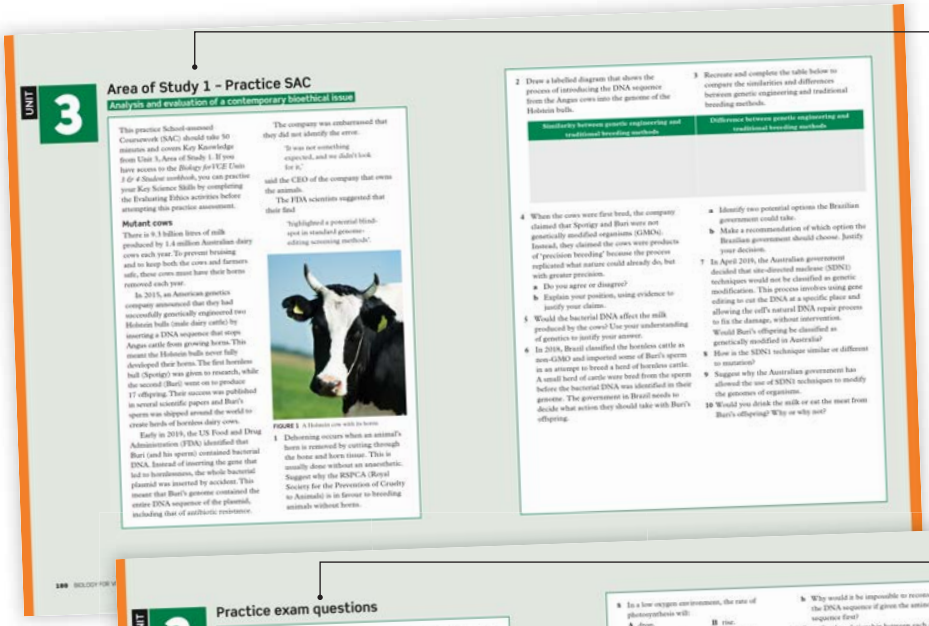
Provides tips and strategies for maximising marks on exam responses.



Think like an examiner

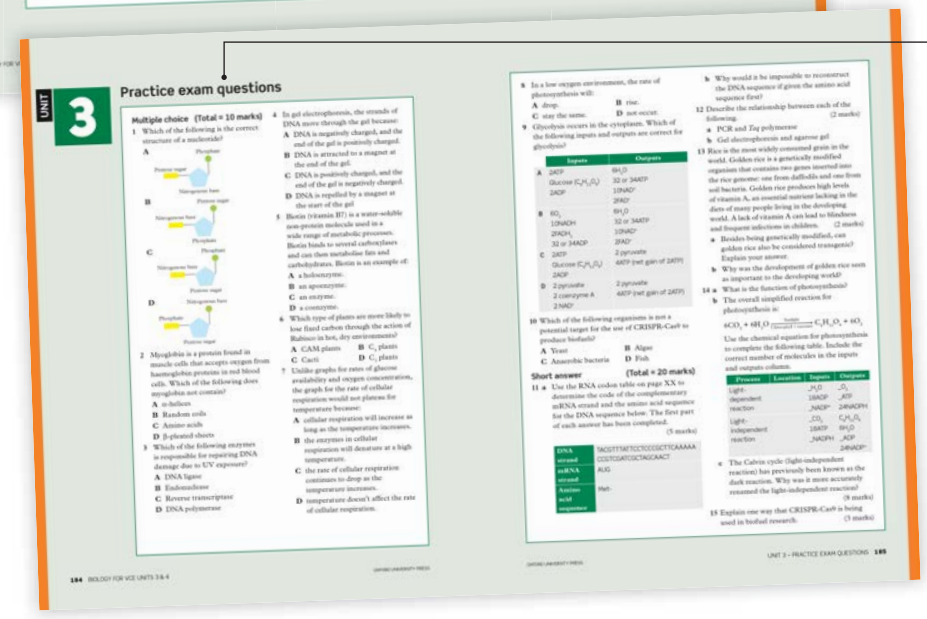
Encourages students to think about how marks are awarded in an exam

Digital links to your obook pro at the point of learning



Practice School-assessed Coursework (SACs)

5 practice SACs to familiarise students with the internal assessments: case study analysis, primary and secondary data analysis, comparison of 3 practicals, bioethical evaluation and student-designed scientific investigation



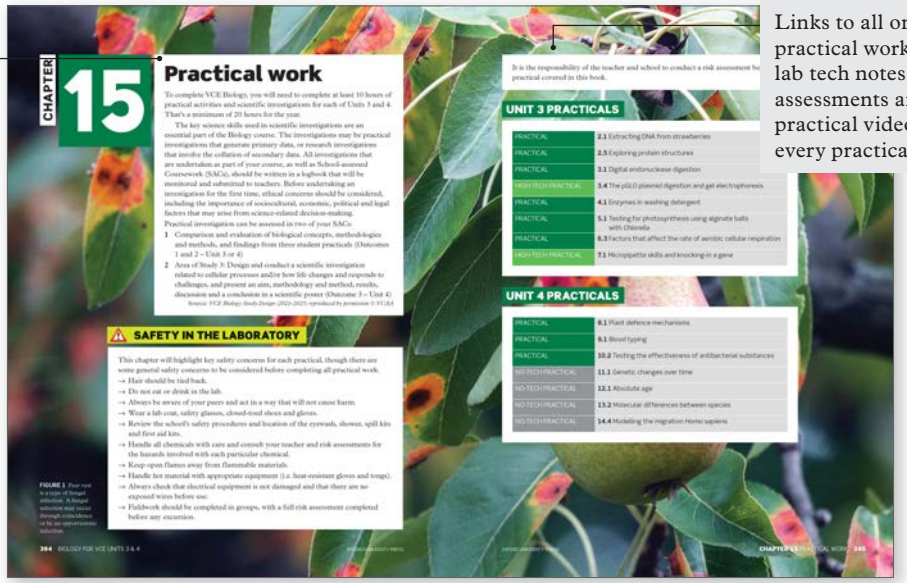
Practice exam questions

Exam-style questions for students to practise writing responses; includes question sets for Unit 3, Unit 4 and combined Units 3 & 4

Practical work

The practical work includes:

- at least one practical per chapter
- safety guidelines for working in a lab
- no-tech practicals that can be completed outside the lab
- standard practicals that can be completed in any school lab
- high-tech practicals that use specialty equipment.



Links to all online practical worksheets, lab tech notes, risk assessments and practical videos for every practical.

Student Workbooks

Biology for VCE Student workbooks are designed to help students succeed in VCE, providing activities and questions to practise for SACs and exams. With an engaging design, full-colour photos and relevant scientific diagrams throughout, these write-in workbooks help students to develop examinable key science skills. The workbooks include:

» a stand-alone **biology toolkit** teaching students how to read and use biological data, how to write and present reports, how to use their logbooks, and how to answer exam questions and read an examiners report

» full **chapter summaries** and chapter checklists for students to self-assess their understanding of Key Knowledge

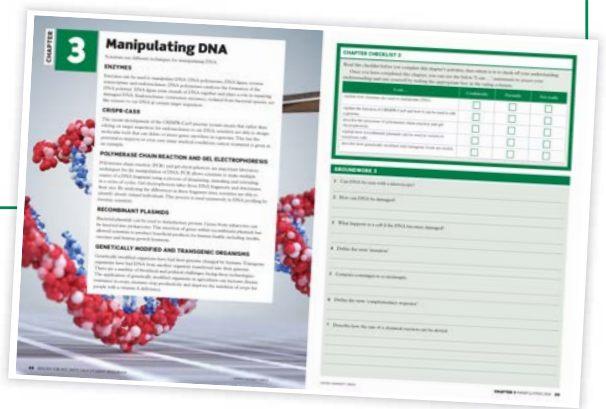
» **support groundwork questions** to assess student understanding

» four activities per chapter – Case cracker, Data drill, Experiment explorer and Evaluating ethics to **practise key science skills** and **prepare for SACs**

» Unit 3, Unit 4 and Units 3 & 4 **practice exam questions**

» write-in **practical worksheets** from the student book

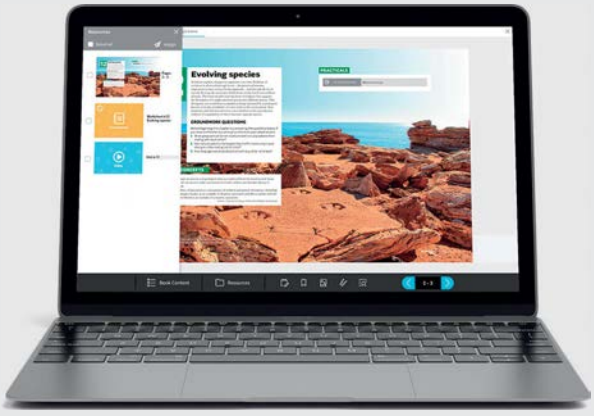
» **answers to all questions**.



Digital resources

obook pro is Oxford's next generation digital learning resource that offers an interactive digital version of the Student book with links to additional resources including videos and online assessments. This digital format can be used to immerse students in digital learning, or as part of a blended print and digital approach to learning.

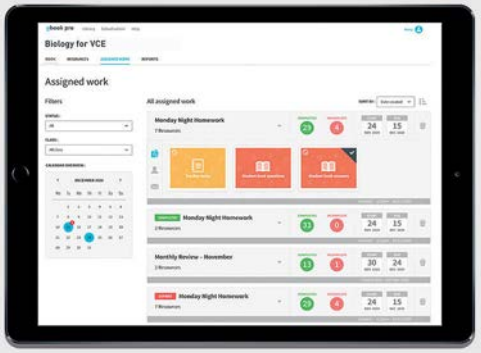
Biology for VCE Units 3&4 Student obook pro is hosted on oxforddigital, the home of Oxford's digital resources.



Student obook pro

Students receive:

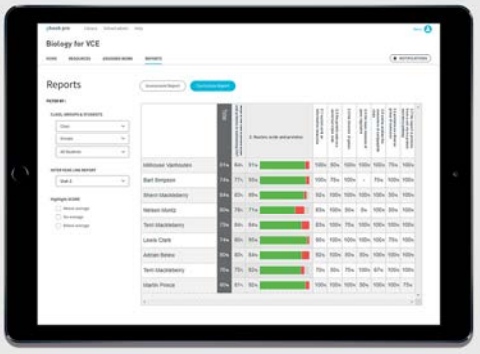
- a complete digital version of the Student Book with interactive note-taking and bookmarking functionality
- integrated Australian Concise Oxford Dictionary look up feature
- targeted instructional videos for key concepts, practicals and worked examples
- groundwork resources to support assumed knowledge
- interactive assessments to consolidate understanding
- auto-marked practice exam question sets
- integrated QuizletLive sets including real-time online quizzes with live leaderboards
- access to their online assessment results to track their own progress.



Teacher obook pro

In addition to the student resources, teachers receive:

- answers to all questions in the Student Book
- detailed planning resources
- practice exams and guidance for Student Assessed Coursework (SACs)
- the ability to assign resources and assessments to students
- reporting functionality that tracks student progress and success.



CHAPTER 1

Biology toolkit

Biology is an evolving science that seeks to understand the diversity of life on Earth. Studying biology provides a continual pathway to a range of careers. Biologists investigate the different types of organisms, their origin, location and interactions with other organisms and the non-living environment. They investigate these interactions through observations, measurements and experiments. Biologists formulate hypotheses that are modified over time, and this process is called the scientific method.

It is important to comprehend the structure of the VCE Biology course so that the interlinking of biological concepts can be understood and applied to new contexts. This chapter contains a toolkit of ideas and skills to refer to throughout your Units 3 & 4 VCE Biology course.

KEY SCIENCE SKILLS

Throughout VCE Biology Units 3 & 4 you will apply the following key science skills:

→ develop aims and questions, formulate hypotheses and make predictions
→ plan and conduct investigations
→ comply with safety and ethical guidelines
→ generate, collate and record data
→ analyse and evaluate data and investigation methods
→ construct evidence-based arguments and draw conclusions
→ analyse, evaluate and communicate scientific ideas.

Source: VCE Biology Study Design (2022–2026) reproduced by permission © VCAA

FIGURE 1 Biology is the study of living things.

1.1 Biology as a subject

KEY IDEAS

In this topic, you will learn that:

- biology is the study of all living things
- there are many career pathways that stem from studying biology
- the VCE Biology course has a clear structure
- Aboriginal and Torres Strait Islander Peoples are the traditional custodians of Australia and have varying methods for taking care of the land and producing medicine.

biology
the science of living things divided into different fields that cover the morphology, physiology, anatomy, behaviour, origin and geographic distribution of organisms

Biology is a diverse and evolving science based on the study of all living things. It explores life by investigating the structure, function, origin, development and geographic distribution of organisms, and their interactions with the non-living environment.

Some of the branches of biology include botany, genetics, immunology, microbiology, pharmacology and zoology, and these can be applied to a wide range of human endeavours.

The five basic principles

All branches of biology are unified by a framework of five basic principles. These principles state that:

- All things are composed of cells, the basic unit of life.
- All living things require energy, and energy flows between organisms and the environment.
- All living things contain genetic material, the 'barcode' for structure and function in organisms.
- All living things must maintain homeostasis – that is, a relatively stable internal environment.
- The concept of evolution unifies all living things.

Biology as a career

Studying biology provides a continuing pathway that can lead to a range of careers. With ongoing advances in technology and biological concepts, career options in biology continue to grow.

These career options include, but are not limited to, the following professions:

- research scientist
- medical practitioner
- allied health professional
- ecologist
- pharmacologist.

This may also include career options in the following areas:

- biotechnology
- bioengineering.

FIGURE 1 Allied health professionals help manage our physical and mental health and need a background in biology to succeed.

There are also career opportunities in cross-disciplinary areas such as bushfire research, environmental management and conservation, forensic science, medical research and many other fields.

All science disciplines, including biology, teach a wide variety of **core skills** that you will use throughout your lives, and these include skills such as:

- problem-solving
- teamwork
- research
- critical thinking
- communication
- attention to detail
- innovation
- resilience.

core skills
key 'employment skills' required in most careers

CASE STUDY 1.1

Meet a scientist

Dr Marguerite Evans-Galea is a molecular biologist, executive and entrepreneur. Marguerite studied VCE Biology and continued a pathway of biology and STEM (science, technology, engineering and mathematics) throughout her professional life. This is her inspirational story:

'As a child, I had an innate, insatiable curiosity and constantly asked "Why?". My thirst for knowledge grew stronger during high school and I was the only student in my senior year to take all three sciences and Maths Methods. I remember each and every one of my science and maths teachers – they were inspiring! At the beginning of Year 11, I read a book called *The Double Helix* and that was when I first fell in love with DNA and molecular biology. The thrill of discovery was exciting.

'I went to the University of Queensland (UQ) in Brisbane after finishing high school. My undergraduate was tough, completing two degrees: a Bachelor of Music and a Bachelor of Science. I was juggling chamber ensembles with tissue culture, and this was essentially the first 5 years of my tertiary education.

'My advice to you is to remember that science allows you to have a positive impact on the world and make a difference to people's lives. STEM qualifications and the skills you gain through studying science can be applied in many professional arenas including academia, industry, education and government. Be open to a wide range of professional roles in your STEM career. We have STEM qualified professionals running companies, overseeing global research, facilitating innovation, ensuring conservation of Australia's flora and fauna, making discoveries in the laboratory, in the field, and developing evidence-based policies for government. STEM experts contribute broadly to improving Australia's health and economy, across a breadth of professional sectors – embrace these opportunities. Dream big and go for it!'
– Dr Marguerite Evans-Galea

FIGURE 2 Dr Marguerite Evans-Galea, PhD in molecular biology, co-founder and CEO of Women in STEMM Australia and honorary research scientist at the Murdoch Children's Research Institute

Structure of the VCE Biology course

Weblink
VCE Biology Study Design

The Victorian Curriculum and Assessment Authority (VCAA) sets the Study Design for each VCE subject, and Biology is one of the five science courses offered in VCE.

VCE Biology provides the opportunity to engage in a range of inquiry tasks to develop key science skills that identify the links between theory, knowledge and practice. By undertaking this course, you will develop an understanding of how life has evolved and how this has shaped the biodiversity of species on Earth.

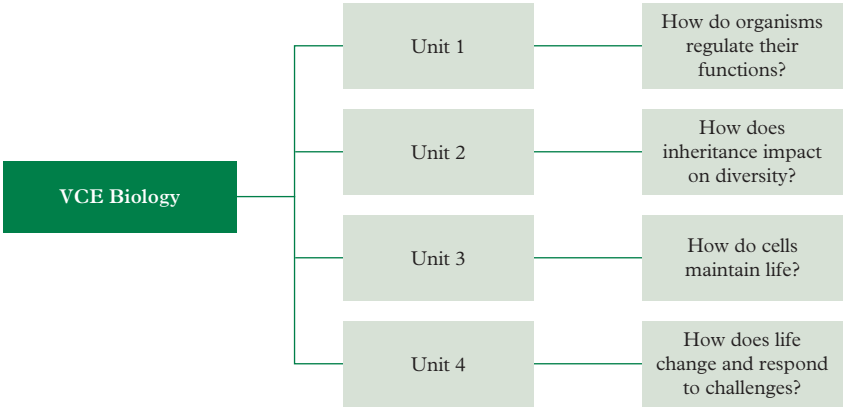


FIGURE 3 Structure of the VCE Biology course

TABLE 1 The VCE Biology course, Units 1–4

Unit	Description
1: How do organisms regulate their functions?	Units 1 & 2 are designed to be the first year of the VCE Biology course. These units build a foundation of biological concepts for Units 3 & 4; however, they are not a prerequisite for Units 3 & 4.
2: How does inheritance impact on diversity?	
3: How do cells maintain life?	Unit 3 is based on understanding life at a cellular level. In this unit, you will investigate the biochemical structure and function of cells, the role of nucleic acids and proteins, as well as the nature of biochemical pathways, specifically photosynthesis and cellular respiration.
4: How does life change and respond to challenges?	Unit 4 considers the continuous changes and challenges for life on the Earth. In this unit, you will investigate the functioning of the immune system and the issues and challenges related to disease. You will also consider that the concept of biological evolution is based on an accumulation of evidence, that speciation can occur through isolation and divergence, and identify evidence of this change through measurements of relatedness between species.

TABLE 2 Units 3 & 4 breakdown of Areas of Study

Unit 3	
Area of Study	**Description**
1: What is the role of nucleic acids and proteins in maintaining life?	In this area of study 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.
2: How are biochemical pathways regulated?	In this area of study 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.
Unit 4	
Area of Study	**Description**
1: How do organisms respond to pathogens?	In this area of study students focus on the immune response of organisms to specific pathogens. Students examine unique molecules called antigens and how they illicit 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.
2: How are species related over time?	In this area of study 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.
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. When undertaking the investigation students are required to apply the key science skills to develop a question, state an aim, formulate a hypothesis and plan a course of action to answer the question, while complying with safety and ethical guidelines. Students then undertake an investigation to generate primary quantitative data, analyse and evaluate the data, identify limitations of data and methods, link experimental results to scientific ideas, discuss implications of the results, and draw a conclusion in response to the question. The presentation format for the investigation is a scientific poster constructed according to the structure outlined on pages 11 and 12 [of the *Victoria Certificate of Education Biology Study Design 2022–2026*]. A logbook is maintained by students for record, assessment and authentication purposes.

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

Aboriginal and Torres Strait Islander Peoples' knowledge, cultures and perspectives

Aboriginal and Torres Strait Islander Peoples
the original inhabitants and owners of the land now known as Australia, inhabiting this land for over 65 000 years

Country
an area (not just geographical) that is traditionally owned and looked after by an Aboriginal (and sometimes Torres Strait Islander Peoples) language group or community; a place that is of spiritual meaning with deep feelings of connection and attachment

Place
a space confined by physical or intangible boundaries occupied and regarded as belonging to individuals or groups of Torres Strait Islander Peoples (and sometimes Aboriginal Peoples); the spaces have varying spiritual meaning to the people

Within the VCE Biology course, the special relationship the **Aboriginal and Torres Strait Islander Peoples** have with waterways, sea, sky and land is acknowledged. It is important to consider and understand the unique history and cultural diversity of the First Peoples of Australia and their ways of being, knowing, thinking and doing. First Peoples' knowledge of the land has been passed on for thousands of years through song and dance, as well as Dreamtime stories, and there are many things to be learnt from the traditional custodians of Australia.

The traditional custodians of Australia are people who are of Aboriginal and/or Torres Strait Islander descent, identify and are accepted as such by the community in which they live. Aboriginal and/or Torres Strait Islander Peoples may also be referred to as Indigenous Peoples, First Nations, First Peoples and/or Traditional Custodians/Owners when referring to a collective group. Indigenous Peoples are also identified by their particular Community/Nation/Tribe or Clan that they/their family and/or community recognise.

The knowledge, cultures and perspectives of over 250 language groups belonging to a particular **Country** or **Place** of Australia are embedded throughout the VCE Biology course, with a specific focus in Units 2 and 4. Unit 2, Area of Study 2, discusses the contribution of Aboriginal and Torres Strait Islander Peoples' knowledge of species in Australian ecosystems. Indigenous Peoples of Australia have a profound relationship with the land – Indigenous Peoples honour and respect biodiversity. Indigenous Peoples' understanding of traditional seasons, farming and fire practices has allowed for occupation and respect for the land for thousands of years. Unit 4, Area of Study, 1 investigates the impact of European arrival on the spread of diseases, particularly on the Aboriginal and Torres Strait Islander Peoples. Unit 4, Area of Study 2, discusses the migration of modern humans with a focus on Aboriginal and Torres Strait Islander Peoples populations migrating into Australia and the connection to Country and Place.

FIGURE 4 The gum that exudes from the bark of the red bloodwood (*Corymbia gummifera*) tree is used as an antiseptic by the Aboriginal and Torres Strait Islander Peoples.

CHECK YOUR LEARNING 1.1

Apply, analyse and compare

1 Investigate a branch of biology that interests you. Research and describe this area of biology and justify your choice of investigation.

Design and discuss

2 After reading Dr Marguerite Evans-Galea's story, discuss the career opportunities that can lead from studying biology and STEM.

3 Choose one of the following to conduct research on.
- **a** The spread of disease with European arrival and its effect on the Aboriginal and Torres Strait Islander Peoples
- **b** Traditional methods of caring for the Australian land
- **c** The migration of Aboriginal and Torres Strait Islander Peoples into Australia

Present your findings to your class.

1.2 Key science skills

KEY IDEAS

In this topic, you will learn that:

+ the key science skills and their application are important to succeeding in VCE Sciences.

scientific method the experimental process of formulating a hypothesis, then collecting data in order to determine whether the hypothesis is supported

The key science skills are central to the VCE Biology course, across Units 1–4 and over all Areas of Study.

The key science skills are important for the planning and conducting of practical investigations, collating and analysing primary and secondary data, organising data in an informed manner, identifying errors and uncertainty, critically evaluating methodology, and researching and communicating scientific ideas. This method of planning and conducting practical investigations is known as the **scientific method**.

Practising the key science skills is important for succeeding in your assessments.

Understanding the key science skills

There are seven key science skills identified by VCAA that should be considered when conducting practical investigations and evaluating research. These are summarised in Table 1.

TABLE 1 Key science skills as outlined by VCAA

Key science skill	VCE Biology Units 1–4
Develop aims and questions, formulate hypotheses and make predictions	• identify, research and construct aims and questions for investigation • identify independent, dependent and controlled variables in controlled experiments • formulate hypotheses to focus investigation • predict possible outcomes
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 • 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 • work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications
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 • apply relevant occupational health and safety guidelines while undertaking practical investigations • demonstrate ethical conduct when undertaking and reporting investigations

TABLE 1 Continued

Key science skill	VCE Biology Units 1–4
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 • record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data • organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs • plot graphs involving two variables that show linear and non-linear relationships
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 • identify and analyse experimental data qualitatively, handing 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 • identify outliers, and contradictory or provisional data • repeat experiments to ensure findings are robust • 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
Construct evidence-based arguments and draw conclusions	• distinguish between opinion, anecdote and evidence, and scientific and non-scientific ideas • 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 • evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis • use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation • identify, describe and explain the limitations of conclusions, including identification of further evidence required • discuss the implications of research findings and proposals
Analyse, evaluate and communicate scientific ideas	• use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement • discuss relevant biological information, ideas, concepts, theories and models and the connections between them • 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 • critically evaluate and interpret a range of scientific and media texts (including journal articles, mass media communications and opinions in the public domain), processes, claims and conclusions related to biology by considering the quality of available evidence • 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 • use clear, coherent and concise expression to communicate to specific audiences and for specific purposes in appropriate scientific genres, including scientific reports and posters • acknowledge sources of information and assistance, and use standard scientific referencing conventions

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Formulating a hypothesis

hypothesis
a testable statement, or proposed explanation for the predicted outcome of a practical investigation, based on scientific reasoning

When conducting scientific investigations, you will be required to develop a hypothesis. A **hypothesis** is developed from a research question. It is written as a testable statement that may include a prediction about the outcome of the investigation.

There is not one correct way to write a hypothesis, but the following steps can be helpful to make sure you include everything you need.

1 Ask a research question. This must be specific and testable.
For example, does mould grow faster if it is not exposed to light?

2 Identify the independent and dependent variables in the research question.
In the above example, the rate of mould growth is the dependent variable and the amount of light is the independent variable.

3 Write an IF, THEN, BECAUSE statement, which provides a possible explanation for the relationship between the independent and dependent variables.

Video
Writing a hypothesis

IF THEN BECAUSE

IF	THEN	BECAUSE
If the independent variable is changed	then the dependent variable will increase/decrease/grow/be larger than/be smaller than etc.	because of scientific reasoning

Study tip

When formulating hypotheses, consider the relationship between the independent and dependent variables and make sure the possible outcome is formed from accurate scientific knowledge.

For example, if the amount of sunlight available is reduced, then the rate of mould growth increases because sunlight evaporates moisture from the air that mould needs to grow.

Resource
Risk assessment template

Risk assessments

risk assessment
a document that outlines the potential risks, hazards and control measures that should be taken to avoid harm

A **risk assessment** is a systematic way of identifying hazards and risk factors that could cause harm, and implement control measures to avoid those risks. There are different formats to generate a risk assessment, and some programs will automatically generate the risk assessment for you. It is important that the control measures outlined in the risk assessment are followed to ensure the safety of all people involved in the practical investigation. You can find a blank risk assessment template on your obook pro.

CHECK YOUR LEARNING 1.2

Describe and explain

1 Explain the importance of complying with safety and ethical guidelines when planning and conducting practical investigations.

2 Why should a hypothesis be written before an experiment is conducted?

Design and discuss

3 An experiment is being conducted to investigate the effect of detergent on a beetroot cell membrane. Sliced beetroot will be placed in different solutions containing increasing concentrations of detergent. The red pigment will leak out of the beetroot cell membrane as it breaks down. The colour of the solution will be measured after 1 minute. Design a hypothesis for this investigation.

4 Design a risk assessment for the practical investigation described in Question 3.

1.3 Scientific investigation

KEY IDEAS

In this topic, you will learn that:

- there are different methodologies that can be used in scientific investigations
- a logbook must be maintained throughout an investigation
- scientific posters have a common layout.

primary data
data collected by the investigator from firsthand sources

secondary data
data collected from another person, not the investigator, which is relevant to the scientific investigation

methodology
the approach used to plan and conduct a scientific investigation with justification

Scientific investigations are an important part of the VCE Biology course and can be the practical investigation of **primary data** or research investigation using **secondary data**. An individual, a small group or a class may undertake a scientific investigation; however, all the work that will be assessed (i.e. logbook and poster) needs to be completed individually.

Scientific investigation methodologies

Scientific investigations can be approached in a variety of ways. The particular **methodology** chosen should consider the aim of the investigation and the research question.

TABLE 1 An overview of the different scientific investigation methodologies and possible research questions

Inquiry method	Inquiry outline
Case study	Case studies are a good choice when the investigation is based on a certain activity, behaviour, event or problem that contains a real or hypothetical situation. Research question example: • Could the 'Genetics of resistance to HIV infection' case study be used to explain why some people remain HIV negative even when exposed to the virus?
Classification and identification	Classification is investigating phenomena and arranging it into smaller, more manageable groups. Identification is recognising whether phenomena belong to a particular set or part of a new set. Research question example: • Can a key be used to categorise the classification of the cave beetle species that were newly discovered in 2018?
Controlled experiment	A practical investigation that looks at the relationship between the independent and dependent variables, where all other variables are controlled. Research question example: • What is the effect of temperature on the rate of liver enzyme activity?
Correlation study	An observational investigation where variables don't have to be controlled. This study investigates the relationship between the variables and identifies factors that have greater importance on those variables. Research question example: • Does smoking have the greatest impact on the degeneration of nerve cells in Alzheimer's disease?

Inquiry method	Inquiry outline
Fieldwork	Selecting a particular environment beyond the laboratory (or classroom) where observations are made and/or experimental investigations are carried out. Common sampling techniques are used to gather qualitative or quantitative data. Research question example: • Does water temperature have an effect on distribution of seaweeds in the rock pools of Rickett's Point Marine Sanctuary?
Literature review	Involves researching, gathering and interpreting secondary data. This may be used in preparation for an investigation or used to help explain observed events. Research question example: • Is there evidence of pheromone cues between *Drosophila* from scientific investigations to explain how species-specific mating occurs?
Modelling	Involves physically, conceptually or mathematically developing a model that could simulate a concept to assist understanding and knowledge of a particular system. Research question example: • What model best predicts the effect of stomach pH on digestive enzyme activity?
Product, process or system development	Involves designing a product, process or system to meet a human need. This should link technological developments to scientific knowledge. Research question example: • Which process would be more more efficient at recording the oxygen output from a photosynthetic plant than by measuring the carbon dioxide used?
Simulation	Uses an existing model to investigate a scientific system by manipulating variables. Simulations are used when variables cannot be controlled in a real system. Research question example: • What if a plant cell observed through a microscope was used to determine the effect of temperature on osmosis?

Logbook

You must keep a logbook for all stages of practical investigations. This logbook can be a physical book maintained in print form, or it can be digital. Check with your teacher to see if it is possible to authenticate that the work in your digital logbook is your own.

A logbook should include the following information: lists of ideas (with advantages and disadvantages); scientific research (including full references); notes on the experimental process; possible means of collecting data; raw data or results; and possible interpretations of data. All items included in the logbook must be documented clearly and dated.

Logbooks provide the opportunity for all information to be in one place when it comes time to write a scientific report or prepare a scientific poster. The student-designed scientific investigation for Outcome 3 of Unit 4 requires a logbook submission for satisfactory completion.

FIGURE 1 Maintaining a logbook is a critical component of practical investigations.

Scientific poster

Unit 4, Area of Study 3 (the student-designed scientific investigation) requires a demonstration of key science skills by presenting findings from the investigation as a scientific poster and logbook entry. The poster can be produced in print form or electronically, and has a maximum word count of 600 words.

When formatting the scientific poster, there's a specific poster structure that should be followed according to the VCAA Study Design. The centre of the poster should be between 20–25% of the poster space and include a summary sentence outlining the outcome of the investigation.

The following sections must be included in the poster.

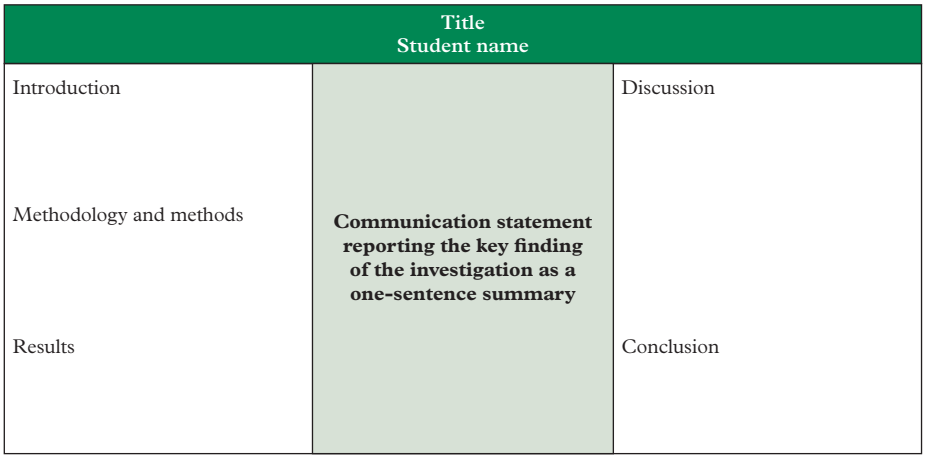

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FIGURE 2 The layout for the student poster

Title and name

The poster title should be written as a question. It should be short and draw interest to the poster. The title should include the issue or inquiry – and any variables – under investigation, the experimental approach and the system (e.g. an experimental set-up, an organism or a model). Don't forget to include your name.

Introduction

aim
the main purpose of the practical investigation

The introduction should include a clear **aim** (one to two sentences) to state the purpose of the investigation. Following this, a hypothesis is included – a statement of the possible outcome of the investigation. The independent and dependent variables should be clearly stated, as well as the direction of the suggested outcome. Descriptions of relevant background concepts will give context to the investigation. This introduction should also include any reliable sources of secondary information, and prior investigations that are relevant to the inquiry. If necessary, definitions and relevant formulas should be included in order to facilitate a greater understanding of the nature of the investigation.

Study tip

Methodology is different to method. **Methodology** is the rationale for your practical investigation or research. A **method** is a tool used to answer your research question/s.

Methodology and methods

The type of investigation, the materials required and the procedures should be described in the methodology section in such a way that another researcher could replicate the investigation. Figures, photos and flowcharts can sometimes be used to demonstrate the method instead of a formal scientific report format.

This section is authenticated by logbook entries, and the detail and accuracy of those entries will affect the reproducibility of the investigation by other researchers. You can read more on the accuracy of the methodology in Topic 1.4.

Results

Data from the logbook is selected and presented in an appropriate format to show the trend, pattern and relationship between the independent and dependent variables.

Since there's limited space on the poster, it's not effective to include both a table and graph that represent the same dataset. It is important to sequentially number all graphs, tables and figures in this section since they are referred to in the discussion section.

The results section also briefly states the usefulness of the results in the context of the experiment and whether or not the hypothesis was supported.

Discussion

The discussion section focuses on examining the data and providing explanations that link to accurate scientific understanding. The examination of the data is used to identify whether it supports, partly supports or refutes the hypothesis (from the introduction).

The results are used to examine the relationship between the independent and dependent variables and then a comparison can be made with the expected results. The discussion section provides the opportunity to explain the significance of the results, draw conclusions and link back to the purpose of the investigation (the aim). A logical explanation is required to describe the appearance of outliers. It should also explain any outliers or other inconsistencies within the dataset.

In the discussion section, there should also be a description linking the results to previous experiments or investigations that were conducted on a similar question. The limitations of the experimental design should be described and include suggestions on how future investigations can provide improvement.

Study tip

Be careful with the use of language in the discussion section. The use of terms such as 'proved', 'correct' and 'disproved' should be avoided. Use words such as 'support', 'indicate' or

Study tip

All sections of the poster should be written in a passive voice (avoid pronouns) and past tense (with the exception of the introduction).

Conclusion

The conclusion describes the main outcome of the investigation. This should be a response to the research question. This brief statement identifies whether the hypothesis was supported and whether the results were linked to the relevant underlying scientific concepts. The conclusion summarises the main limitations of the investigation. Future work that would refine or extend the results obtained from the investigation can also be included. It's important that no new information is introduced in this section.

Study tip

An example poster and activities associated with producing your poster can be found in the *Biology for VCE Units 3 & 4 Student workbook*.

References and acknowledgments

A list of references in an appropriate format (e.g. Harvard or APA) is included. Ask your teacher about the preferred referencing style in your school. These references should be presented in the body of the poster as embedded quotations or sourced content. The acknowledgments provide the opportunity to thank any individuals, groups or organisations for specific contributions to the investigation. The references and acknowledgments section does not count towards the word limit of the poster.

CHECK YOUR LEARNING 1.3

Describe and explain

1 Why is it important to maintain a logbook for practical investigations?
2 What is the purpose of the results section of a scientific poster?
3 What should be in the centre of a scientific poster?

Apply, analyse and compare

4 Contrast primary and secondary data.
5 Compare the scientific investigation methodologies of simulation and modelling.

Design and discuss

6 Design a research question for one of the scientific investigation methodologies.
7 An experiment was set up to investigate the predicted spread of a virus in a Victorian rural town. People were surveyed to gather data about possible symptoms, general health, geographic location, travel over 3 months and cultural background. What kind of methodology is this investigation using? Explain.

1.4 Data, measurement and error

KEY IDEAS

In this topic, you will learn that:

- raw data needs to be presented in a simple and easy to understand way
- data can be continuous or discrete
- experiments must be valid, repeatable and reproducible
- precise data may not be accurate
- errors can be random or systematic.

Scientific investigations are important – they aim to develop explanations for natural phenomena. Evidence needs to be organised and presented in an appropriate manner and then analysed to consider the quality of the data.

Presentation and analysis of data

raw data
measurements or observations of the dependent variable

Raw data are the unprocessed data collected during an investigation. It can be difficult to interpret and needs to be presented in a manner that is easy to analyse so that the reader can draw conclusions.

Study tip

'Data' is always considered plural. There is never one data.

Tables can be used to present qualitative and quantitative data. All tables should have a heading that states what the table is showing. This usually indicates the identity of the independent and dependent variables. Each of the columns must have a heading and if numerical data are being used, the units should also be included. **Qualitative** (non-numerical) data may show trends and are often worth presenting in a table before comparing or contrasting these results in the discussion.

table
a form of organising data systematically into columns

Quantitative (numerical) data are presented in columns that display the numerical values of each of the related variables, but may not give an immediate or clear indication of the relationship between the variables. A table is usually the first step in recording information and guides the selection of the most appropriate graphical representation. Various mathematical applications can be readily applied once the data are in a table.

qualitative
data that tends to be non-numerical and are subjective (e.g. hair colour, outfit choice, etc.)

quantitative
data expressed as numbers (e.g. concentration of a solution, temperature, etc.)

Graphing features

All scientific **graphs** have features in common:

- The information on the graph should be easily identified. This includes the heading, axes titles and numbers.
- The title should be a descriptive statement that includes the independent variable and the dependent variable.
- The points of each axis must be of equal unit size (i.e. scaled). Each axis should also start at zero.
- Each axis should be clearly labelled and include the unit of measurement.
- The data should not be plotted beyond each axis.
- If there are two sets of data on a single graph, two different symbols should be used and/or a coloured key used.

graph
a way of representing data to visually identify the relationship between the variables

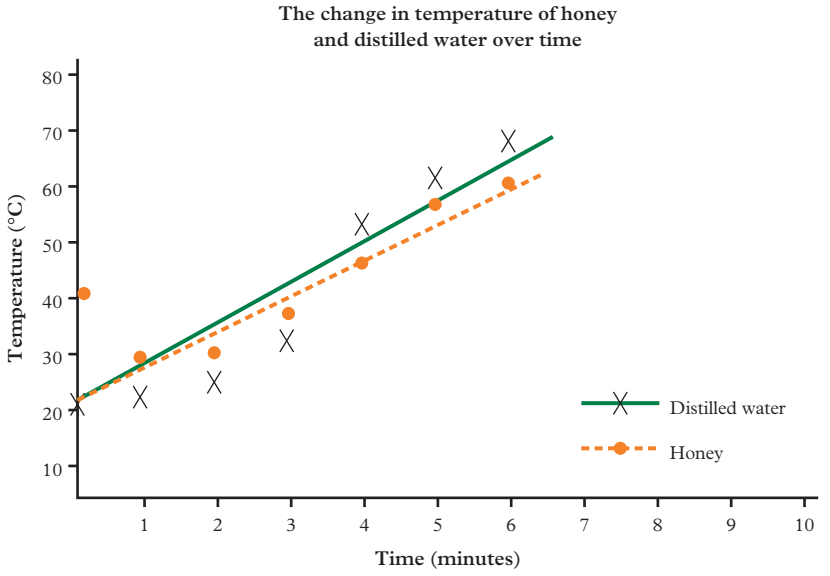
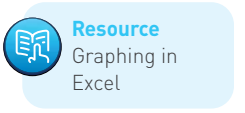


FIGURE 1 Example of a graph with key features, including lines of best fit to the data

Graphing continuous data

The type of graph is chosen to represent a set of data is determined by the type of data.

Line graphs



When there is a relationship or correlation between the independent and dependent variables, the data can be presented as a line graph. If the lines slopes upward (Figure 2a), it means the independent and dependent variables increase together. This is called a positive correlation. If the line slopes down (Figure 2b), the independent variable increases, while the dependent variable decreases. This is known as a negative correlation. If the line is horizontal, then there is no correlation or a neutral correlation between the variables.

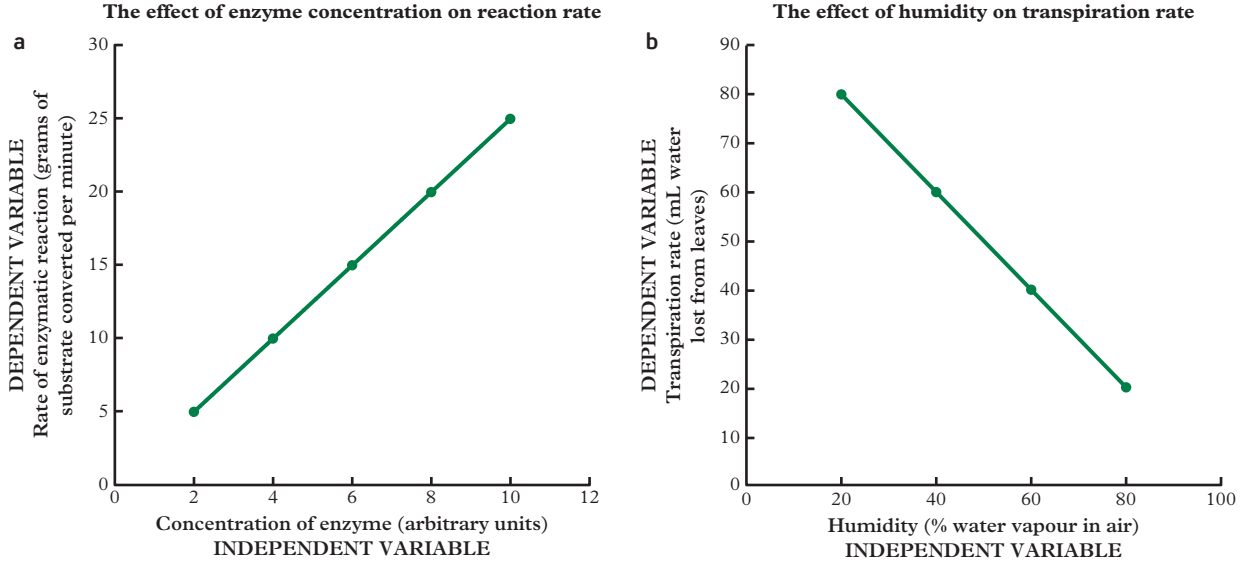


FIGURE 2 **a** An example of a positive correlation in a line graph; **b** an example of a negative correlation in a line graph

Study tip

You can skip values on the axes, to better display your data. You can indicate this on the axes with an axis break. See the two diagonal lines breaking the two axes on Figure 3a.

Scatterplots

If data on a graph are not in a line, then a scatterplot is a better choice of graph. A line of best fit can be drawn by eye or input using Microsoft Excel to show the general trend of the data.

The amount of scatter on either side of a line of best fit indicates the closeness of the variables. The closer the points are to the line of best fit, the stronger the correlation between the variables. When the points are scattered such that a line of best fit cannot be drawn, there is no correlation between the two variables.

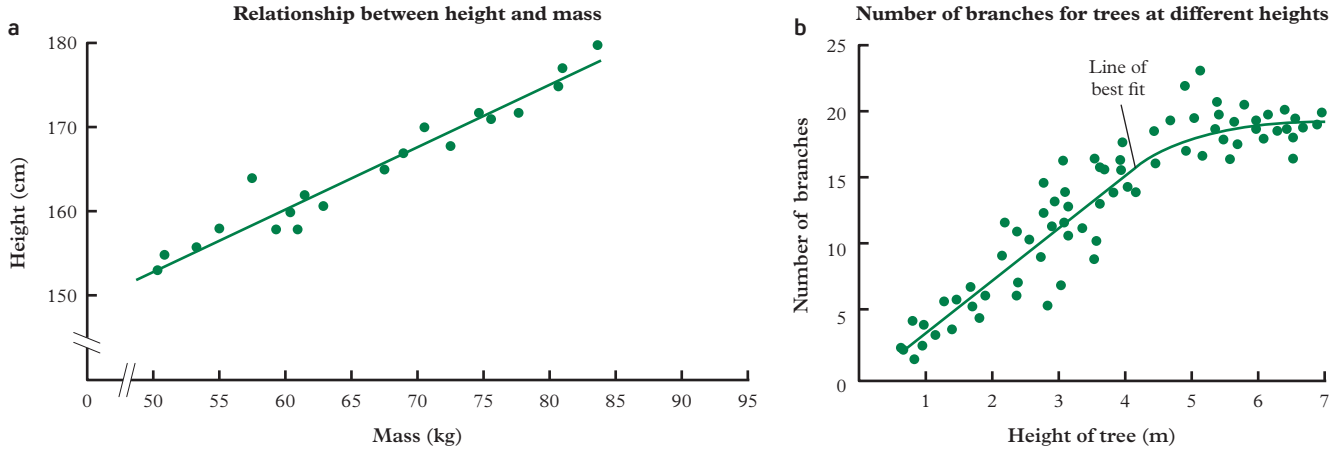


FIGURE 3 Two graphs depicting lines of best fit through a scatterplot

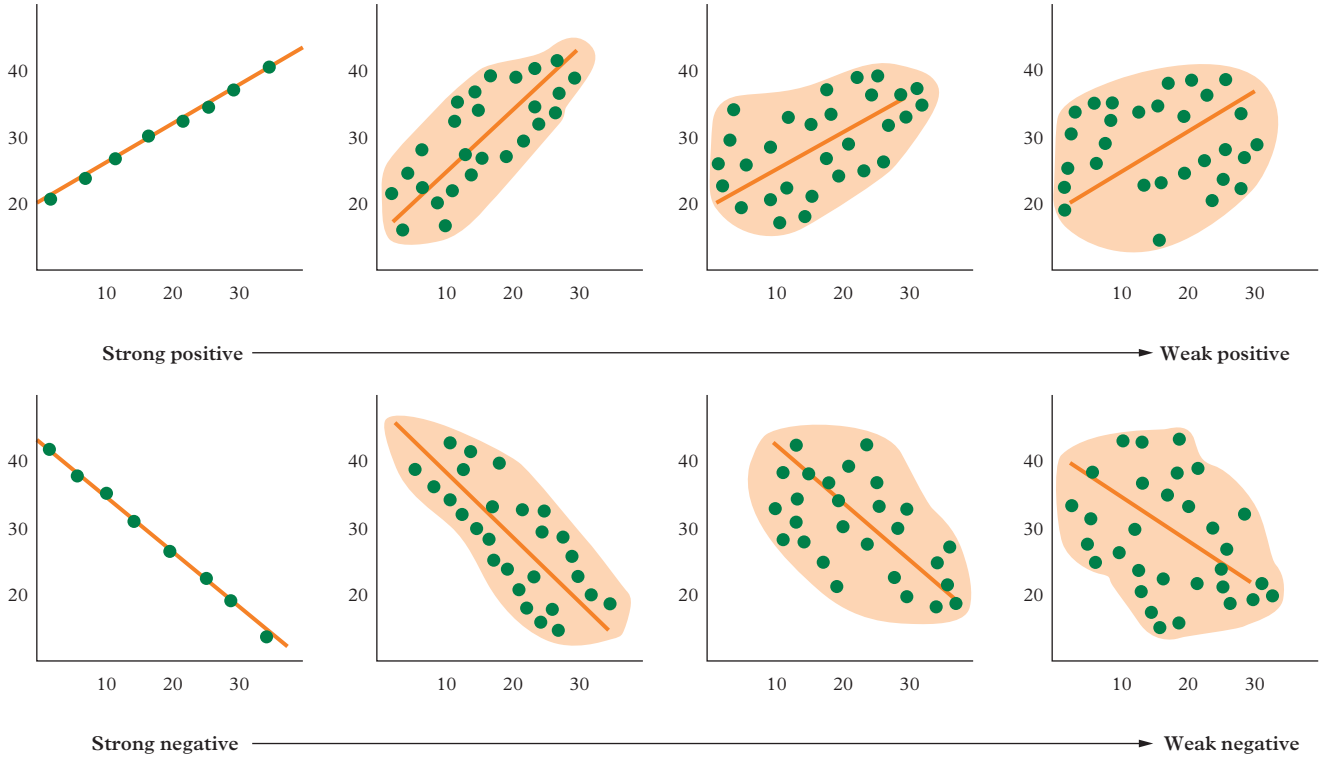


FIGURE 4 Examples of different levels of correlation of data

Interpreting line graphs

When describing a graph, the following should be considered.

- The independent variable and dependent variable
- The type of correlation shown by the graph (e.g. positive, negative or neutral)
- The shape of the graph (i.e. linear or curved)

Although there may be a correlation between the two variables, this does not mean that the independent variable caused the change in the dependent variable. Correlation does not imply causation.

FIGURE 5 A ruler is an essential tool to use when drawing graphs by hand.

Graphing discontinuous data

Several types of graph can be used when the data are discontinuous. Discontinuous data are discrete and not related (e.g. the energy content in different food types or individual recovery rates after exercise).

- A column graph shows the distribution of a distinct characteristic within a population (e.g. human blood groups).
- A histogram represents continuous values of the independent variable that are grouped into classes of equal width (e.g. the recovery time after exercise divided into 2-minute intervals, or the pulse rates of a population).
- A pie graph is useful in showing the relationship of all the parts of a whole.

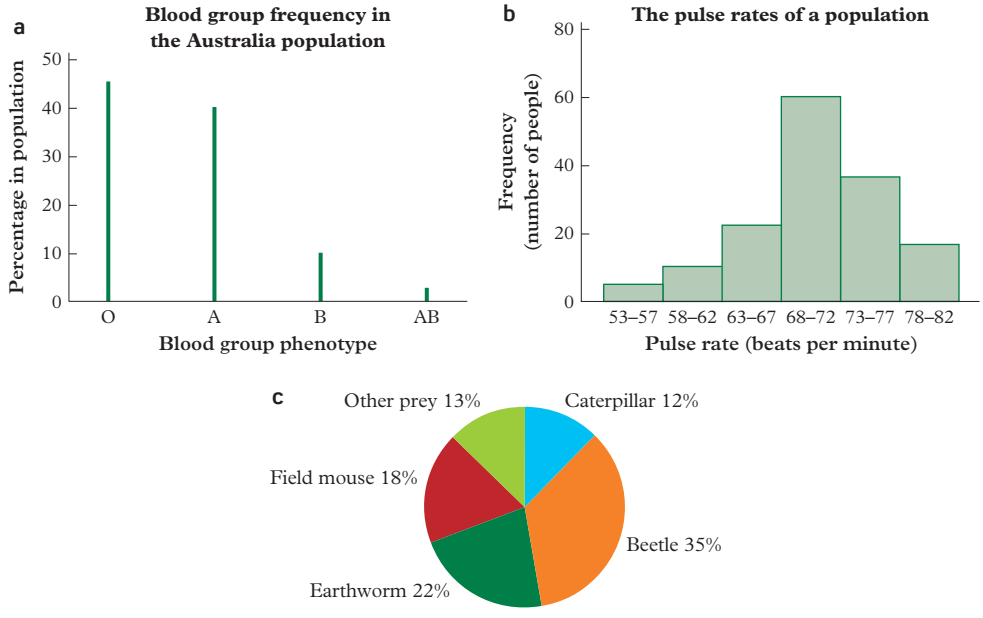


FIGURE 6 Different graphs for discontinuous data: **a** column graph; **b** histogram; **c** pie graph

Different sets of data are often drawn on the same graph using a range of colours or symbols in order to make comparisons.

Data and measurement

When analysing and discussing quantitative data, the accuracy, precision, repeatability, reproducibility, true value and validity need to be considered.

accuracy
a comparison of the experimental data to the true value (the closer the data to the true value, the more accurate it is)

- **Accuracy** describes how close the experimental data are to the 'true' value of the measurement. This can be improved by carefully calibrating the equipment before each experiment.

precision
the closeness of the data in each trial

- **Precision** analyses how close the set of data values are to one another. An experimenter can improve the precision by repeating an experiment or by increasing the sample size.

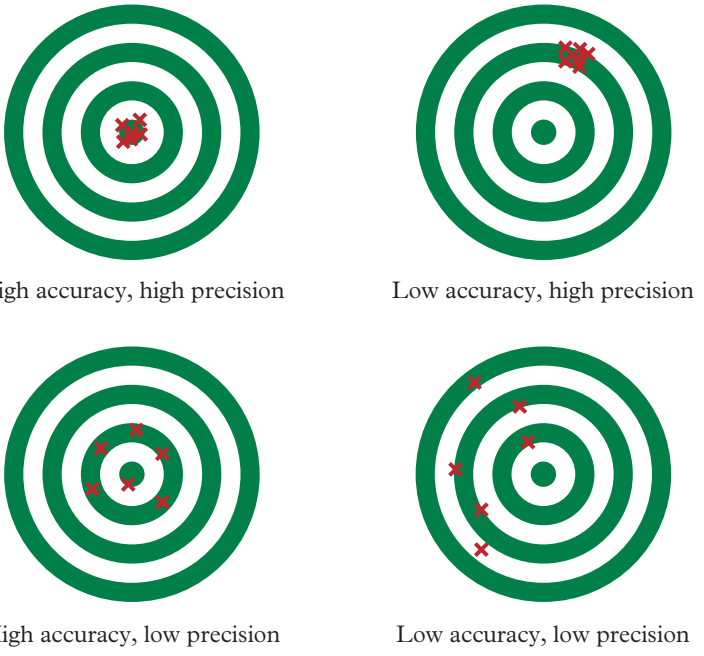


FIGURE 7 Examples of accuracy and precision

repeatability
a measure of achieving the same set of data if the experiment was repeated in the same conditions

- **Repeatability** describes the ability for the same data to be produced again by the same experimenter in the same laboratory under the same conditions.

reproducibility
a measure of achieving the same set of data if the experiment was repeated with a different experimenter in a different laboratory

- **Reproducibility** refers to the ability for the same data to be produced by a different experimenter in a different laboratory. Both repeatability and reproducibility rely on a detailed and informative method with well-defined variables.

true value
the value that accurately represents the measurement if the experiment was conducted perfectly

- The **true value** is the value that would be obtained had the quantity been measured perfectly. The experimental data are compared against the true value to determine the accuracy of the data.

validity
a measure of whether the investigation measures what it is intended to

- The **validity** of the measurement describes whether the experiment will actually answer the scientific question that was asked.

When discussing validity, the experimental design and its implementation should be considered.

Experimental errors, uncertainty and outliers

random error
an error that reduces the precision of the data due to an error in the experimental process that is unpredictable

systematic error
an error that reduces the accuracy of the data by causing the reading to differ from the true value

Experimental errors should not be confused with personal error. Personal or human errors are a result of mistakes that should have been corrected by the experimenter. An experimental error is described as the difference between the measurement and the true value. It is also important not to confuse error with uncertainty. Uncertainty is when a measurement seems unreliable and is associated with doubt.

There are two types of errors to consider in scientific investigations: **random errors** and **systematic errors**.

Random errors

Random errors are those that are unpredictable. They are present in measurements due to an error in the measurement process. Random errors reduce the precision of the data. An example of a random error is parallax error, when an object's measurement has diverged from the true value due to the observer viewing the object at the wrong angle. Random errors can be reduced by doing multiple trials.

Study tip

A mistake due to incorrect measurement is not the same as experimental error – this relates to problems with the experimental design.

Systematic errors

Systematic errors are those that are consistent and repeatable. This type of error reduces the accuracy of the data. Systematic errors are usually caused by faulty equipment or uncalibrated measuring instruments. These errors cause readings to differ from the true value consistently each time. This means that repeating the experiment would not reduce the occurrence of systematic errors. An example of a systematic error would be not making sure the scales are at zero at the beginning of an experiment, and repeating this uncalibrated measurement across all tests.

Study tip

Need more practice on data presentation, measurement and errors? See the *Biology for VCE Units 3 & 4 Student workbook* for associated activities.

Outliers

A data point that is outside the rest of the dataset is called an **outlier**. It is possible that this abnormal data point may be caused by mistakes made by the experimenter or equipment during the experiment, but this is not always the case.

outlier
any value that sits outside the dataset

Outliers should always be plotted on a graph; however, they may be excluded when determining the line of best fit. The experimenter should attempt to explain the cause of the unexpected data in the discussion section of the scientific investigation. Outliers cannot be simply dismissed, but rather they should be investigated and accounted for. Conducting multiple trials can be a useful way to examine outliers.

CHECK YOUR LEARNING 1.4

Describe and explain

1 State the three types of graphs that can be used to represent discrete data.
2 Which error – random or systematic – affects the accuracy of the data? Explain.
3 Describe what an outlier is.
4 State three features common to all graphs.

Apply, analyse and compare

5 Why are outliers excluded from the line of best fit?
6 Compare the following sets of terms.
  a Accuracy and precision
  b Repeatability and reproducibility
  c Continuous data and discrete data
  d Uncertainty and error
7 Explain which type of graph would best represent continuous data that measures the heart rate of an aquatic organism in solutions of different salinities.

Design and discuss

8 Discuss the importance of organising raw data into tables and/or graphs.
9 An experiment was completed on an aquatic plant, testing the effect of temperature on the rate of photosynthesis (measured by oxygen output). Using the following set of data, answer the questions below:

Temperature (°C)	0	5	10	15	20	25	30	35	40
Oxygen output (mL/hr)	0	0.1	3	8	15	21	21	14	2

  a Which type of graph would be used to represent the data?
  b What would be a suitable title for this graph?
  c Draw a graph of the data, including all appropriate graph features.

1.5 Ethics

KEY IDEAS

In this topic, you will learn that:

+ when undertaking research, you must apply ethical understanding
+ ethical approaches help guide discussion and decision-making in research.

Ethical understanding is a principle that should be considered across all VCE sciences. One of the School-assessed Coursework tasks is analysing and evaluating a bioethical issue. This can be completed in either Unit 3 or 4.

Applying ethical understanding can involve: considering the impact of scientific investigations on living things or the environment, conducting investigations with integrity, for example when reporting results or using other peoples' data, making decisions about ethical issues using ethical concepts and considering different values or social, economic, political and legal factors.

Some practical investigations may involve humans as subjects. In these cases, it is the responsibility of your teacher to make sure consideration to ethics is given. Animals are not expected to be used in this course and may only be used if it complies with the law.

Ethical approaches

Ethical approaches can help to guide discussions, thoughts and decision-making.

There are three types of ethical approaches that could be considered.

- A consequences-based approach considers the implication of the decision by maximising the positive outcomes and minimising the negative consequences. (Ask yourself: Does the end justify the means?)
- A duty-based approach means that people have a responsibility to act in a particular way. This approach is not concerned with the consequences of the outcome. (Ask yourself: Did they follow the rules?)
- A virtues-based approach considers a person's virtue or moral character, not the action. It considers good behaviours and actions. (Ask yourself: Did the person mean well?)

integrity
the ethical principle regarding the commitment to the search for knowledge and being honest in the approach

justice
the ethical principle regarding ensuring a fair and equal consideration of all factors

beneficence
the ethical principle regarding a commitment to minimising risk and doing good

non-maleficence
the ethical principle to avoid harm or to decrease the amount of harm inflicted

Ethical concepts

When exploring ethical issues and dilemmas in research, it is important to consider ethical concepts to determine how acceptable particular effects and causes are.

- **Integrity** is the commitment to being honest. During research, it is important to honestly communicate results, whether favourable or unfavourable.
- **Justice** is the moral rightness and commitment to fairly assessing claims, means and actions. It means that all are treated equally to ensure a moral obligation stands.
- **Beneficence** is the idea that the purpose of a person's action should minimise the risk of harm. This involves analysing potential risks against the benefits of the action.
- **Non-maleficence** is to avoid harm. Because scientific research may involve harm, the benefits of the actions in the research results must outweigh the resulting harm.

- **Respect** refers to the intrinsic value of all living things, which takes into consideration the religious beliefs, cultural heritage, views and opinions, customs, as well as the health and safety of an individual or group. This ethical principle ensures that living things have the ability to make their own decisions and when that can't happen, the decision made should be based on empowerment and protection.

respect
the ethical principle that takes into consideration the value of living things and the ability of living things to make their own decision where possible

CASE STUDY 1.5

Three-person babies – a story of ethics

The United Kingdom government has granted permission for 'three-person babies' for women who have a mitochondrial gene mutation that gives rise to a neurodegenerative disorder known as MERRF syndrome. This means that fertility doctors have approval to produce a healthy embryo from a woman with this mitochondrial mutation in a process known as mitochondrial donation therapy.

MERRF (myoclonic epilepsy with ragged red fibres) syndrome is caused by a mutation in mitochondrial DNA (mtDNA) inherited from the mother. Mitochondrial DNA is usually lost from sperm during fertilisation (therefore, human mtDNA is inherited from the mother via the egg cell). This disorder is rare and mainly affects the nervous and skeletal systems. MERRF syndrome worsens over time and often results in early death.

A baby born from mitochondrial donation therapy will essentially have DNA from three parents, inheriting genes from two mothers and a father. The mitochondrial donor would only contribute approximately 0.2% of the total genes to the embryo, with the majority from the two parents' nuclear DNA. The woman who donates her mitochondria would be anonymous and would not have any rights over the child.

Mitochondria are not well understood and the mtDNA may affect inherited traits in unknown ways, and this may have an effect on the child, who did not consent to this procedure. Some scientists believe that mitochondria should be better understood before techniques like mitochondrial donation therapy are used.

FIGURE 1 A scientist removing the nucleus from an egg cell

CHECK YOUR LEARNING 1.5

Describe and explain

1 Explain the importance of having an ethical understanding in science.
2 Describe the three types of ethical approaches.
3 What is the ethical concept of respect?

Apply, analyse and compare

4 Compare the ethical concepts of beneficence and non-maleficence.
5 When would the ethical concept of integrity be important in biology?

Design and discuss

6 Identify and discuss the ethical dilemmas of Case study 1.5 Three-person babies – a story of ethics. Make sure to include the pros and cons of the situation and then write down your decision backed up with evidence.

1.6 School-assessed Coursework

KEY IDEAS

In this topic, you will learn that:

- there are five Outcomes in VCE Biology
- the School-assessed Coursework (SAC) is worth 50% of your study score
- there is a variety of assessment tasks to be completed in the School-assessed Coursework.

School-assessed Coursework (SAC)
an internal assessment, written by the school, to be completed in class time that contributes towards the study score for a subject

study score
the score out of 50 for a subject, calculated by VCAA, using School-assessed Coursework and the end-of-year examination

outcome
the Key Knowledge and skills needed to demonstrate a satisfactory achievement for an Area of Study

There are a total of five Areas of Study in the Units 3 & 4 VCE Biology course. Each Area of Study has an Outcome that is assessed with **School-assessed Coursework (SAC)**.

The two SACs for Unit 3 will contribute to 20% of your **study score** and the three for Unit 4 will contribute to 30% of your study score. Therefore, each SAC will contribute 10% towards your study score. **Outcomes** 1 and 2 for Units 3 & 4 will each be 50–70 minutes in length as a written task, or 10 minutes for a multimodal or oral presentation. Outcome 3 of Unit 4 will be a minimum of 7 hours of class time.

To achieve a satisfactory completion for each Outcome, you must demonstrate the set of knowledge and skills for the relevant Area of Study. Each SAC will assess the appropriate Key Knowledge for the Outcome and may include assessing the key science skills as well.

Unit 3

Outcome 1 (Area of Study 1)

Unit 3 Outcome 1 will assess your understanding of the relationship between nucleic acids and proteins and the tools and techniques used and applied in the manipulation of DNA. This content is covered in chapters 2 and 3 of this book.

Outcome 2 (Area of Study 2)

Unit 3 Outcome 2 will assess your understanding of the structure and regulation of biochemical pathways, focusing on photosynthesis and cellular respiration, and how biotechnology can be used to solve problems related to these pathways. This content is covered in chapters 4–7 of this book.

FIGURE 1 A bioreactor is a type of biotechnology used to grow microorganisms on a large scale.

Unit 4

Outcome 1 (Area of Study 1)

Unit 4 Outcome 1 will assess your understanding of the immune response to specific antigens, the different ways immunity may be acquired, and the challenges and strategies in the treatment of disease. This content is covered in chapters 8–10 of this book.

Outcome 2 (Area of Study 2)

Unit 4 Outcome 2 will assess your understanding of the genetic changes in populations and species, evidence for relatedness between species, and evidence for human change over time. This content is covered in chapters 11–14 of this book.

Outcome 3 (Area of Study 3)

In Unit 4 Outcome 3, you will design and conduct a practical investigation related to cellular processes and/or responses to change over time using the key science skills as outlined in Topic 1.2. Your results will be presented in a poster and logbook entries. See Topic 1.3 for guidelines on how to present the poster and logbook entries. You can seek additional support for poster and logbook skills in the *Biology for VCE Units 3 & 4 Student workbook*.

Outcomes 1 & 2 assessment task types

For Outcomes 1 & 2 of Units 3 & 4, you will complete one of each of the following tasks once in either Unit 3 or 4:

- **Case study analysis and evaluation:** This involves an in-depth examination of a subject of study. You may be provided the case study to read before the assessment, or there may be a stimulus in the assessment for you to read and analyse. There are a number of case studies scattered throughout this book. There are also questions on each case study in the Check your learning questions. These questions can be good practice for this assessment.
- **Data analysis and evaluation:** This involves evaluating given primary data and/or collated secondary data. You could be asked to look at a variety of forms of data and produce graphs or other forms of displaying data. Topic 1.4 on data, measurement and error is a good reference tool to use when preparing for this assessment.
- **Response to three practical activities:** This assessment task will involve completing three practical activities, then responding to questions that reflect upon linked biological concepts, methodologies and findings across all of the practical activities. Completing the course's practical activities and practising your logbook skills are key to doing well in this assessment.
- **Response to a contemporary ethical issue:** Ethical issues are a big part of biological science. You will be required to analyse all of the factors that relate to a contemporary ethical issue. Topic 1.5 on ethics is a good reference tool to use when preparing for this assessment.

Each of these assessments is worth 10% of your final grade for the VCE Biology Units 3 & 4 course.

Study tip

Ask your teacher for a checklist of the Key Knowledge that will be assessed in the SAC so that you can thoroughly prepare. Don't forget that the key science skills may also be assessed in each Outcome.

FIGURE 2 Students need to study for their internal and external assessments.

Practice SACs

Study tip

Studying for your Student-assessed Coursework is as important as studying for your external examination. You can find Practice SACs on pages 180–183 and 376–385.

It is important that before starting your first School-assessed Coursework, you complete practice SACs to practise the key science skills required to do well in these assessments. At the end of each Unit in this book there are practice SACs for each Outcome. These cover concepts on the key knowledge you will be assessed on in your summative assessments. Give yourself 50 minutes to complete each of these practice SACs.

For Unit 3, Area of Study 1, a bioethical issue and associated questions have been provided. For Unit 3, Area of Study 2, a comparison of three practical investigations and associated questions have been provided. For Unit 4, Area of Study 1, a case study and associated questions have been provided. For Unit 4, Area of Study 2, data and associated questions have been provided.

These practice SACs can be completed at any time, but it is suggested that you complete them after you have learned all the Key Knowledge for that Area of Study. The *Biology for VCE Units 3 & 4 Student workbook* has four mini-activities for each chapter that assess the skills required to succeed in each of your assessments. When sitting a practice SAC, it is important to mimic the test environment that you will have in the classroom. Time yourself and sit in silence with no device or other resources distracting you. Once completed, mark your paper and diagnose any knowledge or skills that require further revision.

Video
Tips for the student investigation (AOS3)

Unit 4, Outcome 3 – Student-designed scientific investigation

This student-led assessment involves using the key science skills to state an aim, formulate a hypothesis or hypotheses, plan an investigation, carry out the investigation safely and following the ethical guidelines and then present the results in a poster with accompanying logbook entries.

At the end of Unit 4 in this book, there are detailed steps and tips for completing this assessment. Topics 1.2, 1.3, 1.4 and 1.5 of this chapter will be good reference tools to use when preparing for this assessment.

Assessment

The key knowledge covered in the student-designed scientific investigation are investigation design, scientific evidence and science communication.

Investigation design

Investigation design is all about planning your own assessment. You need to have clear knowledge of biological concepts and key terms from Units 3 & 4. A flash card glossary of all glossary terms in this book can be found on your obook pro. You need to pick your methodology and methods correctly and identify the independent and dependent variables of your investigation. You will need to generate primary quantitative data for your investigation and be aware of accuracy, precision, reproducibility, repeatability and validity of any measurements you make. You will also need to conduct a risk assessment of your investigation and ensure it follows ethical guidelines.

Scientific evidence

When conducting your investigation, you will need to identify evidence that supports or refutes (acts against) your hypothesis(ses). When presenting your data, you will need to organise it in a way that displays patterns and is easy for the reader to follow. You will need to comment on any assumptions, errors and uncertainty in your data, and you will need to record it all in your logbook entries.

Science communication

Part of being a good researcher is the ability to communicate about science effectively. When completing this investigation you will need to consider the terms, symbols, formulas, standard abbreviations and units of measurement that you use to convey your results. You will need to follow the poster layout provided for you and present the poster in an appropriate manner. Key points for poster presentation can be found in the *Biology for VCE Units 3 & 4 Student workbook* and on page 14 of this chapter. When you make concluding statements you will need to address your key findings and their implications.

CHECK YOUR LEARNING 1.6

Design and discuss

1 Discuss ways to prepare for your School-assessed Coursework.

2 Review the different School-assessed Courseworks and if you have any queries, ask your teacher.

1.7 External examination

KEY IDEAS

In this topic, you will learn that:

- there are techniques you can use during reading time in an examination
- there are strategies you can use during the writing time to address different types of examination questions
- there are revision techniques you can used to prepare for assessment.

external examination
a test written by external assessors that determines an individual's knowledge and skills in a subject

The level of achievement for Units 3 & 4 Biology is further assessed by an end-of-year **external examination**. The School-assessed Coursework contributes 50% of the study score, with the examination contributing the remaining 50%.

The end-of-year examination can assess any of the Key Knowledge and key science skills from Units 3 & 4. The date of the examination is published annually by the VCAA. Writing time for the examination is 2 hours 30 minutes, with a reading time of 15 minutes.

Exam techniques

It is important that every minute of your time in the examination is used efficiently.

Weblink
VCAA VCE Biology past examinations

Video
Preparing for the exam

Using reading time

- Scan the examination paper to check the kinds of questions you have been provided and that all the pages of the material are there. This will help you to start planning your writing time.
- **Multiple-choice questions** are normally designed to take approximately 1 minute each. So if there are 40 questions in this section, aim to spend approximately 40 minutes on the multiple-choice questions.
- When reading through the **short-answer questions**, check the mark allocation to get an idea of the time you should allocate (approximately 1 minute per mark). Read each question carefully and assess the question to consider the Key Knowledge and/or key science skills that are being assessed.
- Make a mental note of the questions that may be more difficult, might take more of your time to complete or may need further reflection. It is recommended that you complete those questions last, so this gives you more time to consider the responses and more time to write the responses if you have been able to finish the other questions more quickly.

multiple-choice question
examination-style question that has four possible alternatives in which you are required to select the most appropriate option

short-answer question
examination question that requires a short written response

Strategies for writing time

- Read each short-answer question three times. On the first read, try to understand the overall view of the question, and on the second read find the key information. Do the third read after you have written your answer to make sure that you have included everything that's needed in your response.
- Highlight or underline the key words within the question and reflect on these key words while writing your response or selecting the correct answer.
- For the short-answer questions, consider the concept being addressed and the key scientific terminology you may need to include in your answer.

- Make sure you format your responses as dot points, where each dot point represents a piece of information. It can also be helpful to underline the scientific terminology in your response to demonstrate clear understanding. Do not repeat the stem of the question in your response.
- Be aware of the time to make sure you are keeping to the plan you set during reading time. If you get stuck on a question, circle it and come back to it later.

Different types of questions

- Multiple-choice questions may emphasise a definition or require analysing and interpreting data.
  - Only one option is completely correct and the other three options may be distractors; therefore, you should read all of the options before selecting the best response.
  - If unsure, cross out the options you know are definitely incorrect. This improves your chance of selecting the correct answer.
  - Never leave a multiple-choice question unanswered, because you still have a 25% chance of selecting the correct response. Marks are not deducted for an incorrect response.
- Short-answer questions will most likely take the form of one of the following task words: name, describe/draw, explain how, explain why, media response or experimental design.
  - '**Name**', '**state**', '**list**', '**what is?**' are all examples of questions that would be worth 1 mark. The response should be brief, concise and clear.
  - '**Describe**' and '**draw**' questions require a judgment of the marking allocation to get an idea of the detail required and the time to be spent on the response. If you are asked to draw, you can use a pencil but make sure the drawing is clear and labelled. Do not shade or include irrelevant detail in your drawing. Remember to keep to your time limit with the diagram.
  - '**Explain how**', '**outline how**', '**state the way**' are generally questions worth 2 or more marks. If the question asks for an outline of the process, identify the starting point and finishing point of the process, then think about the step-by-step stages of the process. Make sure you are using dot points to formulate your response. You can also choose to respond with a labelled diagram. Consider the key terminology you should include in your response before writing it.
  - '**Explain why**' and '**justify**' are questions that require an application of the key content knowledge and are generally a cause-and-effect question. These questions often require a higher level of thinking and more detail in the response.
  - '**Compare**', '**contrast**', '**what are the differences/ similarities between**' are questions that require a comparison between concepts or key terms. It is a good idea to draw a line down the centre of the space allocated for the response. On one side of the line, describe one term/concept, and on the other side make the comparison.
  - Media response questions assess the application of content as well as your analytical and literacy skills. Be clear with your response since many students can get off-track and go on tangents.

FIGURE 1 For compare and contrast questions, it is a good idea to write the similarities and differences down as a brainstorming activity.

– Experimental design questions assess your understanding of the experimental processes. If the question asks you to design an experiment, it is a good idea to answer with a flowchart like that shown below.

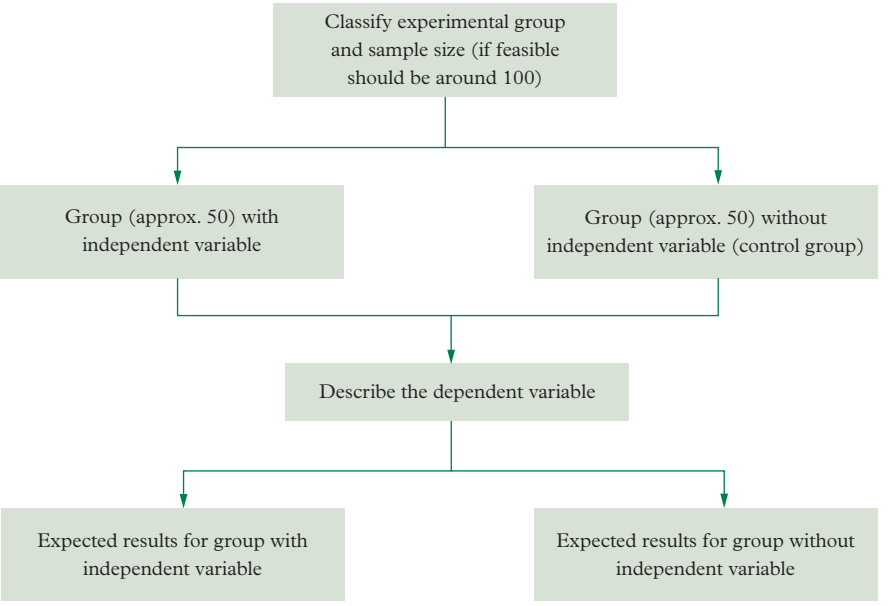


FIGURE 2 Flowchart for planning your experimental design question in the external examination

Short-answer questions may also ask you to 'refer to results' or 'use data'. Make sure that when you are responding to these types of questions you use quantitative or qualitative data from the results provided, which might be in the form of a table or graph, or within an article.

Revision strategies

The key to revising is to start early, which requires organisation and time-management skills. Making a study timetable and goal-setting are two strategies that can assist with those skills. Each individual learner will have a preferred method of revision; however, here are some strategies to consider:

- Use the Pomodoro technique where the revision is in manageable time chunks – such as 20 minutes each chunk. Set a goal and work on that task for a set time (20 minutes) and at the end of that time, stop for a 5 minute break. Start the timer again and begin on a new task after the break. After several sessions you can take a longer break. Using this method can maximise efficiency and productivity because it breaks up the learning into manageable sections.
- Have a study group with peers who are also studying Biology. Make sure the group members are those who will motivate you during study sessions.

FIGURE 3 Focus study groups can be a great way to share your questions and listen to different explanations of key concepts.

- Complete a set of practice questions. You may wish to do these as open book or in exam style conditions. It is always a good idea to time yourself completing questions to get an idea of time allocation and the pace you should be working at or towards.
- Produce a **mind map** or concept map to overview a concept, Area of Study or Unit. This technique helps to make connections, identify big ideas and visualise concepts.
- Translate a concept or key idea into a flowchart or a diagram.

mind map
a graphical way to represent key ideas and relationships between concepts

Work space

Each student will have a space at home where they complete schoolwork and revise. There are important things to consider for making this space an efficient study environment.

Where possible, the environment should be silent (or at least quiet) with adequate light and ventilation. The table should be large enough for all of your resources with a supportive chair. When completing or revising work, it is important to minimise distractions and one of the biggest distractions is a mobile phone. If you know your device distracts you, turn it off, put it on aeroplane mode or leave it in another room.

Study tip

Your school library and local library will have quiet spaces that may provide an environment constructive for learning. Check out these libraries for suitable spaces since you might find you are more productive in this space than at home.

FIGURE 4 Creating a comfortable study space will help boost your mental efficiency.

CHECK YOUR LEARNING 1.7

Describe and explain

1 What percentage of the external examination contributes to your study score for Biology?
2 How long should you spend on each question in the external examination?
3 Describe the Pomodoro study technique.

Apply, analyse and compare

4 Why is a productive workspace important?
5 Analyse the revision techniques/strategies you currently use. What might you do differently, and why?

Design and discuss

6 Discuss important considerations when answering short-answer questions.

CHAPTER 1

Review

Chapter summary

1.1
- Biology investigates the interactions of living things with their environment.
- Biology provides a pathway to a diverse range of career options.
- There are four units in VCE Biology, which are taught over 2 years.

1.2
- The key science skills are essential when undertaking investigations.
- The scientific method is a series of steps of formulating a hypothesis and planning and conducting practical investigations for collection of data to determine whether the hypothesis is supported.
- Risk assessments outline hazards and provide control measures to avoid risks.

1.3
- There are many ways to conduct a scientific investigation.
- Maintaining a logbook is an essential requirement for scientific investigations.
- A student-designed scientific investigation requires a demonstration of key science skills by presenting findings from the investigation as a scientific poster.

1.4
- Tables are usually the first step in formatting qualitative and quantitative data.
- Graphs are used to show relationships between variables.
- Accuracy, precision, repeatability, reproducibility, true value and validity are used to evaluate the data and the methodology of an investigation.
- Quantitative results are numerical data; qualitative results are not numerical data.
- Random errors are unpredictable and reduce the precision of the data. Systematic errors are consistent and repeatable and reduce the accuracy of the data.
- Outliers should not be dismissed, but evaluated, investigated and accounted for.

1.5
- Ethical approaches include consequences-based, duty-based and/or rule-based, and virtues-based approaches.
- Ethical concepts include integrity, justice, beneficence, non-maleficence and respect.

1.6
- School-assessed Coursework (SAC) contributes 50% towards the score for VCE Biology. The Unit 3 SAC contributes 20% and Unit 4 SAC contributes 30%.
- There are four types of assessment task for Units 3 & 4, Outcomes 1 & 2: case study analysis and evaluation, data analysis and evaluation, a response to three practical activities, and a response to a contemporary ethical issue.
- Unit 4, Outcome 3 will be a scientific investigation presented as a scientific poster and accompanying logbook.

1.7
- The external end-of-year examination contributes the remaining 50% of the study score. It will assess Key Knowledge from Units 3 & 4, as well as the key science skills.
- Preparing for the external examination requires organisation, time-management skills and effective revision strategies.

Revision questions

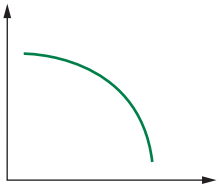
Multiple choice

1 Which of the following would be an appropriate methodology to investigate the following research question: Is there evidence from scientific investigations that suggests genetically modified food causes harm to living organisms?

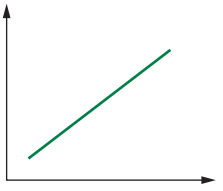
A Modelling
B Controlled experiment
C Literature review
D Simulation

2 A graph is produced to show the relationship between the rate of water loss in plants and temperature. Which of the following graphs shows that as the temperature increases, the rate of water loss increases?

A

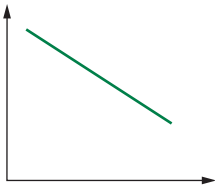


B



C

D



3 An investigation was conducted to test the effect of soil pH on seed germination. The hypothesis stated that if the soil pH is increased, then more seeds will germinate. What is the independent variable?

A The different seeds
B The different pH levels of the soil
C The number of germinated seeds
D The amount of water given to each seed

Short answer

Describe and explain

4 Explain the importance of generating risk assessments for practical investigations.

Apply, analyse and compare

5 Analyse the significance of the knowledge and skills of the Aboriginal and Torres Strait Islander Peoples to VCE Biology.

6 Compare qualitative and quantitative data. Practise answering using the exam technique of drawing a line down the middle of the page and comparing qualitative data on one side and quantitative data on the other.

Design and discuss

7 Molly undertook an experiment to investigate the effect of temperature on yeast growth. She set up three test tubes, each containing dry yeast with added water and sugar (providing an environment for the yeast to survive and reproduce). The initial heights of the test-tube contents were recorded. One test tube was placed in a water bath set to 25°C, another at 30°C and another at 35°C. The final heights of the test-tube contents were recorded after 15 minutes.

a What is the aim of the investigation?
b Write a hypothesis.
c What would be three controlled variables in this experiment? Justify each one.
d Since there was only one trial at each temperature, explain why precision cannot be analysed.
e Design a different experiment to investigate whether sugar affects yeast survival and reproduction. Make sure to include the variables (independent, dependent and controlled), a control group and expected results. You can use the flowchart outlined in Figure 2 in Topic 1.7.

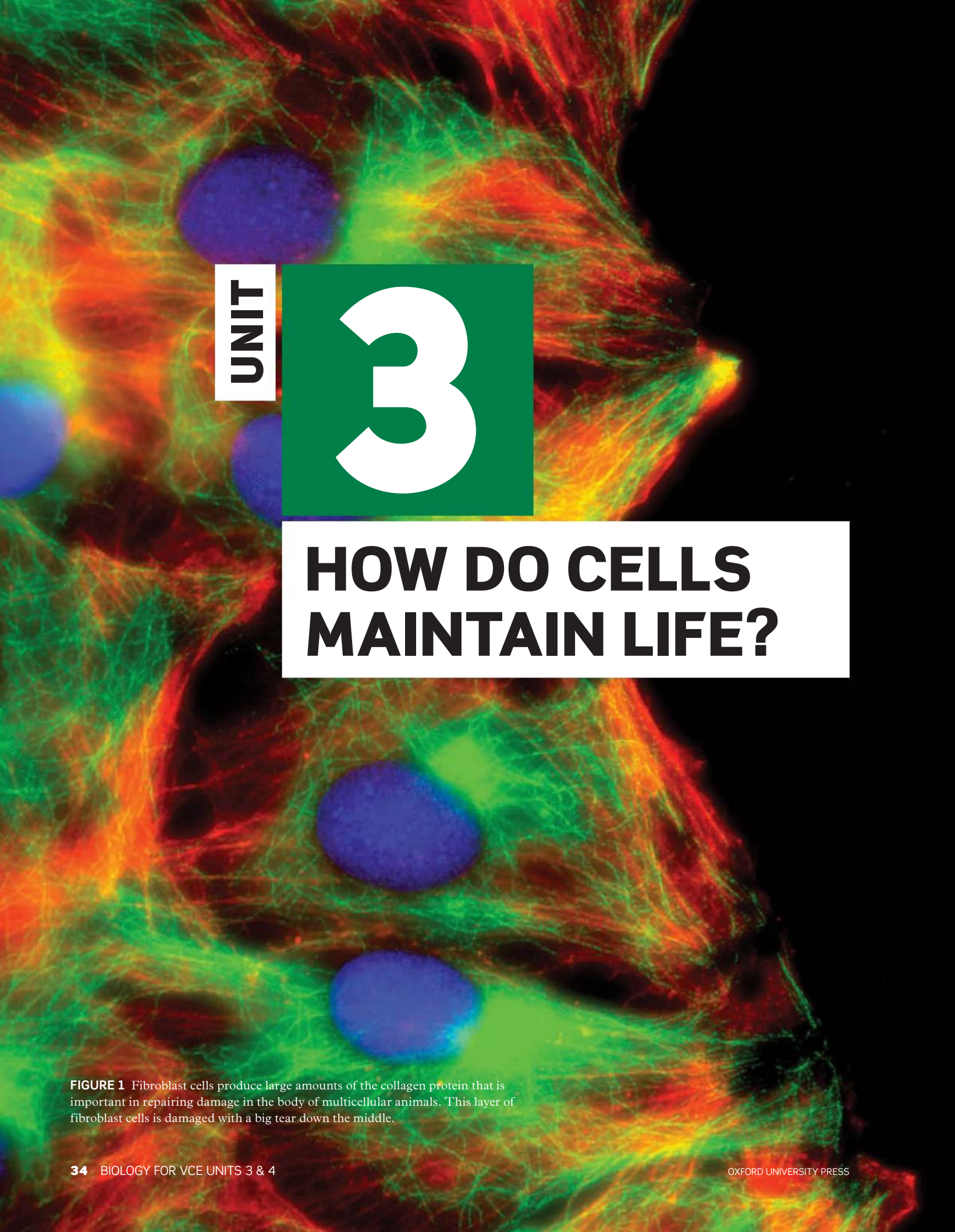
Check your Student obook pro for these digital resources and more:

QuizletLive
Compete in teams to test your knowledge.

Chapter quiz
Check your understanding of this chapter.

Check your Teacher obook pro for these resources and more:

QuizletLive
Launch a quiz for your students on key concepts in this chapter.



UNIT 3

HOW DO CELLS MAINTAIN LIFE?

FIGURE 1 Fibroblast cells produce large amounts of the collagen protein that is important in repairing damage in the body of multicellular animals. This layer of fibroblast cells is damaged with a big tear down the middle.

The smallest living part of any organism is the cell. Cells come in all shapes and sizes, from the smallest 10 micrometre bacteria, to the approximately 25-metre-long basal ganglion cell of a blue whale. Cells contain a set of genetic instructions in the form of DNA that enable them to survive.

Just like other multicellular organisms, the cells in your body each contain an identical set of genetic material, known as genes. If the DNA found in your fingertip is identical to that found in your eye, why then don't you grow an eye on the end of your finger? This suggests that every cell in your body must have a way of controlling which genes are switched on and which are switched off. Each gene is responsible for the way a cell functions, from making specific proteins to controlling other genes. Every cell will produce a range of proteins to carry out key functions.

Enzymes are proteins that control the chemical reactions in a cell. Some of these enzymes are responsible for controlling energy production in cells. For example, enzymes in plant cells facilitate the process of photosynthesis. The disruption of a single enzyme has an effect on the rest of a biochemical pathway and may have a domino effect on other reactions.

Increasing our understanding of how our genes control the production of proteins enables humans to look at techniques that manipulate cell function. The ability to manipulate DNA by adding, disabling or removing genes allows us to potentially improve the quality of life for a range of organisms. We can cure or prevent diseases, improve the efficiency of photosynthesis, or increase crop productivity and the nutrients in food.

Outcomes

On completion of this unit, students should be able to:

→ analyse the relationship between nucleic acids and proteins, and evaluate how tools and techniques can be used and applied in the manipulation of DNA

→ analyse the structure and regulation of biochemical pathways in photosynthesis and cellular respiration, and evaluate how biotechnology can be used to solve problems related to the regulation of biochemical pathways.

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

Area of Study 1	What is the role of nucleic acids and proteins in maintaining life?	Chapters 2–3, pages 36–105 Practice SAC, pages 180–181
Area of Study 2	How are biochemical pathways regulated?	Chapters 4–7, pages 106–179 Practice SAC, pages 182–183

CHAPTER 2

Nucleic acids and proteins

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are types of nucleic acids found in the cells of all living organisms. Nucleic acids provide the instructions for every structure and function of an organism, including DNA replication and protein synthesis. The genetic code within the sequence of nitrogenous bases of DNA contains the blueprint for all life processes. Gene expression and regulation varies between prokaryotes and eukaryotes due to differences in cell structure as well as differences in the location, size and structure of their DNA and RNA.

KEY KNOWLEDGE

- → nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three forms of RNA (mRNA, rRNA, tRNA) and a comparison of their respective nucleotides
- → 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
- → the structure of genes: exons, introns and promoter and operator regions
- → the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of the regulatory process
- → amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein
- → proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways
- → the role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

FIGURE 1 The double helix structure of DNA is the building block for all life on the Earth and even influences architecture such as the Helix Bridge in Singapore.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

2A Draw a nucleotide and label the phosphate, sugar and nitrogenous base.

2A Groundwork resource
Nucleotides

2B Explain why DNA is often referred to as the universal blueprint of life.

2B Groundwork resource
The role of DNA

2C Use your knowledge of DNA to define the term 'gene'.

2C Groundwork resource
Genes

2D Describe exocytosis.

2D Groundwork resource
Exocytosis

PRACTICALS

PRACTICAL	**2.1** Extracting DNA from strawberries
PRACTICAL	**2.5** Exploring protein structures

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

2.1 The structure of nucleic acids

KEY IDEAS

In this topic, you will learn that:

- the nucleic acids DNA and RNA are information molecules in the cells of prokaryotes and eukaryotes
- nucleotides are comprised of a pentose sugar, a phosphate and one of five nitrogenous bases
- DNA is a double-stranded molecule coiled around histone proteins that condenses into chromatin and forms chromosomes
- the three forms of RNA are messenger RNA, ribosomal RNA and transfer RNA.

protein
a long chain of amino acids folded into a specific shape that determines its cellular function

Nucleic acids are macromolecules found in the cells of all living organisms. Nucleic acids store genetic information, enable the production of **proteins** and allow for the transfer of genetic information from one generation to the next.

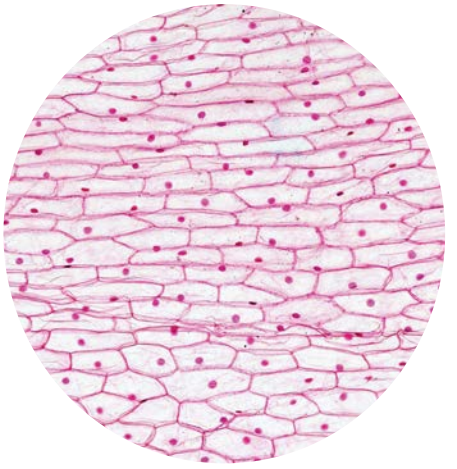


FIGURE 1 Cells are the building blocks of life. These cells are from an onion and have been stained pink.

The structure of life

The smallest structure of life is the cell. The cells of all living organisms share four common features:

- a plasma membrane that controls what goes in and out of a cell
- cytoplasm that contains all the fluid and material (except the nucleus) inside a cell
- nucleic acids that contains the genetic code for making proteins
- ribosomes that are responsible for making proteins.

These structural components are required by living organisms and ensure their survival by carrying out the cellular life processes of growth, repair and reproduction.

prokaryotic
a cell with no membrane-bound organelles

eukaryotic
a cell containing a nucleus and other membrane-bound organelles

All living organisms can be categorised as either **prokaryotic** or **eukaryotic** based on their cellular components.

Despite these four common features, prokaryotes and eukaryotes have some important differences in the location and structure of their nucleic acids. As a result, the process of protein synthesis is different between prokaryotes and eukaryotes.

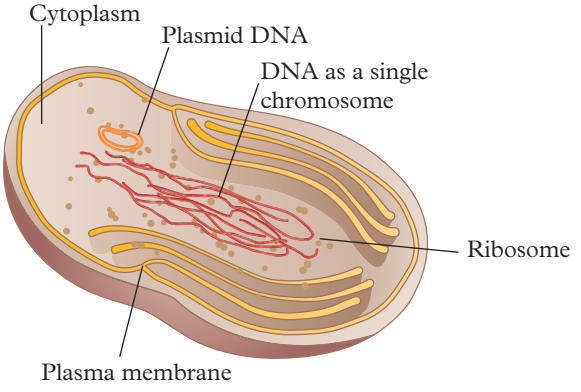


FIGURE 2 A 3D model of a prokaryotic cell

Prokaryotic structure

unicellular
a living organism composed of a single cell

Prokaryotes are microscopic, **unicellular** organisms from the bacteria and archaea kingdoms or domains. They lack a membrane-bound nucleus and other

specialised **organelles**. The nucleic acid is a single chromosome made of deoxyribonucleic acid (DNA) that floats freely as part of the cytoplasm along with proteins, ribosomes and other cellular components.

The DNA in prokaryotes is:

- a circular, double-stranded chromosome
- not bound to **histone proteins**.

Eukaryotic structure

Eukaryotes can either be unicellular (e.g. yeast) or multicellular organisms (e.g. plants and animals). They have a distinct nucleus surrounded by a nuclear membrane as well as membrane-bound organelles that carry out specialised functions. For example, the **endoplasmic reticulum** and **Golgi apparatus** are involved in protein transport, modification and packaging. These are discussed more in Section 2.5. The DNA is found within the nucleus, separate to other organelles.

The DNA in eukaryotes is:

- linear, double-stranded
- bound to histone proteins.

organelle
a specialised membrane-bound structure that carries out a specific function within eukaryotic cells

histone protein
a type of protein that eukaryotic DNA coils around to form nucleosomes

endoplasmic reticulum
an organelle involved in the production, modification and packaging of materials such as proteins, lipids and steroids

Golgi apparatus
an organelle involved in the packaging and secretion of cell products

Video
Cell structure

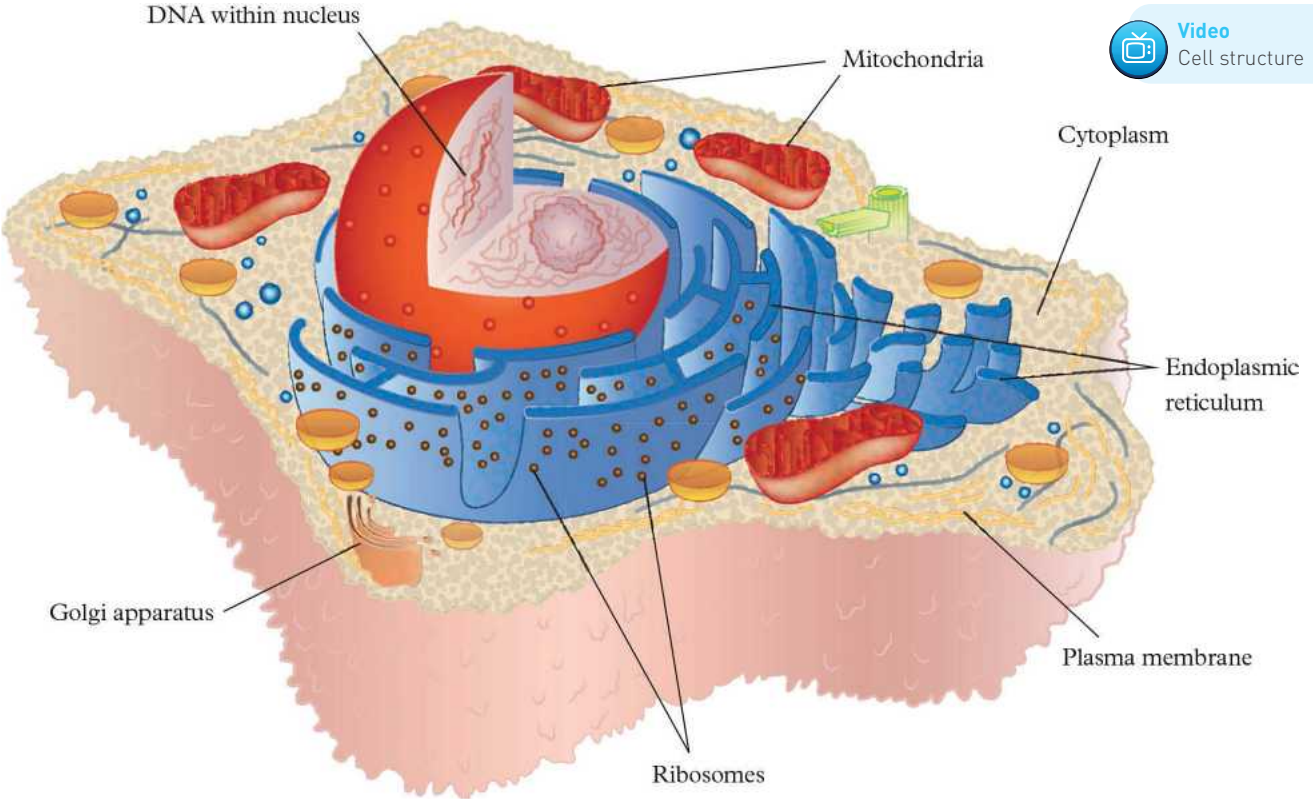


FIGURE 3 A 3D model of a eukaryotic cell

Nucleotides

nucleotides
the building blocks of nucleic acids; comprised of a sugar, phosphate and nitrogenous base

Nucleic acids are polymers, consisting of smaller, repeated units (monomers) called **nucleotides**. All nucleotides consist of a:

- 5-carbon (pentose) sugar
- phosphate group
- nitrogenous base.

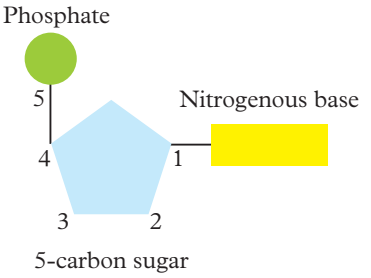
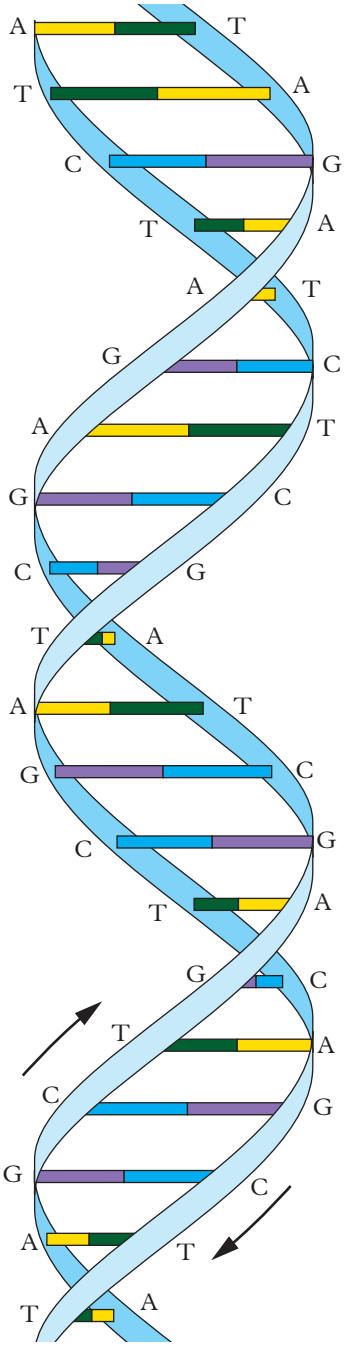


FIGURE 4 Basic structure of a nucleotide; the sugar is a deoxyribose pentose sugar.

deoxyribonucleic acid (DNA)
a type of nucleic acid that stores the genetic instructions for all life processes

ribonucleic acid (RNA)
a type of nucleic acid involved in protein synthesis



Types of nucleic acids

There are two types of nucleic acids: **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**. They differ in their chemical composition, structure and length. This structure is directly related to their functional roles within a cell.

The structure of DNA

DNA is often called the 'blueprint of life'. It is responsible for the shape of a cell and all the processes that a cell carries out. DNA does this through the production of proteins. DNA is also responsible for passing on genetic information to daughter cells through the process of DNA replication, cell division and reproduction.

DNA is a double-stranded nucleic acid. Each strand has alternating pentose sugars and phosphates that form the backbone of the molecule. The nitrogenous bases attach the two backbones together at the centre. The pentose sugar in DNA is a deoxyribose sugar (meaning a sugar with one less oxygen).

The nitrogenous bases join the two single DNA strands together by weak hydrogen bonds that form complementary base pairs at the centre of the two strands.

The complementary base pairing in DNA is:

- adenine (A) pairing with thymine (T) using two hydrogen bonds
- cytosine (C) pairing with guanine (G) with three hydrogen bonds.

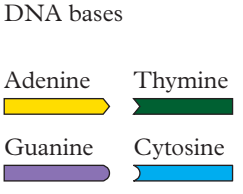


FIGURE 5 The structure of DNA with complementary base pairing

DNA is antiparallel

DNA consists of two polymer nucleotide strands. These are held together by the bonding of the nitrogenous bases between adjacent strands. The two strands are **antiparallel**, meaning that one strand starts with a (3') sugar, while the other strand is facing the opposite direction and has a (5') phosphate at the same end. This gives a ladder-like structure to the DNA molecule, which twists on its axis to form a double helix.

antiparallel where the two strands of DNA run in opposite directions to each other

Remember each sugar molecule has five carbons. These are labelled 1' through to 5' (or one prime through to five prime). The nitrogenous base is always joined to the 1' carbon (Figure 4), while each phosphate joins to the 3' carbon of one sugar and the 5' carbon of the next.

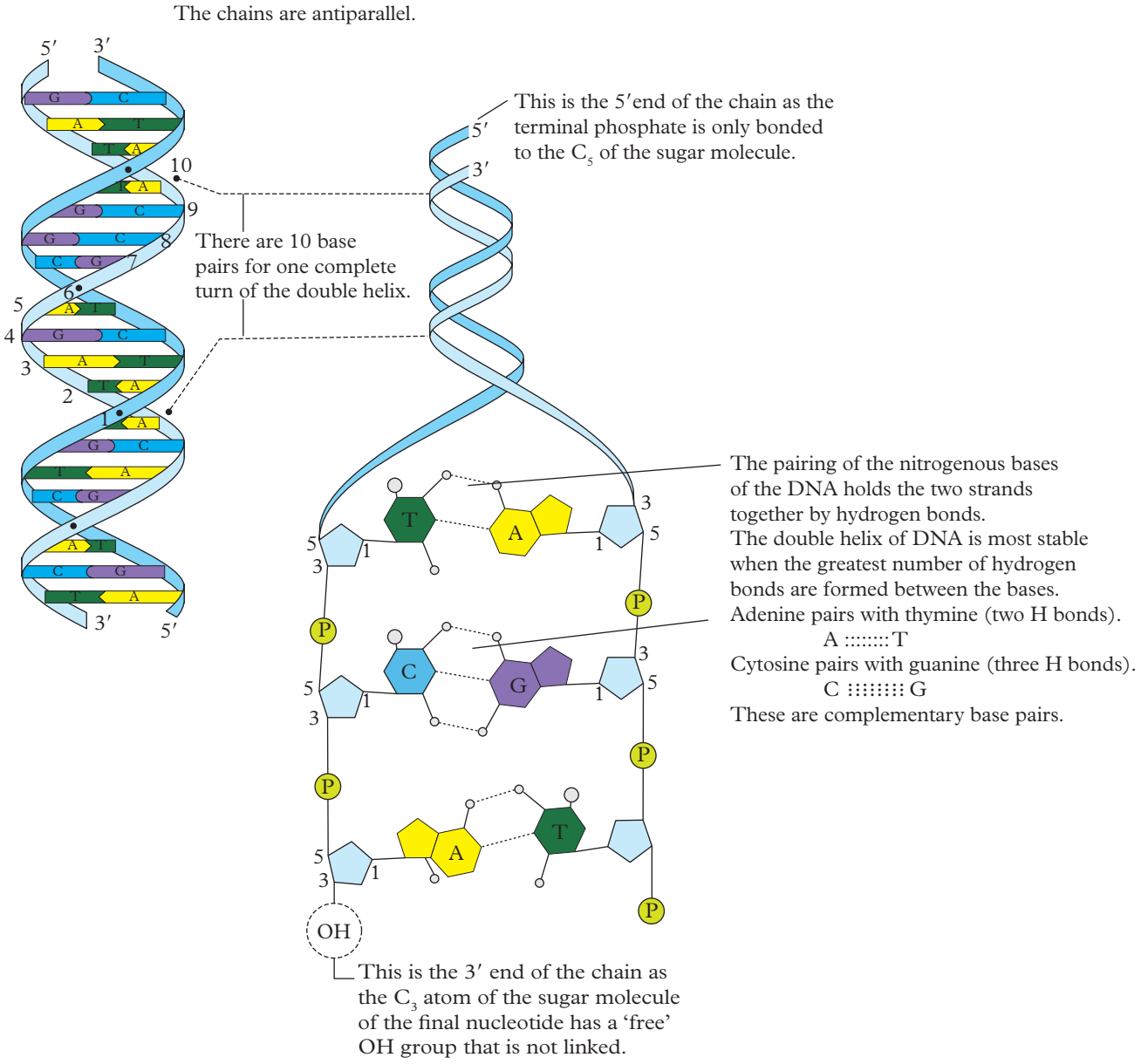


FIGURE 6 The antiparallel structure of a DNA molecule and structure of the hydrogen bonds

DNA coils to form chromosomes

The average human cell contains almost 2 metres of DNA. To organise the DNA and prevent it from tangling, the DNA is wound around histone proteins to form **nucleosomes**.

nucleosome
a complex formed when short sections of DNA coil around histone proteins

Each nucleosome is comprised of eight histone proteins with a short length of DNA (less than 200 base pairs) coiled around it.

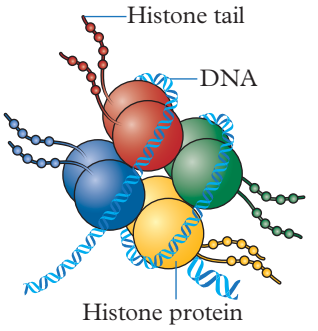
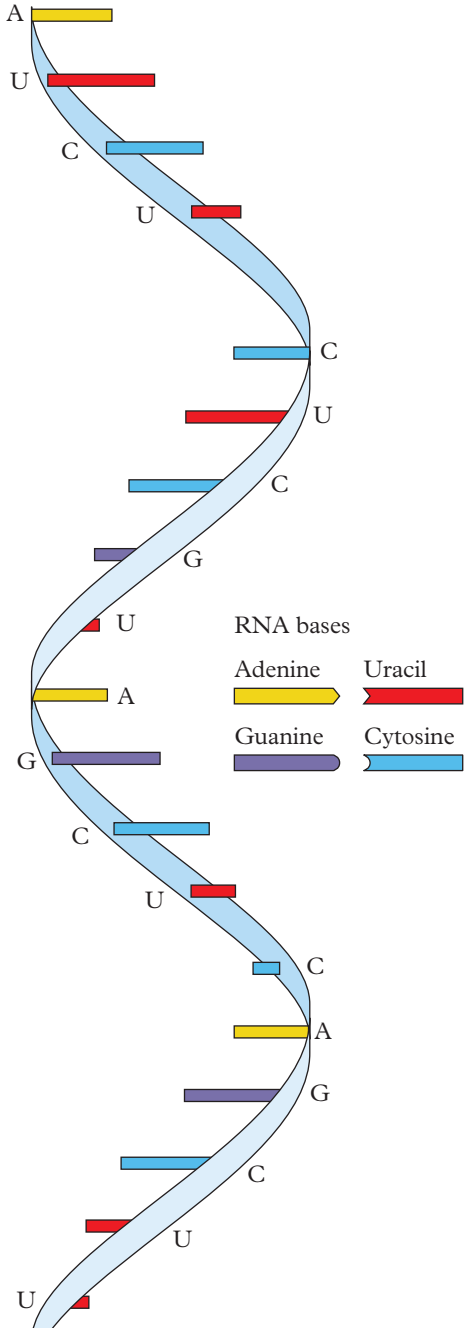


FIGURE 7 The coiling of DNA around histone proteins to form a nucleosome



amino acids
the building blocks of all proteins

FIGURE 8 The structure of RNA with complementary base pairing

The structure of RNA

Ribonucleic acid (RNA) is a type of nucleic acid directly involved in protein synthesis, biochemical reactions and complex cellular regulation processes. Like DNA, RNA is an information molecule that carries the same information as the DNA template; however, it is not involved in the long-term storage of the genetic code.

RNA is a single-stranded molecule consisting of a sugar–phosphate backbone with exposed nitrogenous bases (Figure 8). The pentose sugar in RNA is a ribose sugar (rather than the deoxyribose sugar in DNA) (Figure 9). The exposed bases are available for complementary pairing with template DNA or other RNA molecules and **amino acids** during protein synthesis. Some RNA molecules have looped regions that also form double-stranded RNA.

The complementary base pairing in RNA is similar to DNA with the exception of the amino acid uracil, which takes the place of thymine.

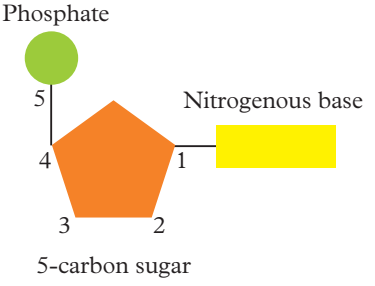


FIGURE 9 The basic structure of the RNA nucleotide with a ribose pentose sugar

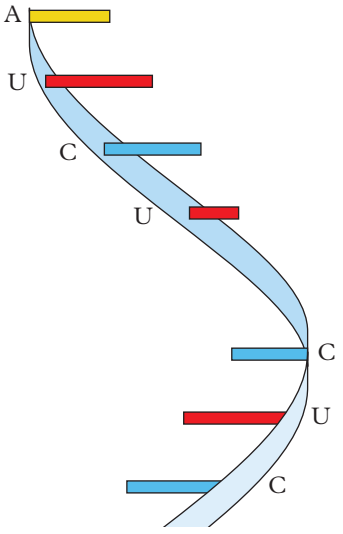
The complementary base pairing in RNA is:

- adenine (A) pairing with uracil (U) using two hydrogen bonds
- cytosine (C) pairing with guanine (G) using three hydrogen bonds.

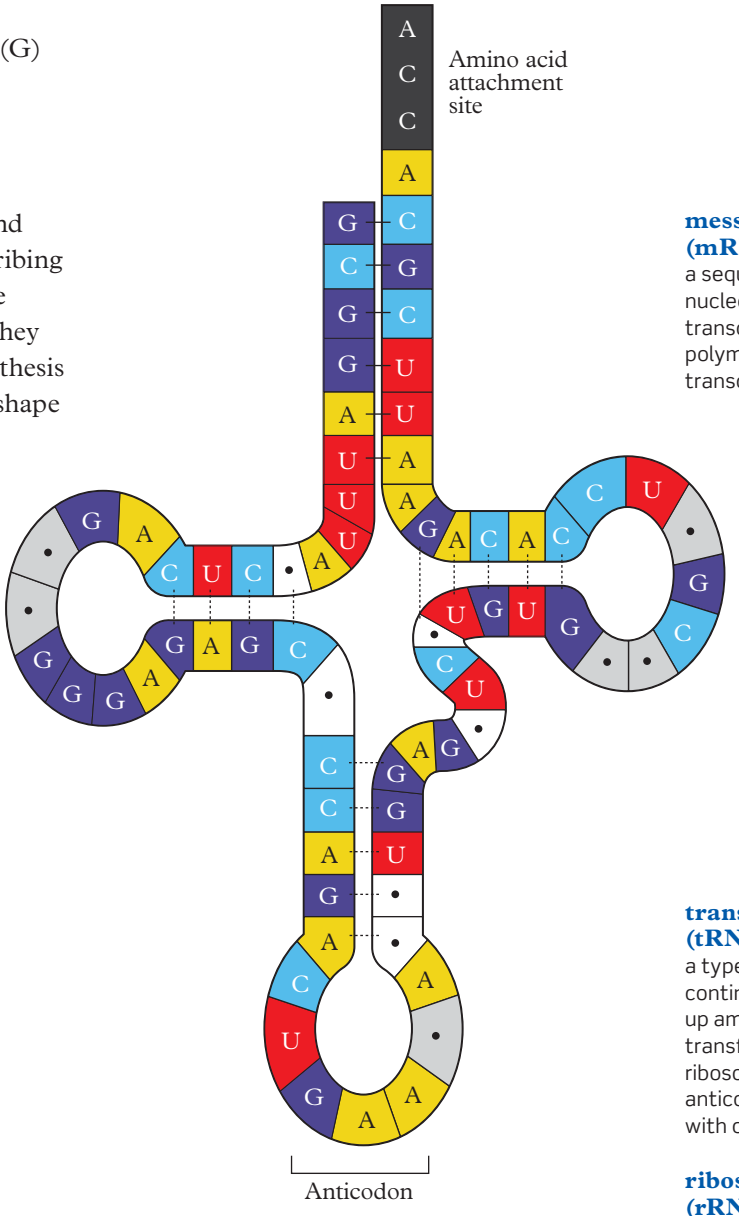
Types of RNA

There are three major types of RNA (**messenger RNA**, **transfer RNA** and **ribosomal RNA**), involved in transcribing and translating the DNA code into the proteins that are needed by the cell. They carry out different roles in protein synthesis and therefore have a different length, shape and location within a cell.

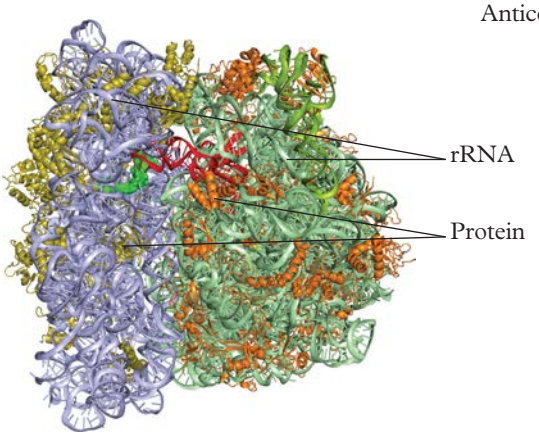
a Messenger RNA (mRNA); single-stranded and linear



b Transfer RNA (tRNA); coiled cloverleaf shape



c Ribosomal RNA (rRNA); spherical shape that holds the ribosome together



messenger RNA (mRNA)
a sequence of nucleotides transcribed by RNA polymerase during transcription

transfer RNA (tRNA)
a type of RNA that continuously picks up amino acids and transfers them to the ribosomes using its anticodon to match with codons on mRNA

ribosomal RNA (rRNA)
a component of ribosomes involved in the process of translation

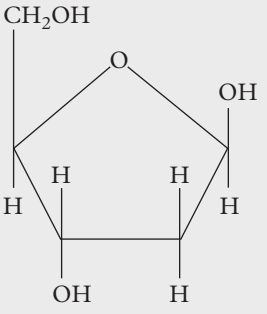
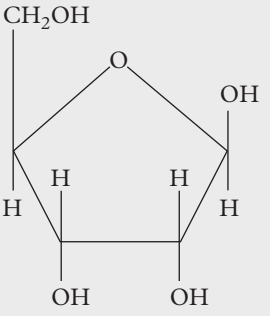
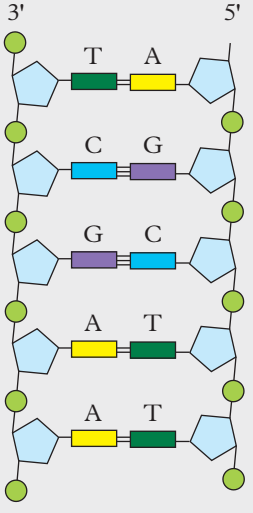
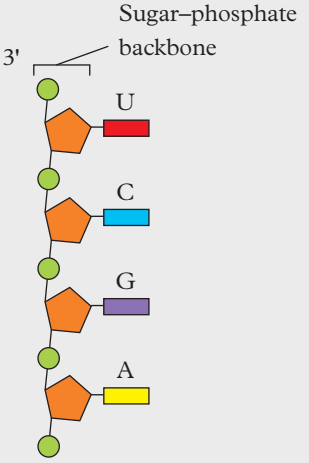
FIGURE 10 The structure of the three types of RNA: **a** messenger RNA; **b** transfer RNA; and **c** ribosomal RNA

Comparison of DNA and RNA

DNA and RNA are both information molecules that carry the code for the synthesis of proteins. While DNA maintains and stores the genetic information to encode proteins, the three types of RNA transfer and convert this code into a polypeptide chain.

Although DNA and RNA are both nucleic acids comprised of simple monomer nucleotides, there are several chemical and structural differences in the molecular components and the nucleic acids themselves.

TABLE 1 Summary of the differences between DNA and RNA

	DNA	RNA
Relative length	Long	Short
Nitrogenous bases	Adenine (A), Guanine (G), Cytosine (C), Thymine (T)	Adenine (A), Guanine (G), Cytosine (C), Uracil (U)
Base pairing	Adenine–Thymine Cytosine–Guanine	Adenine–Uracil Cytosine–Guanine
Stability	Very stable	Unstable
Sugar	Deoxyribose 	Ribose 
Strands	Double 	Single 
Information storage	Long-term, permanent storage within the nucleus of a cell	Short-term, temporary storage and transfer of information from the nucleus

CASE STUDY 2.1

Rosalind Franklin's contribution to the structure of DNA

English biochemists Rosalind Franklin and Maurice Wilkins spent many years analysing the DNA molecule using crystallographic studies. By 1935, Franklin determined the components and their proportions in the DNA molecule and from X-ray diffraction images stated that DNA was a helix shape.

American geneticist James Watson had previously worked with eminent chemist Linus Pauling, who had been studying DNA structure. Pauling (working with dried DNA) thought the molecule was triple stranded, but Franklin (working with fresh DNA) found it was double-stranded.

Watson travelled to the United Kingdom and started working with Francis Crick, a physicist at Cambridge University. Without Franklin's knowledge, Wilkins passed on her findings to them. Watson and Crick never did experiments themselves. Instead they reinterpreted other people's data. As a result, they were able to determine the double-helix structure of DNA in 1953.

Watson, Crick and Wilkins were awarded a Nobel Prize for this finding in 1962. Posthumous Nobel Prize awards are not granted and so Franklin was not credited for her massive contribution in her lifetime or after her death.

FIGURE 11 Rosalind Franklin was the first person to identify that DNA was a helix shape.

CHECK YOUR LEARNING 2.1

Describe and explain

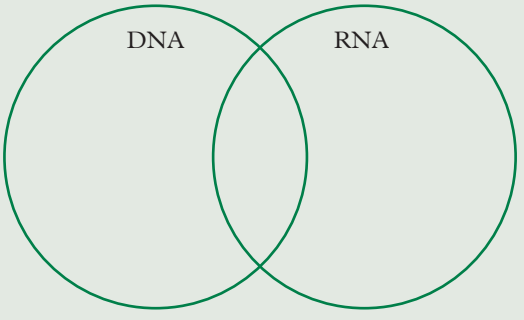
1 Identify the five nitrogenous bases and determine which are present in DNA and which are present in RNA.

2 Explain the role of RNA in a eukaryotic cell.

3 Describe how two metres of DNA is able to fit within the nucleus of a human cell.

Apply, analyse and compare

4 Create a Venn diagram to compare and contrast the similarities and differences between DNA and RNA.



5 A section of DNA contains 200 base pairs. There are 64 cytosine bases in total. How many thymine, guanine and adenine nucleotides would this DNA contain? Show your working.

Design and discuss

6 Discuss the importance of histone proteins to the permanent storage of DNA within the nucleus of a eukaryotic cell.

7 Design a simple method that you could follow to determine whether an unknown microscopic organism was prokaryotic or eukaryotic. What identifying structure or structures would you need to observe to be certain the organism was eukaryotic?

2.2 The genetic code

KEY IDEAS

In this topic, you will learn that:

- the genetic code is a degenerate, universal triplet code
- transcription involves mRNA synthesis from template DNA
- RNA processing in eukaryotic cells splices introns from pre-mRNA to produce mature mRNA
- translation converts the code on mature mRNA into a chain of amino acids brought together in the rRNA subunits of a ribosome by tRNA molecules.

genome
the complete set of genes for an organism

gene
a section of DNA that has a specific function

polypeptide
a chain of amino acids that forms the primary structure of a protein

triplet
a three nitrogenous base sequence in DNA that corresponds to codons on mRNA

Study tip

Polypeptides are simply proteins that have not yet achieved their full shape or function.

degeneracy
the redundancy of the genetic code, meaning more than one codon codes for the same amino acid

Every cell within a multicellular organism contains an identical **genome** in their DNA, with the exception of red blood cells and gametes. Short segments of DNA (from 200 to 2 000 000 base pairs) that have a specific function in an organism are called **genes**. Some of these genes carry the code for the production of proteins. The DNA molecule is responsible for controlling the actions and processes of each cell through protein synthesis.

The triplet code

DNA stores the information for protein synthesis within the sequence of nitrogenous bases for each gene. Like DNA, proteins are polymers – long chains of repeating monomer units called amino acids. While DNA has only four types of monomer nucleotides, proteins have 20 amino acids that can be arranged in a multitude of combinations and lengths to form **polypeptide** chains of different ordered amino acids.

Nucleotides in DNA are grouped into threes, or **triplets**. Each triplet contains the code for a single amino acid. The four different nitrogenous bases in DNA can be arranged into 64 possible triplet combinations ($4^3 = 64$). All but three of these triplets code for an amino acid. This triplet code forms the basis of the genetic code and is typically universal for all organisms found on Earth.

Degeneracy of the code

Most amino acids are specified by more than one triplet, and therefore the genetic code shows **degeneracy**. Sixty-four possible triplet codes are available for the 20 amino acids. For example, in the case of leucine, there are six possible triplet codes, while methionine (Met) and tryptophan (Trp) have only one possible triplet. Degeneracy most commonly results from a change in the last nitrogenous base of the triplet. For example, the triplets AAA and AAG will both code for the amino acid phenylalanine (Phe).

The roles of RNA in protein synthesis

Most biological activities are carried out by proteins, and accurate protein production is required for the proper functioning of cells and organisms. DNA stores the instructions for protein synthesis within sequences of nucleotides. However, it is the RNA molecules that translate these instructions into the unformed proteins called polypeptide chains. The assembly of amino acids into their carefully ordered polypeptide chains, as encoded by the DNA, is vital to the production of functional proteins.

Three kinds of RNA work together to produce the polypeptide chains that make up functional proteins. Each has a different role in protein synthesis, as described here.

- **Messenger RNA (mRNA)** carries a copy of the genetic information from the DNA to the ribosome on the surface of the **rough endoplasmic reticulum** or floating freely in the cytoplasm. Each triplet on the DNA is converted into a **codon** (three nucleotides) on the mRNA.
- **Transfer RNA (tRNA)** molecules have an open end to which a single, specific amino acid can bind in a reaction that requires a specific enzyme and ATP. At the other end of the tRNA molecule is a triplet of unpaired bases called an **anticodon** that joins to codons on the mRNA by complementary base pairing.
- **Ribosomal RNA (rRNA)** associates with a set of proteins to form the large and small subunits of a ribosome. These physically move along an mRNA molecule and catalyse the assembly of amino acids into polypeptide chains.

rough endoplasmic reticulum
organelle involved in the synthesis, packaging and transport of proteins

codon
a sequence of three nucleotides on mRNA that codes for a specific amino acid

anticodon
a sequence of three nucleotides on tRNA that corresponds to a complementary codon on mRNA

Transcription

DNA contains the genetic code for protein synthesis. This information is permanently stored within the nucleus of a eukaryotic cell. Proteins are synthesised on ribosomes within the cytoplasm, and therefore the code must be transcribed (copied) into mRNA, which can exit the nucleus via a nuclear pore. The process of transferring the code from DNA to mRNA is called **transcription**.

The process of transcription occurs in three stages: initiation, elongation and termination.

transcription
the process of copying the genetic information in DNA into mRNA

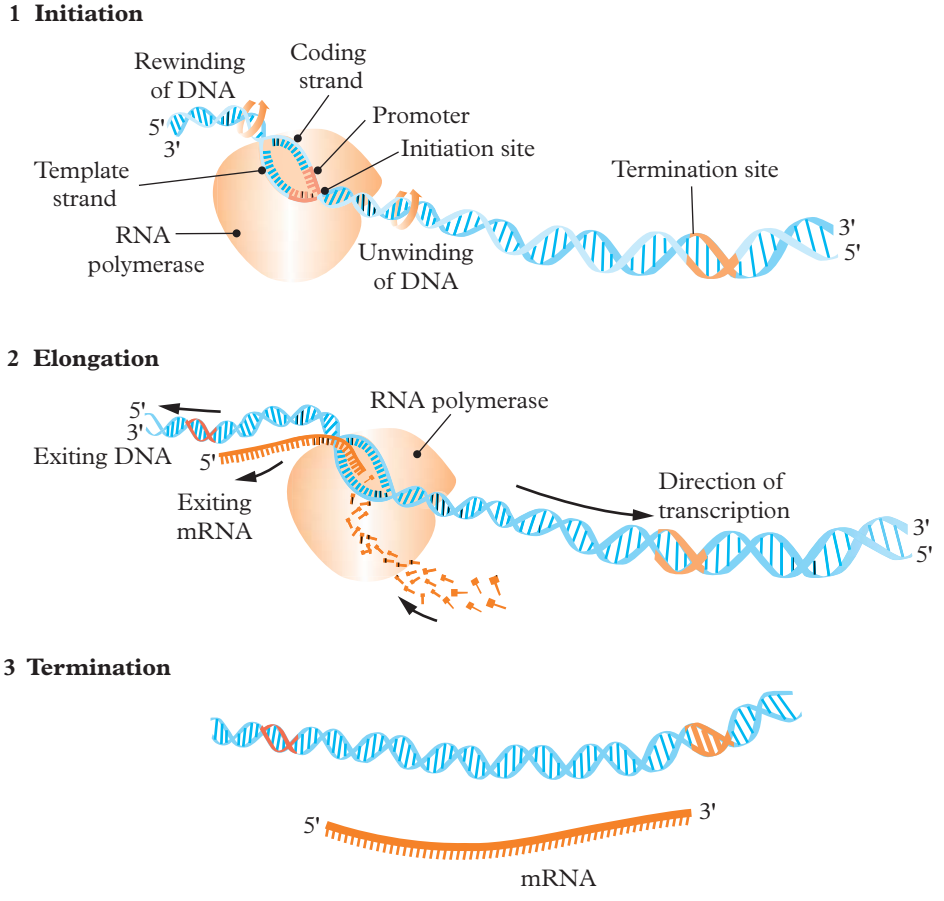


FIGURE 1 RNA polymerase attaches RNA nucleotides to the DNA to form mRNA.

Stage one: Initiation

enzyme
a biological catalyst that increases the rate of a chemical reaction

template strand
the strand used to attach complementary bases during transcription

RNA polymerase
an enzyme that transcribes DNA into mRNA during transcription

Enzymes bind to the DNA and unwind the double helix and break the hydrogen bonds between the nitrogen bases. DNA acts as the **template strand**. Each gene on the template strand has a specific recognition sequence of nucleotides (a promoter) that binds to an enzyme called **RNA polymerase**. The template strand is read from 3' to 5' and the mRNA strand formed is antiparallel 5' to 3'.

Stage two: Elongation

RNA polymerase moves along the exposed gene, joining together free RNA nucleotides (A, C, G and U). Each new nucleotide is added to the 3' end of the previous nucleotide in the 5' to 3' direction. Each mRNA nucleotide is complementary to a nitrogenous base on the DNA. Unlike complementary DNA strands, the mRNA contains uracil, instead of thymine. Either strand of the DNA may serve as a template for RNA as some genes 'run' in one direction while others 'run' in the opposite direction. RNA polymerase joins the RNA sugar–phosphate backbone together with strong covalent bonds in condensation polymerisation. Condensation reactions use ATP and produce a water molecule when two nucleotides join together. As it does this, the RNA polymerase moves along the DNA template strand, producing a growing mRNA strand.

Stage three: Termination

When the mRNA strand is complete, the RNA polymerase reaches a termination sequence and is able to detach. The two DNA strands then re-join by complementary base pairing. The triplets on the template DNA have now been transcribed into codons on the mRNA strand.

DNA base sequence (triplets): 3' GTG ACC TAT CGA 5'

mRNA base sequence (codons): 5' CAC UGG AUA GCU 3'

This initial strand of mRNA needs modification before it leaves the nucleus.

intron
a section of DNA that does not code for polypeptides, but may have other regulatory functions

exon
a region of DNA expressed during protein synthesis

5' cap
an altered nucleotide at the 5' end of mature mRNA

poly-A tail
a chain of adenines added to the end of the mature messenger RNA during post-transcriptional modification

Post-transcriptional modification

In eukaryotes, DNA and mRNA can contain non-coding regions, called **introns**, that are not required to produce a functioning protein. The sequence of mRNA that contains introns is called pre- or primary mRNA. These introns are removed (spliced) from the mRNA by enzymes in the nucleus either during transcription or immediately after the pre-mRNA is produced. The remaining coding regions, called **exons**, are re-joined to form mature mRNA.

Capping and tailing

An altered guanine (methyl-guanine) called a **5' cap** is added to the start of the mRNA and a long 'tail' of adenines called a **poly-A tail** is added to the other end. This process is called capping and tailing and is thought to:

- aid the binding of the mature mRNA to the ribosomes to initiate translation
- make the mature mRNA more stable
- inhibit any other free RNA nucleotides from joining.

Translation

translation
the process of converting the codon on mRNA into a polypeptide chain of amino acids

Translation is the process of converting the order of codons on mature mRNA to a sequence of amino acids. The mature mRNA exits the nucleus via a nuclear pore and becomes associated with ribosomes on the rough endoplasmic reticulum. These ribosomes are comprised of two subunits of rRNA and protein that attach to either side of the mature mRNA. The ribosome contains a region within it that 'reads' each codon, one at a time, as it moves along the mRNA in the 5' to 3' direction.

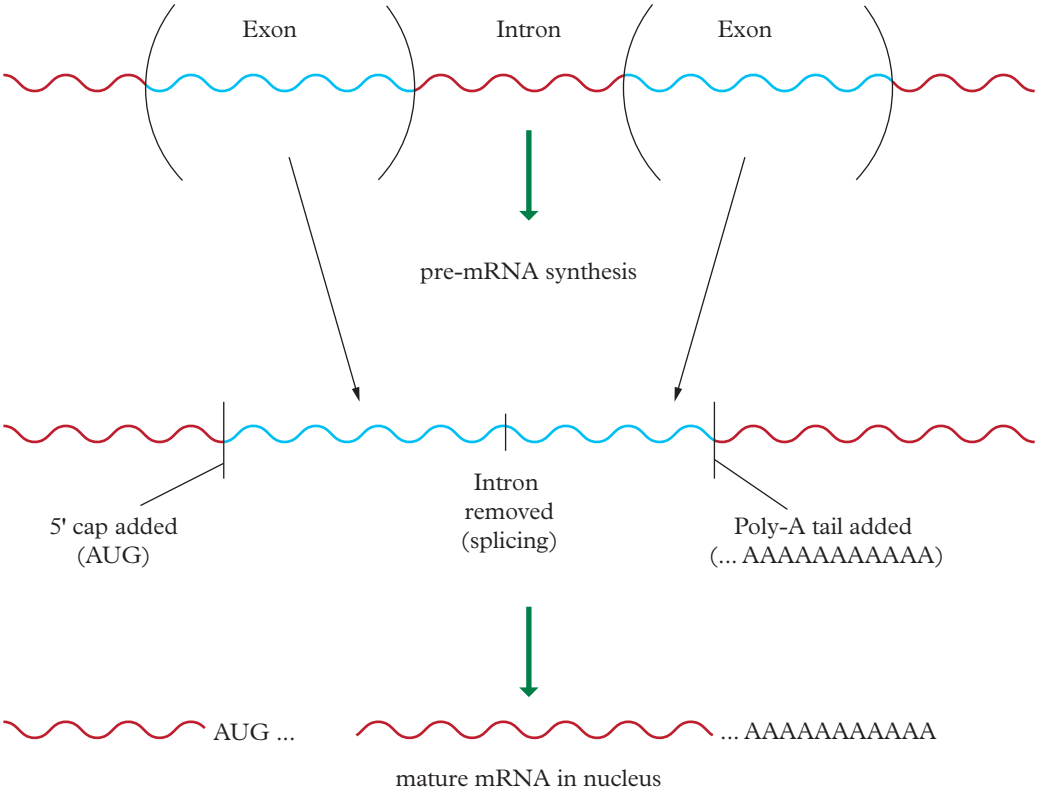


FIGURE 2 Post-transcriptional modification involves capping, tailing and intron splicing to form mature mRNA.

Reading the mRNA

Translation is initiated when the ribosome identifies the codon AUG. Free tRNA molecules are continually combined with their corresponding amino acids. Each tRNA can only carry one type of amino acid. If the tRNA's anticodon is complementary to the codon on the mRNA, they are bound to each other by complementary base pairing.

While the tRNA is still attached to the ribosome and mRNA, the ribosome moves along the mRNA to 'read' the next codon. This process is repeated so that two codons and their complementary tRNA molecules with their anticodons are always bound to each other within the ribosome.

Condensation polymerisation of amino acids

The ribosome holds the two amino acids attached to the tRNA molecules in place so that a **peptide bond** forms between them in a condensation polymerisation reaction using ATP and producing H_2O. The formation of the peptide bond releases the first tRNA, which can be reused, joining with another specific amino acid in the cytoplasm. As the ribosome moves along the mRNA, more and more amino acids are added to the growing polypeptide chain. This process is repeated until the ribosome reaches one of three stop codons – UAG, UAA or UGA – which stops the ribosome 'reading the message'. The ribosome separates from the mRNA and releases the polypeptide chain.

Polysomes

A cluster of ribosomes (called **polysomes**) moves along the mRNA, simultaneously synthesising a polypeptide chain. This enables a large number of polypeptides to be assembled on a single mRNA strand in a comparatively short time. After a period of time, the mRNA is broken down and production of that specific polypeptide ends.

Study tip

When defining tRNA you must say 'specific' amino acid, otherwise you won't be awarded the marks on the exam.

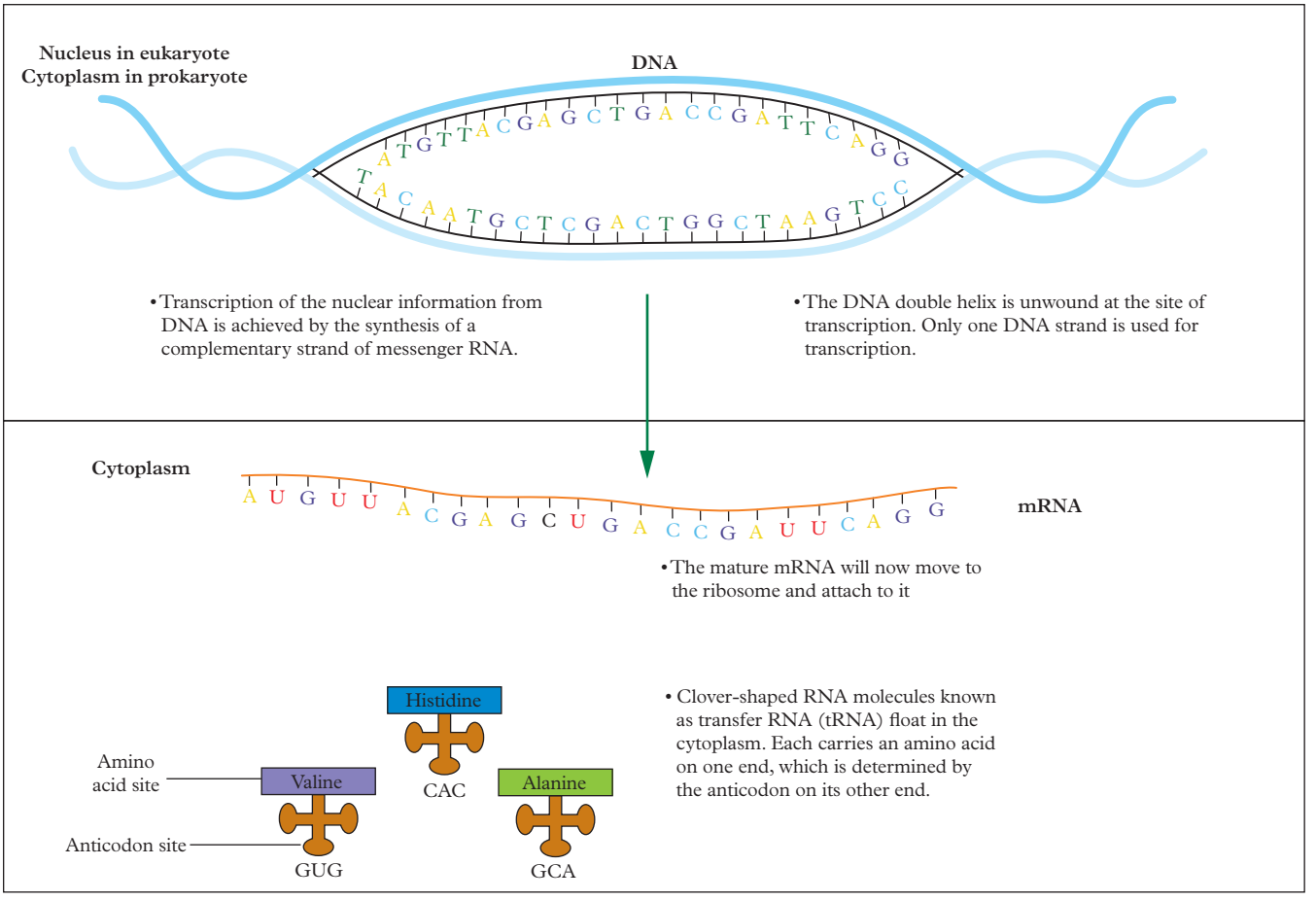
Video
Transcription and translation

Study tip

One way to remember the term 'condensation polymerisation' is to break down the word. Condensation refers to water (water as a by-product of the reaction) and polymer means a chain of a repeating subunit.

peptide bond
a strong covalent bond that joins amino acids together in a polypeptide chain

polysome
a cluster of ribosomes that translates a strand of mRNA



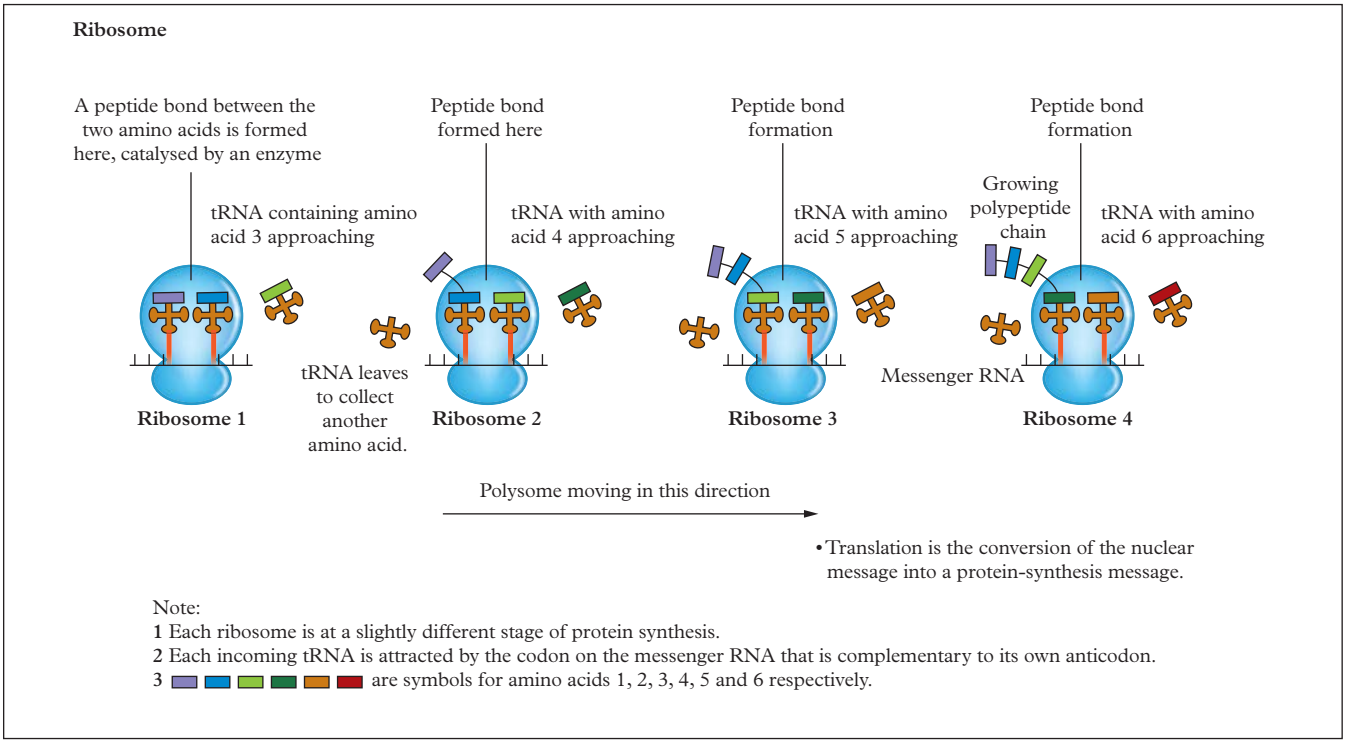


FIGURE 3 An overview of transcription and translation

CASE STUDY 2.2

The transcriptome

Eukaryotic cells have a range of mRNA molecules within them, called a **transcriptome**. While every cell within a multicellular organism contains the same genetic information, its transcriptome is dependent on cell type and function. Pancreatic cells that produce insulin contain transcripts for insulin, while nerve cells that produce neurotransmitters contain transcripts for neurotransmitters. Scientists can determine which genes are being expressed in particular cells and tissues by analysing the collection of mRNA sequences present in the cell (the transcriptome). This field of study is called transcriptomics and often uses RNA sequencing technology to determine the level of expression of cancer-causing genes by comparing the RNA products of healthy and cancerous cells.

transcriptome the entire range of mRNA molecules within a specific eukaryotic cell

FIGURE 4 Axon of a neuron with neurotransmitters being released

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G
	Amino acids				

Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp: Aspartic acid
Cys: Cysteine
Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine
Len: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
Thr: Threonine
Trp: Tryptophan
Tyr: Tyrosine
Val: Valine

FIGURE 5 Codon table, showing the codons for each of the 20 amino acids

Video
Worked example 2.2: Interpreting a codon table

WORKED EXAMPLE 2.2

INTERPRETING A CODON TABLE

Each nitrogenous base within the codon is read as a first, second and third letter to identify the amino acid from the chart.

Locate the amino acid for the codon CUU.

SOLUTION

1 Find the C in the first column to identify the correct row.

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

2 Within this row, locate the second nucleotide U. This gives you a specific box of four amino acids.

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

WORKED EXAMPLE 2.2 (continued)

3 The last nucleotide U gives you the correct amino acid for this codon. CUU codes for the amino acid leucine (Leu).

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

CHECK YOUR LEARNING 2.2

Describe and explain

1 Explain how a ribosome 'reads' the mRNA sequence.

2 Identify and describe one process that requires a condensation polymerisation reaction within a cell.

3 There are six different codons that code for the amino acid lysine. Describe the significance of this feature of DNA to protein synthesis.

Apply, analyse and compare

4 Apply your understanding of transcription to explain how RNA polymerase is able to detach from the mRNA at exactly the correct location to prevent further nucleotides from joining.

5 **a** Write the mRNA sequence for the following exposed nucleotide sequence of DNA:
3'TTA CCG GTG TAA ATA CTG GTT GGA5'

b Use the RNA codon table (Figure 5 on page 51) to work out the order of amino acids for the mRNA strand in Question 5a.

6 Analyse Figure 6.

a Determine which diagram shows the pre-mRNA and which shows the mature mRNA.

b Identify and label the following structures: introns, exons, poly-A tail, 5' cap.

FIGURE 6 Pre-mRNA and mature mRNA

Design and discuss

7 Construct a concept map to compare and contrast the processes of transcription and translation.

8 Mutations are permanent changes to the sequence of nucleotides in DNA. Discuss the possible implications for a pancreatic cell if a mutation produced a codon that ends translation in the middle of an mRNA strand that encodes for the production of insulin.

2.3 Eukaryotic gene expression

KEY IDEAS

In this topic, you will learn that:

- the structure of genes in eukaryotic cells includes exons, introns and promoter regions
- regulatory genes are responsible for controlling gene expression.

Although all cells in an organism contain the same genetic code, only the genes involved in basic metabolic functions required for life are constantly transcribing polypeptides. Other genes are 'switched off' and only expressed if and when they are required in a specialised cell.

For example, the actively expressed genes of muscle cells only transcribe the polypeptides that are essential for muscle cell operation. Genes that are important in nerve cells are not expressed in a muscle cell. If **gene expression** was continuous for all genes, the muscle cell would waste a lot of energy and resources to produce a range of unnecessary proteins. This control of gene expression is called **gene regulation** and it is important for cells to differentiate into specialised cells.

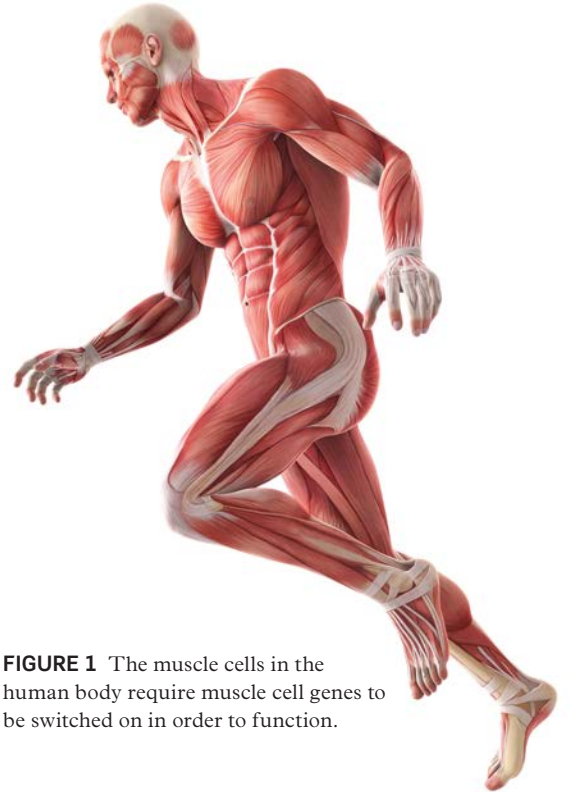


FIGURE 1 The muscle cells in the human body require muscle cell genes to be switched on in order to function.

gene expression
the transcription and translation of a gene into a polypeptide chain

gene regulation
the process of controlling gene expression and therefore the amount and type of proteins produced

Gene structure

The genes of eukaryotic organisms are comprised of alternating regions of introns and exons (as mentioned in Topic 2.2) flanked on either side by **promoters** and **terminator** regions.

promoter
a region of DNA that RNA polymerase binds to, initiating transcription

terminator
a region of DNA at the end of a gene to stop transcription

Exons

Exons are the coding regions of DNA that are expressed during protein synthesis. They combine to form the mature mRNA that is translated into a polypeptide chain.

Introns

These are non-coding sections of DNA that do not produce polypeptide chains. After transcription has occurred, they are removed from the primary mRNA by splicing enzymes in the nucleus. These introns provide a variety of important functions in gene expression, including regulating gene expression. Some introns are responsible for controlling structural genes. The mRNA they produce can change the expression and survival of other genes.

Study tip

You need to be able to compare and contrast the structure of a eukaryotic gene to that of a prokaryote gene – this is outlined in the next topic.

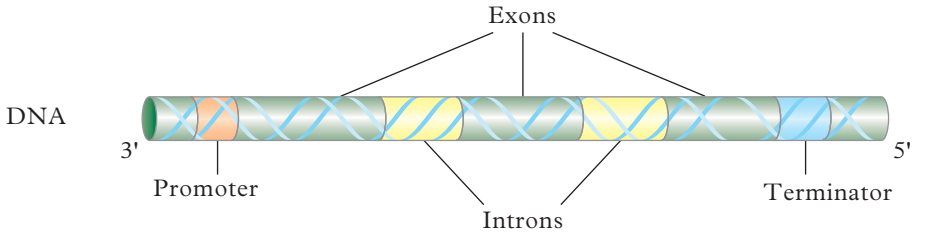


FIGURE 2 Gene structure

Gene regulation

There are two main types of genes present in cells. **Structural genes** are involved in protein production, and **regulatory genes** are responsible for controlling gene expression.

structural gene
a gene involved in the production of a protein

regulatory gene
a gene that produces factors involved in controlling the expression of genes

If protein synthesis was not regulated, RNA polymerase enzymes would continually bind to each promoter and transcribe genes. Gene regulation occurs so that each polypeptide is produced in the correct amount, if and when they are required. Genes can be regulated at different stages: pre-transcription, post-transcription and during translation.

Pre-transcriptional gene regulation

Pre-transcriptional gene regulation occurs during the initiation stage of transcription. RNA polymerase is only able to bind to the promoter with the help of **transcription factors**. These are RNA or protein molecules produced by regulatory genes. These can act as:

- activators, regulating the rate at which a gene is being expressed
- repressors, blocking the attachment of RNA polymerase, preventing gene expression.

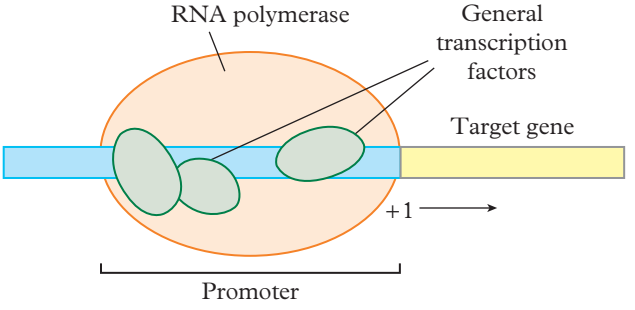


FIGURE 3 RNA polymerase binds to the promoter with the help of transcription factors.

transcription factor
protein or mRNA produced by regulatory genes that help regulate gene expression

Gene activation

Activators bind to the regulatory genes (or enhancer regions) of DNA. This induces bending in the DNA, allowing the regulatory genes to interact with the promoter (often located thousands of bases away). Other transcription factors bind to the promoter region and encourage the RNA polymerase to bind. Together, all the transcription factors initiate the interaction of the promoter region and regulatory genes. This allows RNA polymerase to bind to the DNA and activate transcription of the structural genes if, and when, they are needed.

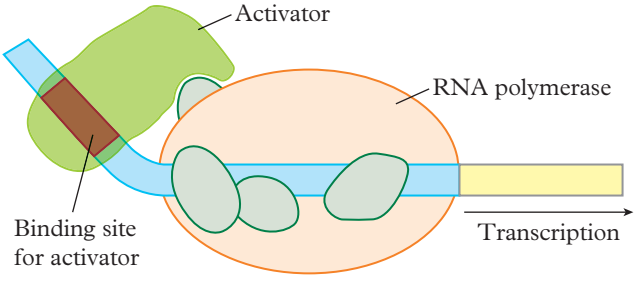


FIGURE 4 An activator binds to the regulatory gene.

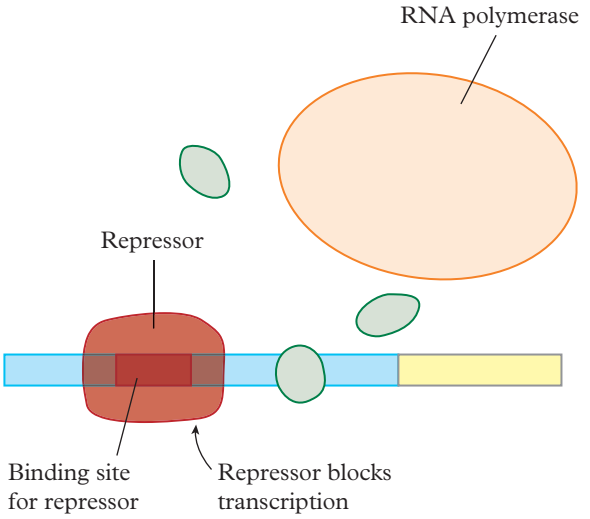


FIGURE 5 Repressor binds to the gene, blocking the RNA polymerase and transcription factors.

Gene repression

repressor
a protein that can bind to DNA and prevent transcription, inhibiting the expression of a gene

Other transcription factors, called **repressors**, respond to external stimuli by binding to specific regions of DNA to block transcription when gene expression is not required. They prevent transcription factors such as activator proteins from binding, so transcription cannot occur.

In some situations, gene expression is altered by the environment. The presence of a large amount of glucose, for example, can send a signal that results in an increased expression of the gene coding for insulin. In contrast, minimal amounts of glucose will send a signal that blocks insulin production.

Post-transcriptional gene regulation

Once the pre-mRNA has been produced, enzymes catalyse the splicing (removal) of introns. The mature mRNA consists of exons, which are connected to one another through the splicing process as the introns are removed. Alternative splicing results in the exons being joined back together in a particular order depending on the protein being produced. This means that a single pre-mRNA strand can produce a variety of functional proteins depending on the order in which the exons have been joined.

Translational gene regulation

Ultimately, mRNA will eventually be broken down by enzymes. Gene expression can be regulated by controlling the length of time the mRNA survives before it is degraded. Many different proteins and amino acids are involved in the translation process. By controlling the amount of these molecules, the rate of gene expression can be regulated.

Even after a polypeptide chain has been formed, chemical modification is required before the final tertiary or quaternary protein is produced. Again, the availability of additional chemical groups, or enzymes, will affect the complete expression of the gene.

The epigenome

If you studied VCE Biology Units 1 & 2, you may already be familiar with how epigenetic factors affect phenotypes. This topic discusses how the epigenome affects the structure of genes in eukaryotic cells.

The **epigenome** is a set of factors that affect which part of the DNA is activated. These factors may occur as a result of stimuli within or surrounding the cell, by neighbouring cells, or entirely by the environment to which the organism is exposed. Each cell type in an individual's body has its own epigenome. Muscle cells (that only express the genes they need) can only replicate to form muscle cells.

epigenome
the set of chemical modifications that affects which regions of DNA are expressed

Throughout an individual's life, the DNA in all cells remains constant (excluding chance mutations), but the **epigenetic factors** in each cell may change. This may be due to puberty, the stage of the menstrual cycle, or as a result of environmental stress or lifestyle. The epigenome acts by producing chemical tags (methyl or acetyl groups) that do not change the DNA 'blueprint', but determine which genes are expressed and which are repressed. This can occur at several levels.

epigenetic factor
any external factor that modifies DNA that may influence gene expression or repression

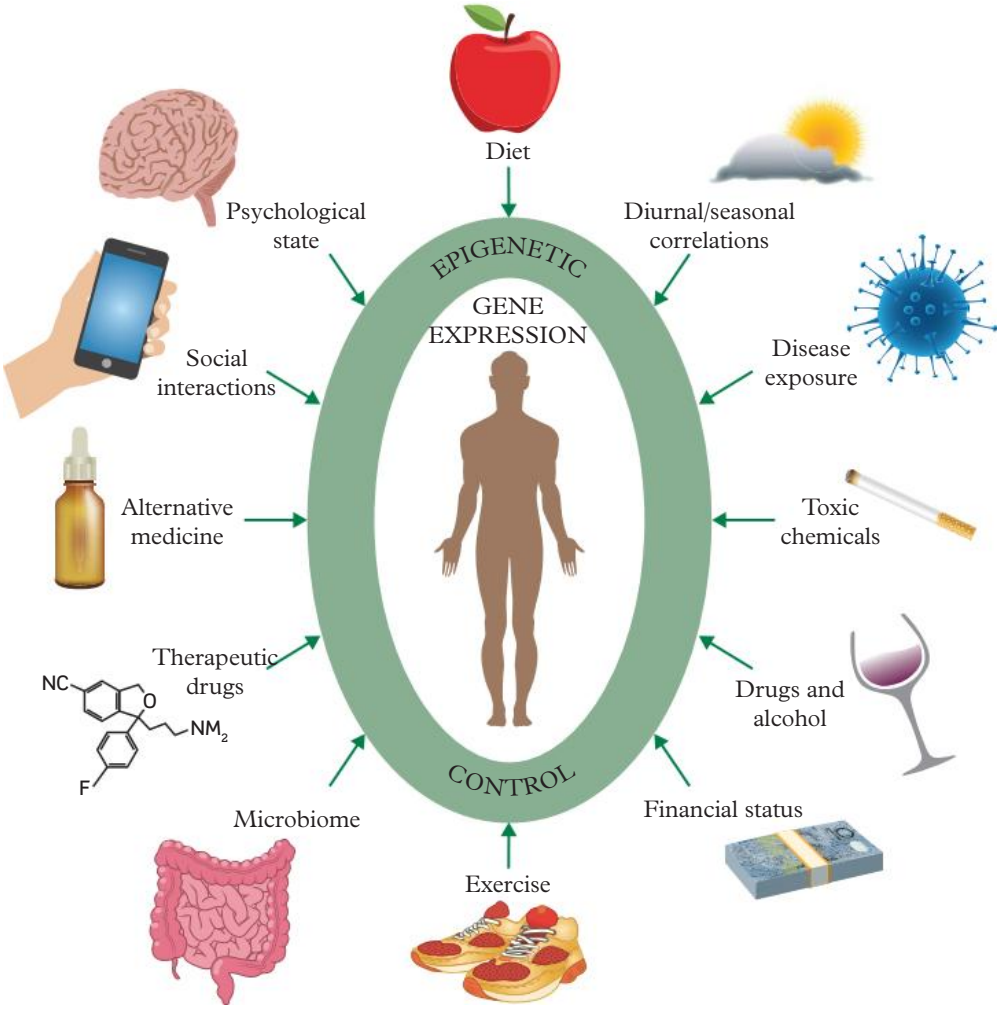


FIGURE 6 Environmental factors affecting epigenetic control in humans

Histone modification

In a non-dividing cell, the DNA molecules are wrapped around histone proteins called nucleosomes. These nucleosomes can be spaced tightly together or far apart. Chemical tags on the tails of the histone proteins control the spacing between the nucleosomes.

When an acetyl group tag attaches to specific amino acids on the tails of the histone molecules in a nucleosome, the histone structure relaxes, and the nucleosomes move further

chromatin
the condensed, coiled nucleosome complexes of eukaryotic DNA

histone modification
change to histone proteins that enables gene expression to be controlled through altering the coiling of DNA (and therefore availability of a gene for transcription)

apart. This section of the DNA is more easily accessed for transcription and the gene can be expressed. Methyl group tags on other histone tails coil the **chromatin** tighter and bring nucleosomes closer together. The DNA between the nucleosomes cannot be accessed and any genes contained in those sections are repressed.

Histone modifications can be reversed according to the chemical environment of the cell at any particular time. Studies have suggested that tags associated with histone modification are not inherited.

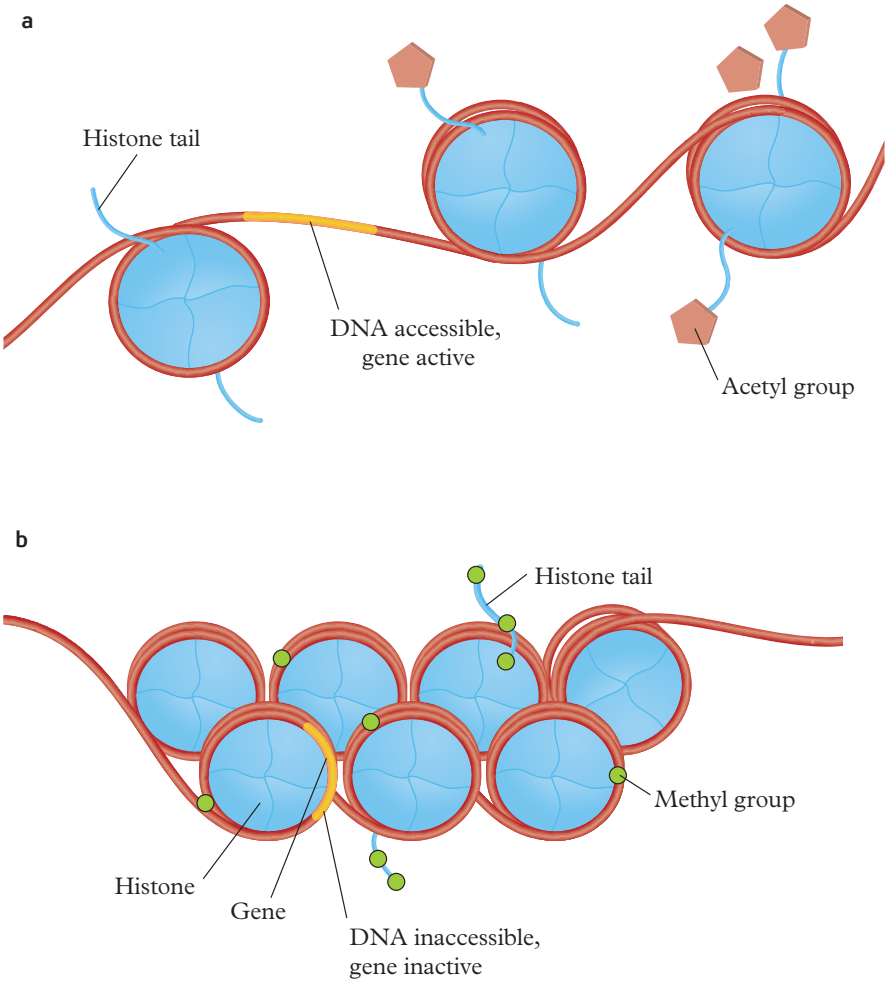


FIGURE 7 Histone modification: **a** when one chemical tag (an acetyl group) attaches to histone tails, the nucleosome coiling is loose and the gene can be expressed; **b** when another chemical group (a methyl group) attaches to the histone tails, the nucleosomes pack tightly together to bring about gene inactivation.

DNA tags

DNA methylation
chemical tags attached to histone proteins, coiling the nucleosomes either tightly or far apart, allowing gene expression to be controlled

A chemical tag (**DNA methylation**) can also be attached to the start of a functional gene. By blocking the attachment of RNA polymerase, the gene cannot be transcribed. Some tags are induced by the environment, but generally the pattern of DNA tags is set in early development (a stable epigenetic marker).

CASE STUDY 2.3

Inheritance of epigenetic factors

During World War II, Nazi troops occupied the Netherlands, and from 1944 to 1945 a German blockade was used to cut off food supplies to the country. It resulted in many pregnant women almost starving. It has been found that children conceived during this time had increased rates of coronary heart disease and obesity compared with children whose mothers were not exposed to famine during pregnancy. These problems were found to be associated with a reduction in DNA tagging of insulin-like growth factor II in both the mothers and the children they carried. This epigenetic control has been shown to be inherited over at least three generations.

Similarly, a study of children born after World War II to Holocaust survivors with post-traumatic stress syndrome (PTSS) found the children were more likely to develop PTSS or depression compared with children born to adults who had not experienced PTSS.

The children shared epigenetic tags with their parents that made them more reactive to stress. It is not exactly clear how these epigenetic tags are inherited across generations, but since the basic DNA tags are laid down during development, the environment or diet of the pregnant mother can potentially have subtle effects on the DNA tagging patterns of the epigenomes during pre-natal and early post-natal development.

It is also likely that environmental consequences to the mother's epigenomes can affect the germ cells of the developing foetus (e.g. from changes in their eating patterns). In females, the lifetime supply of eggs is created in the foetus before birth, as are the sperm stem cells of a male. The activity of a pregnant woman could, therefore, affect the lives of her grandchildren.

FIGURE 8 Studies suggest that some epigenetic factors may be passed down through generations.

CHECK YOUR LEARNING 2.3

Describe and explain

1 Describe the structure of a eukaryotic gene.
2 Describe the function of a eukaryotic promoter region.
3 Describe three environmental factors that can affect epigenetic control in humans.
4 Explain how chemical tags can block the transcription of a gene.
5 Describe how the following can impact transcription:
   a gene repressors
   b gene activators.

Apply, analyse and compare

6 Contrast the role of transcription factors, gene repressors and gene activators.
7 Compare gene expression and gene regulation.
8 Explain why gene expression needs to be regulated.

Design and discuss

9 Use Case Study 2.3 to discuss how different epigenetic factors affect the expression of genes in humans.
10 Discuss how cells are capable of controlling the amount of protein produced after transcription.

2.4 Prokaryotic gene regulation

KEY IDEAS

In this topic, you will learn that:

+ gene regulation in prokaryotes is carried out by the action of allosteric repressor proteins.

operon
a group of prokaryotic genes with a single promoter, operator and terminator region

The nutrients available within the environment of a single-celled prokaryotic organism are continually changing. Prokaryotes need to be able to alter their gene expression based on the type and quantity of nutrients available at any given point in time. They achieve this by grouping their structural genes together as components of **operons**. This means that the groups of structural genes that make up the operons can be switched on or off to regulate an entire biochemical pathway. They do this by either breaking down or synthesising substances when specific nutrients are available. Every gene involved in a biochemical pathway (e.g. the genes encoding for the enzymes involved in each step) can be transcribed and controlled together since they are either all needed at the same time, or not required at all.

FIGURE 1 Prokaryotes living alongside the tube worms in this deep sea hydrothermal environment experience constant changes in the availability of nutrients. They need to be able to alter their expression of certain genes to survive when there are no nutrients.

Some prokaryotic genes remain unregulated and are continually expressed because they are vital to cellular functioning. These include enzymes involved in DNA replication and metabolism as well as the proteins involved in gene regulation.

Because prokaryotic DNA is not located within a nucleus and does not contain introns, transcription and translation occur simultaneously. As the mRNA strand is being produced during transcription, ribosomes attach and begin to translate the mRNA into a polypeptide chain.

Operon structure

Operons are a section of DNA containing groups of genes that have a similar function and are regulated together by a single promoter. Operons are comprised of:

- a single promoter that RNA polymerase binds to, initiating transcription
- an operator that transcription factors such as **allosteric repressors** bind to, regulating transcription
- a group of functionally related genes that are transcribed together when the gene is being expressed
- a terminator region containing a triplet that ends transcription.

allosteric repressor
a type of transcription factor produced by regulatory genes that controls transcription by changing shape, enabling it to attach or release from the operator of an operon

In prokaryotes, regulatory genes produce a transcription factor (often RNA or protein) called an allosteric repressor. This regulatory gene is not part of the operon. Instead, the gene controls the expression of the operon's genes and therefore the proteins that are produced.

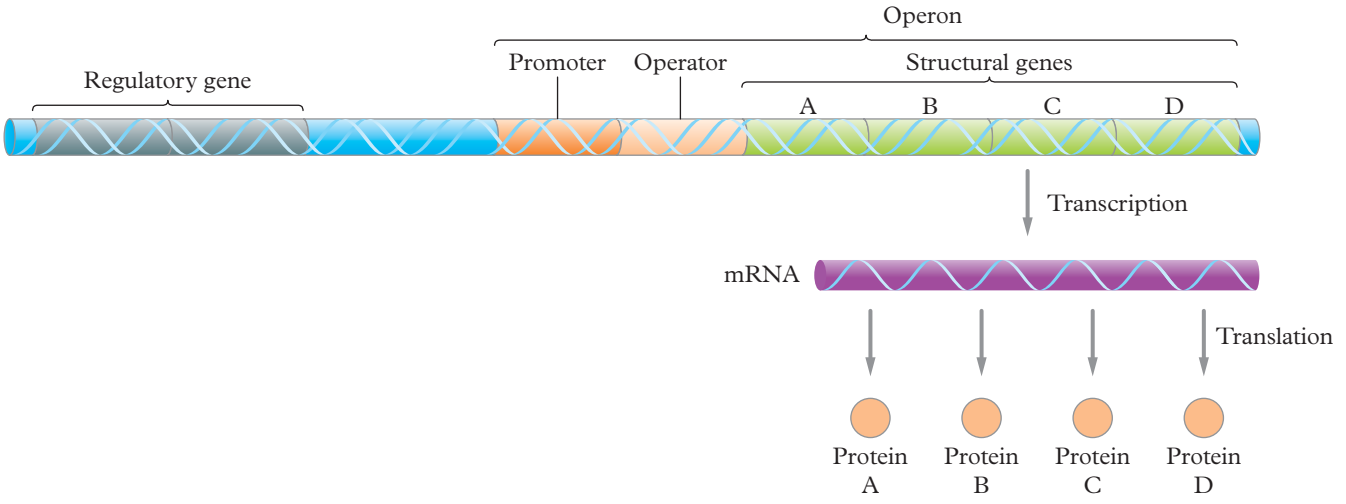


FIGURE 2 Operon structure of a prokaryote showing regulatory genes, promoter, operator structural genes and terminator regions

Repressible operons

Repressible operons are normally in a 'switched on' state. The structural genes of a repressible operon usually produce enzymes that are part of a **biosynthetic pathway**. They act on a substrate that is continually present by the cell to produce an end product that is always required by the cell. Once the end product begins to accumulate past a specific level, the operon is 'switched off' by the repressor blocking the operator region. Once the product that blocks the operator is used up by the cell, the operon is free to be expressed again.

repressible operon
an operon that is usually expressed and is only repressed when the biochemical product is in excess

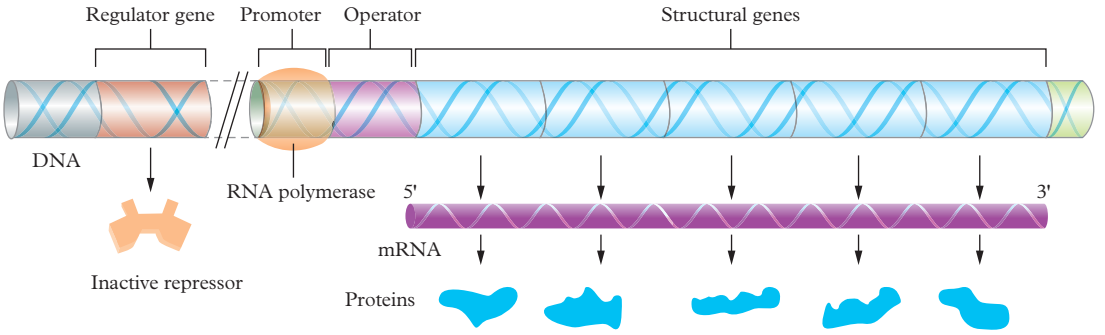
The *trp* operon

The ***trp* operon** is an example of a repressible operon found in *E. coli*. This operon is responsible for the production of the amino acid tryptophan. The biosynthesis of tryptophan is a five-step pathway controlled by five enzymes, produced by the trp operon. Tryptophan is generally found at very low levels and the operon is continuously expressed to produce the enzymes continuously.

Regulation of this operon is determined by the concentration of tryptophan within the cell. When tryptophan levels become too high, it becomes a co-repressor, attaching to the repressor molecule. This tryptophan–repressor complex binds to the operator, preventing RNA polymerase from any further transcription until the levels of tryptophan fall. This causes the repressor to be released and gene expression starts once again.

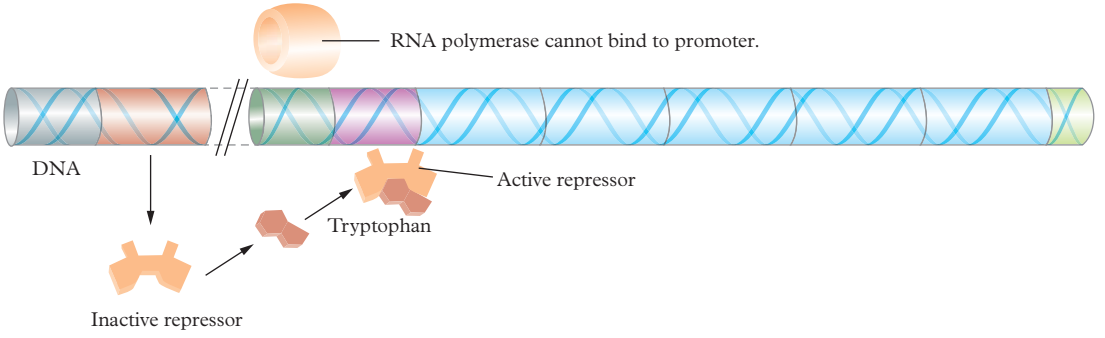
biosynthetic pathway
a series of ordered reactions controlled by enzymes that produce an organic substance (e.g. the amino acid trp is produced this way)

***trp* operon**
a repressible operon that continually produces the amino acid tryptophan until it accumulates within the cell and the operon is switched off



a Tryptophan absent – Enzymes needed to synthesise tryptophan are produced.

Video
Prokaryotic trp operon



b Tryptophan present – Presence of tryptophan prevents production of enzymes used to synthesise tryptophan.

Study tip

The *trp* operon is the only operon that you need to use as an example when explaining how repressible operon systems work in prokaryotes.

FIGURE 3 Structure of the *trp* operon showing: **a** gene expression when tryptophan is in low levels; **b** gene repression when tryptophan levels are in excess

CHECK YOUR LEARNING 2.4

Describe and explain

1 Describe the structure and location of DNA within prokaryotic cells.
2 Name the region of an operon that repressor molecules bind to in prokaryotes.
3 Explain the advantage of using repressible operons in biosynthetic pathways.

Apply, analyse and compare

4 Suggest why not all genes are part of an operon system in prokaryotes.
5 Compare and contrast gene regulation in prokaryotes and eukaryotes.

Design and discuss

6 Discuss how gene regulation is achieved in the *trp* operon of *E. coli*.
7 Suggest the importance of a biosynthetic pathway being controlled by a repressible operon in prokaryotes. Give an example in your response.
8 Not all operons are repressible. Some are inducible, meaning they are normally in a 'switched off' state and only switched on when they are requried. Infer why this may be an advantage to a prokaryotic cell such as bacteria.

2.5 Protein structure

KEY IDEAS

In this topic, you will learn that:

- some proteins are exported out of a cell via the protein secretory pathway, which involves the rough endoplasmic reticulum, Golgi apparatus and vesicles
- amino acids are the monomers of a polypeptide chain
- hierarchical levels of structure within a polypeptide give rise to a functional protein
- proteins are a diverse group of molecules that collectively comprise an organism's proteome
- enzymes are a group of proteins that act as catalysts in biochemical pathways.

The sequence of amino acids in a protein determines how it is folded. Even if two proteins only differ by a single amino acid, the positioning of this amino acid can have a very different effect on the coiling and folding of the polypeptide that determines its final shape. Because the overall three-dimensional shape is important to how that protein functions, it is vital that the order of amino acids is correct. The folding, spiralling and modification of a protein occurs within the cytoplasm, rough endoplasmic reticulum and Golgi apparatus. The final destination of each protein depends on its function. It is either used within the cell or exported out of the cell via the protein secretory pathway.

Video
The protein secretory pathway

The protein secretory pathway

Proteins that require secretion are transferred from the rough endoplasmic reticulum to the Golgi apparatus where they are further processed and folded. Here, they are enclosed by membranes to form secretory **vesicles**. These vesicles bud off from the end of the Golgi apparatus and move through the cytoplasm towards the plasma membrane. The vesicle membrane fuses with the plasma membrane to release the contents (in this case, proteins) out of the cell. This process is known as **exocytosis** and requires cellular energy (ATP) to occur. The role of the endoplasmic reticulum, Golgi appartus and associated vesicles is summarised in Table 1.

vesicle
a small fluid-filled structure bound by a membrane that transports fluids around a cell

exocytosis
active transport of substances out of a cell using a vesicle

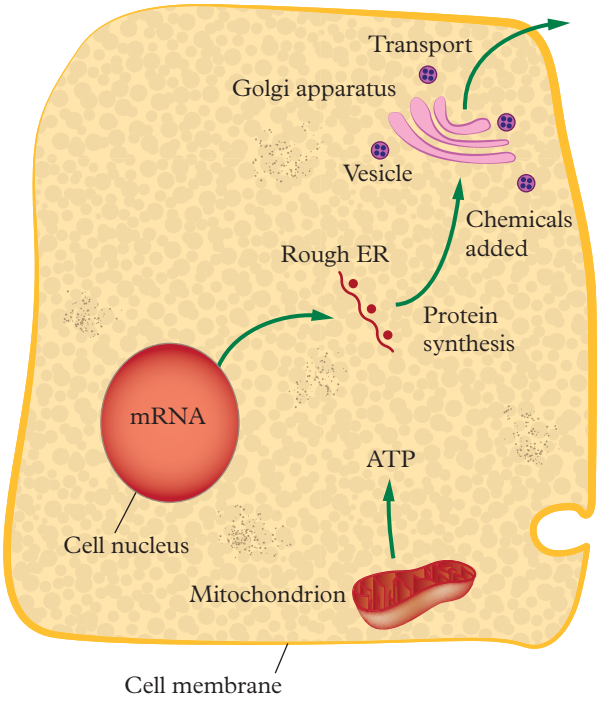
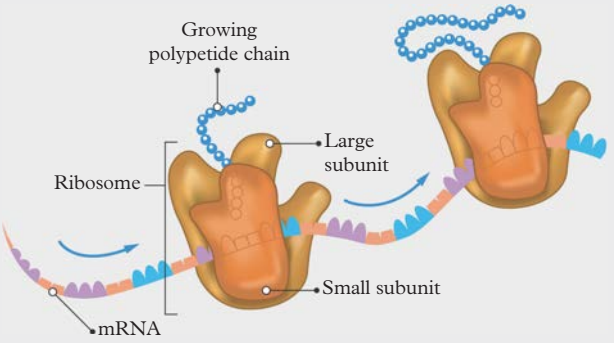
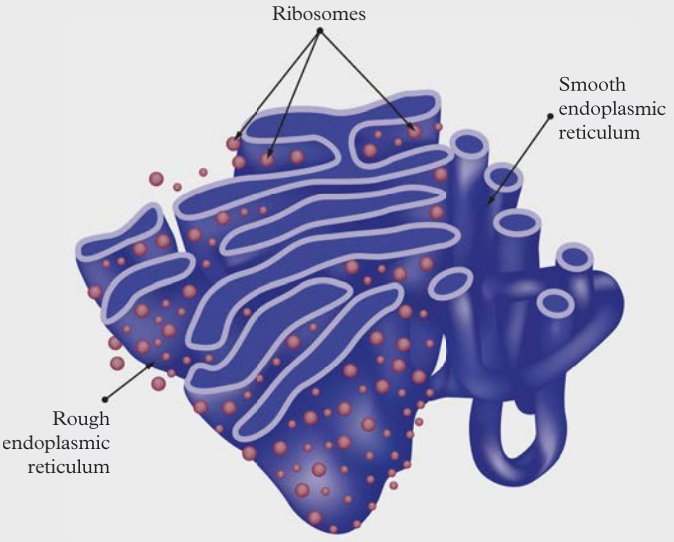
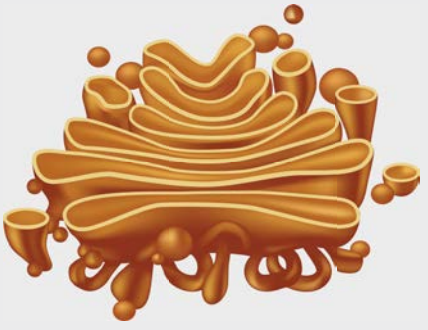
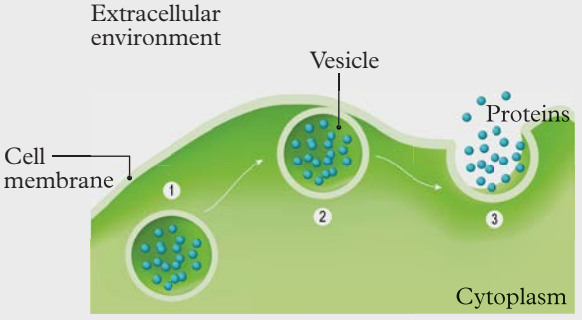


FIGURE 1 Secretory proteins exit the cell via exocytosis.

TABLE 1 Key organelles in the protein secretory pathway

Organelle	Role in the export of proteins
Ribosomes  	– Proteins are synthesised at ribosomes. – Newly translated proteins that have a signal peptide (i.e. a specific amino acid sequence) are moved to the endoplasmic reticulum. – Proteins that don't have a signal peptide stay in the cytosol.
Rough endoplasmic reticulum (Rough ER)  	– Proteins captured by the endoplasmic reticulum fold into their correct shape before being transported in a vesicle to the Golgi apparatus
Golgi apparatus 	– The protein is modified. Each region of the Golgi apparatus contains different protein modification enzymes that can add or remove sugars, or add sulfate or phosphate groups.
Vesicles  	– Final proteins are packaged in secretory vesicles. – Secretory vesicles export the proteins from the cell via exocytosis.

Amino acid structure

All proteins are made up of small organic monomer building blocks called amino acids. There are 20 amino acids that code for thousands of different proteins.

All amino acids are comprised of a central carbon atom that is joined to:

- an amino group (NH_2)
- a carboxyl group (COOH)
- a hydrogen atom
- an R-group.

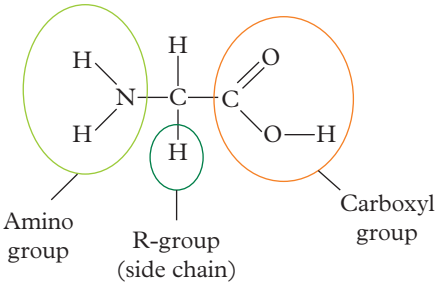


FIGURE 2 The basic structure of all amino acids

R-groups

The **R-groups** of each amino acid are variable side chains, making the 20 amino acids different from each other. R-groups have different chemical properties that are vital to protein structure as they bond with one another within the polypeptide chain. A single change to one amino acid within a polypeptide chain could completely alter the folding, coiling, three-dimensional structure and function of the final protein. Because of side chain interactions, the sequence and location of amino acids in a particular polypeptide guides how it bends and folds.

R-group
variable side chain on amino acids that gives the amino acids their specific chemical properties

Chemical properties of amino acids

As the polypeptide chain moves through the rough endoplasmic reticulum and Golgi apparatus, the amino acids within the chain come into close proximity to each other and bonds can form between the R-groups of different amino acids. This results in the formation of loops, coils, folds and eventually the three-dimensional shape of the functional protein. The chemical properties of the different R-groups include:

- polar
- charged
- non-polar
- disulfide bonds.

Study tip

You don't need to know the chemical properties of each specific amino acid, but it's important to know the general structure common to all amino acids and that variations occur due to the chemical structure of their R-group.

Condensation polymerisation of amino acids

Amino acids are joined together by condensation polymerisation reactions that create peptide bonds. The hydrogen from the amine group (NH_2) of one amino acid joins with the hydroxide from the carboxyl (COOH) group of the next amino acid to to release a water molecule. A strong peptide peptide bond is created in the process. This process continues, forming a polypeptide that has a strong backbone where amino acids have been joined.

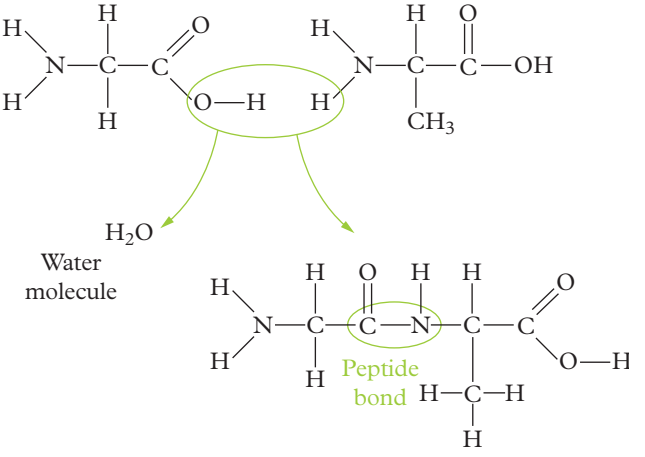


FIGURE 3 Condensation polymerisation reactions join amino acids with peptide bonds to form polypeptide chains.

Hierarchical organisation of proteins

The function of each protein is directly related to its three-dimensional shape, which in turn reflects the order of amino acids within its linear polypeptide structure. There are four levels of folding, coiling and modification that describe the formation of a functional protein. These are the primary, secondary, tertiary and quaternary structures (Figure 4).

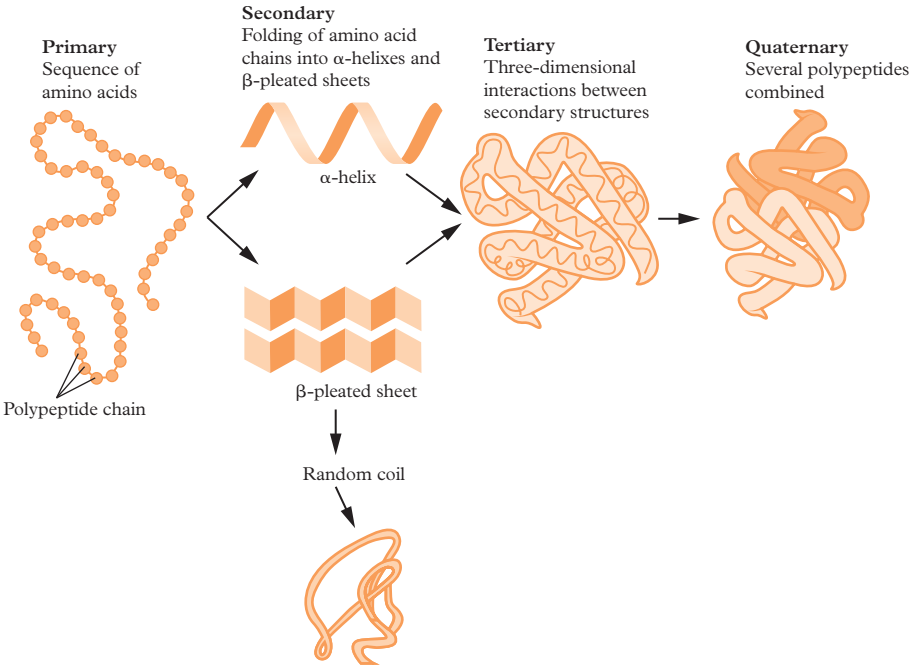


FIGURE 4 The hierarchical organisation involved in folding proteins

Video
Protein structure

Study tip

Use the full term 'beta pleated sheets'. 'Beta sheets' is not accepted on the exam.

Primary structure

The primary structure is the linear order of amino acids within the polypeptide chain. This sequence of amino acids is specific to each protein. Polypeptide chains can be as short as 50 amino acids or as long as 1000 amino acids. If a chain is shorter than 50 amino acids, it is not classified as a polypeptide chain, but rather a **peptide**.

peptide
a small polypeptide chain that is less than 50 amino acids in length

Secondary structure

The secondary structure of a protein is when the polypeptide chain is locally folded or coiled. This happens along the sugar–phosphate **backbone**, and as a result the secondary structure formation does not involve the R-groups. All secondary structure patterns are held in shape by hydrogen bonds that form between the oxygen of one amino acid's carboxyl group and the hydrogen of the amino group of another.

backbone
the carboxyl-amine backbone of a polypeptide, without the exposed R-groups

The three secondary structures are:

- alpha helices (α-helix)
- beta pleated sheets (β-pleated sheet)
- random coils.

Alpha helices

The **α-helix** is a stable, coiled strand with R-groups that face outwards from the coil. The R-groups are exposed, allowing them to interact with each other. Hydrogen bonds create a coiled, spiral structure.

α-helix
a type of secondary structure of a polypeptide chain with a coiled, spiral structure

Sheep's wool is an example of a fibrous protein that consists of many alpha helices, supercoiled together like a rope to give strength – these coiled helices also give wool its elastic nature.

Beta pleated sheets

The **β-pleated sheet** forms where sections of the polypeptide chain are arranged as strands that line up side by side. The hydrogen bonds occur between adjacent strands on either side of the sheet structure. This bonding also causes bending within the sheet, resulting in a zig-zag, pleated appearance.

β-pleated sheet
a type of secondary structure of a polypeptide chain with a sheet structure

Fibroin is an example of a fibrous protein excreted by spiders as long, thin fibres that are used in the construction of spider webs. Fibroin consists of stacked beta pleated sheets that give strength to these thin fibres.

Random coils

Other patterns formed by single polypeptide chains are random and do not conform to a regular pattern. Polypeptides that form in this way are called **random coils**.

Tertiary structure

The tertiary structure of a protein is the overall three-dimensional folded structure of a single polypeptide chain that occurs when the R-groups interact with each other. The tertiary structure includes all the secondary α-helices, β-pleated sheets and random coils as well as the different chemical bonds that can form between the exposed R-groups of the polypeptide chain.

The different interactions of the R-groups that cause specific folding of a tertiary level protein include:

- disulfide bonds between the sulfur atoms of two adjacent cysteine amino acids
- hydrogen bonds between polar R-groups
- hydrophobic interactions between non-polar amino acids that cluster together within the centre of a protein away from water
- ionic bonds that occur between two oppositely charged amino acids
- R-groups with the same charge that repel each other.

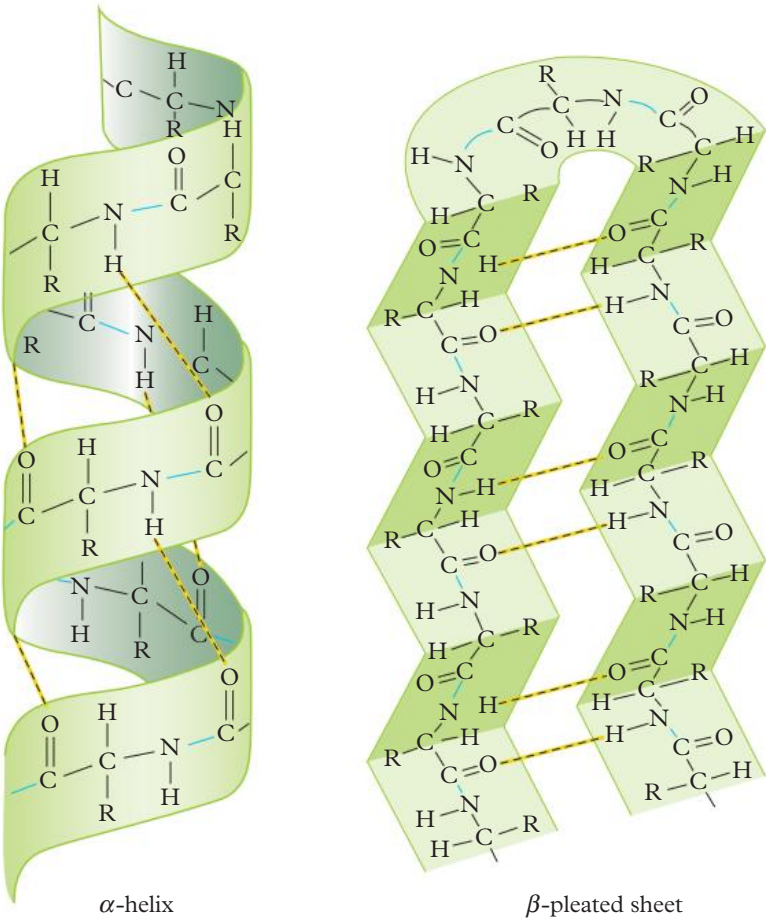


FIGURE 5 Secondary protein structures: α-helix and β-pleated sheet

random coil
a type of secondary structure of a polypeptide chain that does not conform to a regular pattern

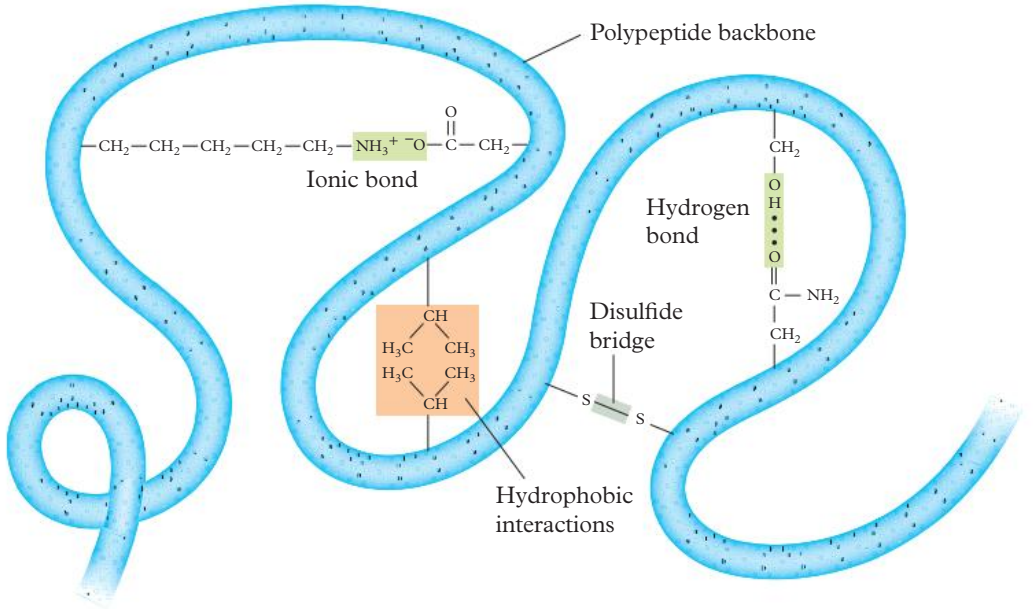


FIGURE 6 Tertiary folded structure of a polypeptide chain

chaperone protein
a protein that sometimes assists in tertiary protein folding

Tertiary folding can occur spontaneously; however, it often requires the assistance of specialised proteins called **chaperone proteins**. For some proteins, this tertiary level of folding will be their final structure.

Quaternary structure

subunit
a polypeptide within a quaternary protein

Some proteins are made up of more than one polypeptide chain that have each been folded into a tertiary structure. These are called quaternary proteins and are made up of tertiary polypeptide **subunits** that are held together by chemical bonds such as weak hydrogen bonds or strong disulfide bonds. Insulin and haemoglobin are examples of quaternary proteins. Insulin has two tertiary peptide subunits, while haemoglobin has four larger tertiary polypeptide subunits.

CASE STUDY 2.5

Hierarchical organisation of insulin

Insulin is a small quaternary protein made up of two peptides, A-chain and B-chain. Its small size relates to its function as a hormone, attaching to receptors on the surface of cells to deliver chemical messages. Insulin's small size makes it an excellent example for studying proteins.

A-chain structure

The primary structure of the peptide A-chain consists of 21 amino acids. The specific order of amino acids allows specific coiling and folding within the chain that form its secondary and tertiary structures. This includes two small alpha helices with a flat ribbon-like random coil that provides a bend in the peptide chain between the two alpha helices. This provides stability and a more compact shape, allowing tertiary bonds to form between the alpha helices. A disulfide bond forms between two of the four cysteine amino acids within the peptide A-chain.

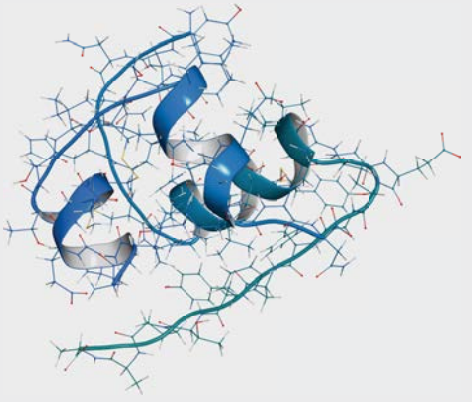


FIGURE 7 Insulin is a quaternary protein made up of two subunits joined together by chemical bonds.

B-chain structure

The peptide B-chain is 30 amino acids in length and consists of a single long alpha helix with a longer random coil at one end. The tertiary interaction of two glycine amino acids near the end of the alpha helix provides a V-shaped bend in the coil.

Insulin structure

The quaternary structure of insulin is created when chemical bonds occur between peptide A and B. These forces hold the two chains together, wrapping the B-chain around the A-chain. The interior of the insulin is non-polar, while the outside is generally polar.

The proteome

Proteins are organic macromolecules that carry out important roles within the cells of all living organisms involving growth, development and reproduction. The entire set of proteins

of an organism, encoded by the genetic code, is called the **proteome**. The proteome is much larger than the genome of an organism. This is because the mRNA produced by a single gene can be spliced differently, resulting in more than one protein.

proteome
the entire set of proteins of an organism

Protein functional diversity

Each protein has a different role in the function of an organism, such as cell communication, transport or triggering responses. Some of these functions occur within the cell in which they were produced, while others are exported out of a cell and work in new cells. For example:

- Enzymes – proteins that act as **catalysts** in biochemical reactions within a cell, such as polymerase enzyme, that speed up or slow down specific cellular reactions. The polymerase enzymes speeds up condensation polymerisation reactions of nucleic acids, forming strong covalent bonds between nucleotides to produce DNA and RNA molecules.
- Structural functions – proteins that form the structural components of an organism's cells, organs and tissues that provide structural support. Keratin is an example of a fibrous protein that forms a protective covering such as skin, hooves, fur and nails.
- Hormonal functions – proteins that are exported out of a cell via a protein secretory pathway (such as insulin) and enter the bloodstream. These proteins then bind to receptors located on the surface of target cells in order to trigger a cellular response.

catalyst
a substance that increases or decreases the rate of reaction without being used up

metabolism
the total set of biochemical reactions occurring within a cell

Enzymes catalyse biochemical reactions

Biochemical reactions within a cell are regulated by enzymes. Each enzyme controls only one (or one type of) reaction. The total set of biochemical reactions that occurs within the living cells of an organism is known as its **metabolism**.

You will learn more about enzymes and how they catalyse biochemical pathways in Chapter 4.

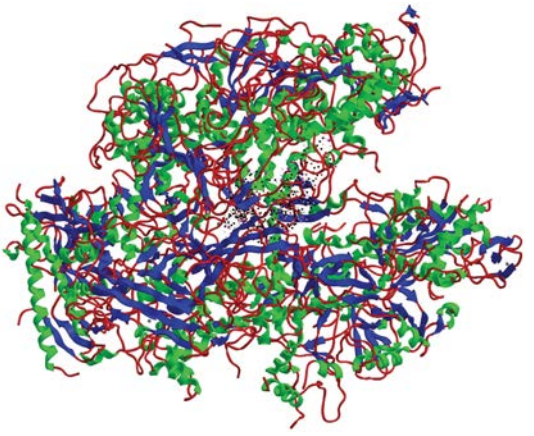


FIGURE 8 RNA polymerase enzyme

CHECK YOUR LEARNING 2.5

Describe and explain

1 Name the type of bond formed between amino acids during condensation polymerisation reactions of proteins.
2 Draw the general structure of an amino acid.
3 What structural component of the 20 amino acids makes them different from one another?
4 Explain the protein secretory pathway. How are the rough endoplasmic reticulum, Golgi apparatus and vesicles involved in this process?.
5 Define each of the following terms.
   a Primary structure
   b Secondary structure
   c Tertiary structure
   d Quaternary structure
   e Proteome

Apply, analyse and compare

6 Identify the level of protein organisation that is shown by the purple structure below and name this specific structure.
7 Compare and contrast the proteome and the genome of a multicellular organism.

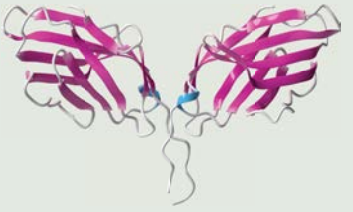


FIGURE 9 A protein structure

Design and discuss

8 Create an image or diagram that shows how condensation polymerisation reactions of amino acids form proteins.

CHAPTER 2

Review

Chapter summary

2.1

- DNA and RNA are types of nucleic acids found in the cells of all living organisms.
- The nucleotide subunits of nucleic acids are comprised of a pentose sugar, phosphate group and nitrogenous base.
- DNA is a double-stranded polynucleotide that is coiled around histone proteins to form chromosomes, while RNA is a single-stranded polynucleotide that has three forms: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA).

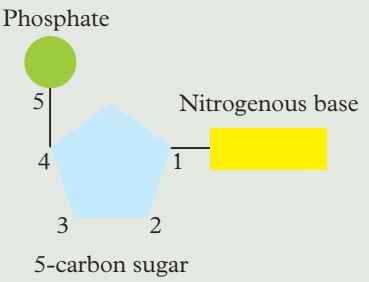


FIGURE 1 The basic structure of a DNA nucleotide

2.2

- The four nitrogenous bases on DNA can be arranged into 64 different codons on mRNA that code for 20 different amino acids.
- RNA polymerase copies template DNA into pre-mRNA during transcription. Specific triplet codes initiate the start and end of transcription for each gene.
- Once pre-mRNA is produced, it is modified by capping, tailing and splicing the introns from the exons to produce mature mRNA.
- Mature mRNA exists in the nucleus where the rRNA of ribosomes converts the code on mRNA into a chain of amino acids through translation. This is done by complementary base pairing of codons with anticodons.

2.3

- The genes of eukaryotic cells are comprised of alternating regions of introns and exons flanked in either side by promoters and terminator regions. These flanking regions assist in the regulation and expression of a gene.

2.4

- Most genes within prokaryotes are contained within operons that are regulated and expressed as a group. Some operons, such as the *trp* operon, are repressible, and the genes are only switched off when they are no longer required.

2.5

- Amino acids are made up of a central carbon atom joined to an amino group, carboxyl group, hydrogen atom and an R-group.
- Amino acids are monomer building blocks of polypeptides that are joined by peptide bonds during condensation polymerisation reactions.
- The hierarchical organisation of protein structure includes primary, secondary, tertiary and quaternary structures.
- The proteome is the entire set of proteins of an organism. Proteins include hormones, enzymes, transport proteins and structural components of cells.
- Enzymes catalyse biochemical reactions.
- Some proteins are secreted via the protein secretory pathway of a cell. This involves vesicles containing secretory proteins that bud off from the Golgi apparatus and transport them to the plasma membrane, where they fuse and release their proteins.

Revision questions

Multiple choice

1 The four nitrogenous bases of DNA are:
- **A** AGCT.
- **B** AGBT.
- **C** UAGC.
- **D** SUAT.

2 During prokaryote transcription of the *trp* operon, the inactive repressor molecule is produced by:
- **A** the regulatory gene.
- **B** the promoter.
- **C** the operon.
- **D** the *trp* structural genes.

3 The component of all amino acids that gives them their different chemical properties is the:
- **A** carboxyl group.
- **B** amino group.
- **C** R-group.
- **D** peptide.

4 Consider the hierarchical organisation of proteins. A dipeptide bond forming between two amino acids is an example of:
- **A** primary structure.
- **B** secondary structure.
- **C** tertiary structure.
- **D** quaternary structure.

5 A portion of template DNA has the sequence:
TAT CCG CAT TGC GAA.
The complementary DNA strand would be which of the following?
- **A** TTC GCA ATG CGG ATA
- **B** AUA GGC GUA ACG CUU
- **C** ATA GGC GTA ACG CTT
- **D** UUC GCA AUG CGG AUA

6 The nucleotide monomers of RNA consist of:
- **A** a deoxyribose sugar, a phosphate and a nucleotide base.
- **B** a ribose sugar, a phosphate and a nitrogenous base.
- **C** a ribose sugar, a peptide and a nucleotide base.
- **D** a deoxyribose sugar, a phosphate and a nitrogenous base.

7 The pathway from production of a protein to its final secretion out of a cell would be which of the following?
- **A** Nucleus → Golgi apparatus → rough endoplasmic reticulum → mitochondria
- **B** Golgi apparatus → rough endoplasmic reticulum → mitochondria → plasma membrane
- **C** Nucleus → rough endoplasmic reticulum → Golgi apparatus → mitochondria
- **D** Nucleus → rough endoplasmic reticulum → Golgi apparatus → plasma membrane

8 Polypeptide chains are formed during the process of:
- **A** DNA synthesis.
- **B** transcription.
- **C** translation.
- **D** splicing.

9 DNA methylation can:
- **A** increase the rate at which proteins are secreted out of a cell.
- **B** change the sequence of nucleotides within a gene.
- **C** alter the order of amino acids during translation, forming alternative proteins.
- **D** block the attachment of RNA polymerase, preventing transcription.

10 When nucleotides are joined to form RNA, the by-product produced is:
- **A** water.
- **B** oxygen.
- **C** carbon dioxide.
- **D** hydrogen.

11 The part of a molecule referred to as an anticodon is found in:
- **A** DNA.
- **B** ribosomal RNA.
- **C** messenger RNA.
- **D** transfer RNA.

Short answer

Describe and explain

12 Draw the basic structure of the DNA nucleic acid. Make sure to label the following:
- Phosphate
- Nitrogenous base
- 5-carbon sugar

13 Why is DNA considered antiparallel?

14 **a** Draw a labelled diagram showing the three subunits of an RNA nucleotide.
b Explain how RNA nucleotides join together to form an RNA polymer.

15 Describe one similarity and one difference between the structure of an alpha helix and a beta pleated sheet of a protein.

16 Describe three differences in the structure of DNA and RNA.

17 Use the codon table on page 51 to locate the amino acid for the following codons:
a ACA
b CUC
c UUU
d GCC

18 Describe the stages of transcription.

19 What is a polysome and what is its role in translation?

20 What are exons and introns?

21 Explain how a repressor acting on an operator can inhibit gene expression.

22 Compare the nucleotides found in RNA and DNA.

23 'The genetic code shows degeneracy.' Explain what is meant by this statement.

24 Describe the role of RNA polymerase in the transcription process.

25 Describe the role of ribosomes in translation.

26 Describe the structure of a eukaryotic and prokaryotic gene, using the terms exons, introns, promoter, terminator and operator regions.

27 Explain how a repressor acting on an operator can inhibit gene expression.

28 Explain how the expression of a single gene can produce different proteins.

29 What is an organism's proteome?

30 Describe the protein secretory pathway, including the role of the Golgi apparatus, the endoplasmic reticulum and vesicles.

Apply, analyse and compare

31 Analyse the short sequence of DNA below.
3' TAC GGA CTA TTA GTA CTA AAG CGC TAA 5'
5' ATA TAT ATG CCT GAT AAT CAT GAT TTC GCG ATT 3'
a Identify the template strand and explain how you determined which strand this was.
b Transcribe the DNA into mRNA.
c Translate the mRNA into amino acids using the codon table on page 51.

32 Outline how and why pre-mRNA is modified to form mature RNA before it leaves the nucleus.

33 Analyse the diagram below and outline how histone proteins can be modified to regulate gene expression.

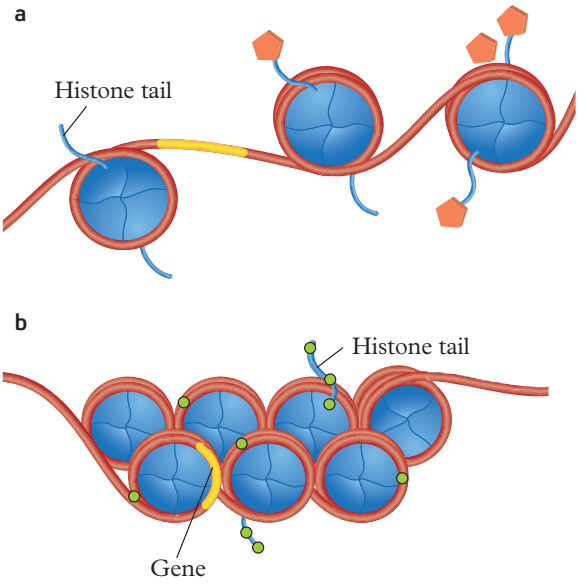


FIGURE 2 Histone protein modification

34 Compare and contrast the process of transcription in prokaryotes and eukaryotes.

35 Contrast the monomers in a DNA molecule and a protein molecule.

36 Salivary amylase is an enzyme in saliva that catalyses the breakdown of carbohydrates

into smaller molecules like sugars. It is encoded by the gene Amy1 and is produced in, and transported by, acinar cells.

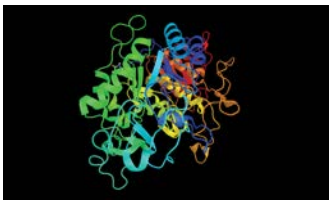


FIGURE 3 A 3D rendering of a salivary enzyme

a Outline the steps of translation in the synthesis of salivary amylase.

b Identify the monomer of the amylase enzyme molecule.

c Explain how the synthesised salivary enzymes are excreted from acinar cells.

d Examine the image of salivary amylase in Figure 3 and explain its structure.

Design and discuss

37 Discuss the roles of the different RNAs in the process of protein synthesis. Include in your answer how their structure relates to their functional roles.

38 The *trp* operon is an example of a repressible operon. Discuss what this means for prokaryotic gene expression.

39 Haemoglobin is a quaternary protein consisting of four tertiary folded subunits. Outline how this uniquely folded quaternary protein is produced to ensure it can carry out its highly specialised function, accounting for the different stages.

40 Explain the part of all amino acids that gives them their chemical properties. Give an example of one in your answer.

41 A study conducted in 2019 investigated the effect of alcohol breakdown on gene expression. When alcohol breaks down in the liver, it produces a by-product called acetate. The acetate circulates in the blood and travels to the brain where it adds chemicals called acetyl groups to histones in neurons. The study found that this can result in key memory genes being turned on, affecting behaviour and learning.

The study used mice to model the effect of alcohol breakdown in humans. The researchers divided mice into groups. One group of mice had acetyl groups added via an enzyme called ACSS2. Another group had the amount of ACSS2 in the brain reduced.

To begin, the groups of mice were exposed to 'neutral' stimuli and alcohol in separate compartments. After this training period, the researchers allowed the mice free access to either compartment. The researchers recorded the mice's preferences and how much time they chose to spend in each compartment.

The study found that mice with normal ACSS2 activity spent more time in the alcohol compartment following the training period. Mice with lowered ACSS2 showed no preference for the alcohol compartment.

a Suggest a hypothesis for the study.

b Identify the dependent and independent variables.

c Suggest one controlled variable for this experiment and describe how it could affect results if not controlled.

d Evaluate the validity of this study based on the information given.

e Suggest how the results of this study could be made more reliable based on the information given.

f Describe one ethical issue and one social implication that could arise from this study.

g Discuss the findings of the study in terms of epigenetic factors influencing gene expression.

Exam essentials

Responding to questions

In your exam, you may be expected to give thorough responses that are also concise.

Be detailed but direct

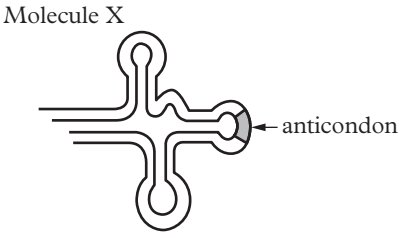
In your short-answer questions, an examiner is looking to see that you have directly answered the question with a suitable amount of detail. Make sure you provide all the necessary detail, but don't waste time writing everything you know.

The following question is taken from the 2016 VCE Biology Examination. Read the question carefully, then consider whether the responses are detailed and direct.

QUESTION 6a (2016 Biology Written Examination)

The hormone insulin is a relatively small protein. Researchers studying the production of insulin in the cells of the pancreas noted that one of the early steps in this process was the formation of a polypeptide called preproinsulin.

Researchers noted that the formation of this polypeptide required repeated use of different types of Molecule X, shown on the right.



a i What is the name of Molecule X? 1 mark

ii How does Molecule X play a role in the production of preproinsulin? 3 marks

Source: 2016 Biology Written Examination Question 6a, Short answer, reproduced by permission © VCAA

Response 1

i tRNA

ii tRNA carries a specific amino acid to a ribosome. The anticodon of the tRNA binds to the codon on the mRNA at the ribosome. When this occurs, the amino acid is released from the tRNA and a chain of amino acids forms that will go on to produce a polypeptide such as preproinsulin.

Suitable abbreviations, such as tRNA, are accepted on the exam. If you aren't sure of the correct abbreviation, write it out in full.

This is a key word when describing tRNA.

In the response, you can refer directly to tRNA instead of Molecule X since you have already named it in part i.

This response is detailed and directly relates to the question asked, describing the role of tRNA. The sentences are concise and to the point. Full marks would be awarded for this response.

Response 2

i Transfer RNA

ii Molecule X plays a large role in the production of preproinsulin. Molecule X, also known as transfer RNA, is one of three types of RNA that are involved in formation of polypeptides. tRNA has an amino acid that is added to a growing chain of amino acids at a ribosome during translation. This growing chain of amino acids is known as the primary structure of protein folding. The ribosome reads the mRNA codons and the amino acids added from the tRNA are specific to those codons. Once the ribosome reads the entire mRNA strand, the polypeptide is formed. This polypeptide continues folding and develops into a protein.

A paraphrase like this wastes time and space in the exam and would not be awarded any marks.

Information does not relate to the question.

Describes the role of the ribosome, not tRNA.

Not concise.

This response is long and does not directly relate to the question.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

Question 1b (2019 Biology Written Examination)

Ten amino acids that form part of a protein are shown below.

-phe-val-asn-gln-his-leu-cys-gly-ser-his-

The section of an RNA molecule found in the nucleus of the cell associated with the translation of these 10 amino acids was found to contain over 300 monomers.

Explain how there can be over 300 monomers in this section of the RNA molecule but only 10 amino acids translated. 2 marks

Some monomers do not code for amino acids, and these are known as introns. This explains how a long section of monomers translates only to 10 amino acids.

Source: 2019 Biology Written Examination Question 1b, Short answer, reproduced by permission © VCAA

Marking guide

Question 1 b 2 marks	- 1 mark for identifying that some monomers make up introns that do not code for amino acids. - 1 mark for identifying that one amino acid is produced from three monomers.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

CHAPTER 3

Manipulating DNA

The DNA in every organism is structurally and chemically identical. The only difference is the order of the individual nucleotides. The discovery of molecules that act as tools to select, cut, move and glue together sections of DNA has given us the opportunity to change the genetics of any organism. Sections of DNA from a plant or animal can be selected, modified and inserted into the genome of other organisms, including humans. We can use these skills to manipulate DNA to secure food sources, use bacteria to make drugs, and cure diseases.

KEY KNOWLEDGE

- → the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA
- → the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome
- → 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
- → the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin
- → the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease.

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

FIGURE 1 The green fluorescent protein that illuminates this jellyfish (*Aequorea victoria)* can be inserted into organisms of other species to make them glow.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

3A Draw a labelled diagram of the chemical structure of DNA. In your diagram, label the following key features: nucleotides, antiparallel, phosphate, deoxyribose sugar and double helix.

3A Groundwork resource
DNA Structure

3B Describe and/or draw the processes of transcription and translation.

3B Groundwork resource
Transcription and translation

3C Describe the difference between a genome and a gene.

3C Groundwork resource
The human genome

PRACTICALS

PRACTICAL	**3.1** Digital endonuclease digestion
HIGH-TECH PRACTICAL	**3.4** The pGLO plasmid digestion and gel electrophoresis

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

3.1 Manipulating DNA with enzymes

KEY IDEAS

In this topic, you will learn that:

- enzymes can be used to manipulate DNA
- DNA polymerase synthesises new DNA
- DNA ligase facilitates linkage of the sugar–phosphate backbone in DNA
- endonucleases recognise and cut DNA at specific recognition sites.

enzyme
a biological catalyst that increases the rate of a chemical reaction

When an engineer designs and builds a structure, they need a set of tools to cut, copy or attach the pieces of material together. Genetic engineering, like other forms of engineering, requires a set of molecular tools (or **enzymes**) to cut, copy or stick together the sections of DNA. Since DNA is structurally and chemically identical in all species, the enzymes that manipulate DNA in one species can also manipulate the DNA of other species. This means the enzymes that operate on bacterial DNA can also be used to manipulate the DNA of plant and animal species.

FIGURE 1 A genetic engineer, just like any other engineer, needs specific tools to cut, copy and stick different sections of DNA together.

Enzymes

Enzymes are usually tertiary structure proteins that make a chemical reaction more likely to occur – they increase the rate of the reaction. The substrates in a chemical reaction bind to the enzyme, making it easier for the molecules to collide. This results in a reaction proceeding faster than when an enzyme is absent. Each cell in an organism produces many enzymes, each of which are specific to a particular chemical reaction. Without these enzymes, the chemical reactions would occur too slowly for the cell to stay alive.

Some of the enzymes found in a cell repair damage that may have occurred as a result of the environment. Other enzymes in bacterial cells cut up stray pieces of DNA that might have been injected into the cell by a virus. It is these enzymes that have been isolated by scientists and are now used as tools in DNA manipulation.

DNA polymerase
an enzyme that facilitates covalent bonding of the sugar–phosphate backbone when replicating DNA

DNA polymerase

Polymerases are a group of enzymes that join the phosphate of one nucleotide to the ribose sugar of another nucleotide, forming a long DNA polymer. The two main types of polymerase are **DNA polymerase**, which is important in the process of DNA synthesis, and RNA polymerase, which is used to form messenger RNA during transcription (see Chapter 2).

Study tip

There are DNA and RNA polymerases and ligases. Specify which one you are talking about when answering a question.

Molecular biologists who manipulate DNA use DNA polymerase to produce new strands of DNA that can then be inserted into bacterial cells, or used to replicate enough DNA so it can be used for forensic testing.

DNA ligase

DNA ligase
an enzyme that facilitates bonding between two fragments of DNA

Many cells have ligases to repair either DNA or RNA. **DNA ligase** is responsible for repairing fragments of DNA that have been damaged by UV radiation, background radiation or chemicals found in the environment. Most commonly the damage may be a break in the bond between the phosphate and ribose sugar of two nucleotides (the DNA backbone). Other times, the nitrogenous base of a nucleotide may be damaged and needs to be replaced. This is the responsibility of DNA ligase.

Endonuclease

Endonucleases (or restriction enzymes) were originally discovered in prokaryotes (specifically bacteria). Endonucleases are the DNA scissors in the molecular scientist's toolbox. They are used to cut sections of DNA so that they can be inserted into a cell's chromosome. They are part of the bacteria's natural defence system against DNA or RNA injected by viruses. Endonuclease acts like molecular scissors that cut the DNA at specific recognition sequences. Each type of endonuclease will only recognise one small section of DNA. Each recognition sequence is a palindrome – a length of letters that reads the same forwards and backwards (see Table 1, page 80). For example, the endonuclease EcoRI (from the *Escherichia coli* RY13 strain) moves along DNA until it finds the recognition sequence 5'_G*AATTC3'_ (Figure 2).

endonuclease
an enzyme (isolated from prokaryotes) that is used to cut DNA at specific recognition sequence sites

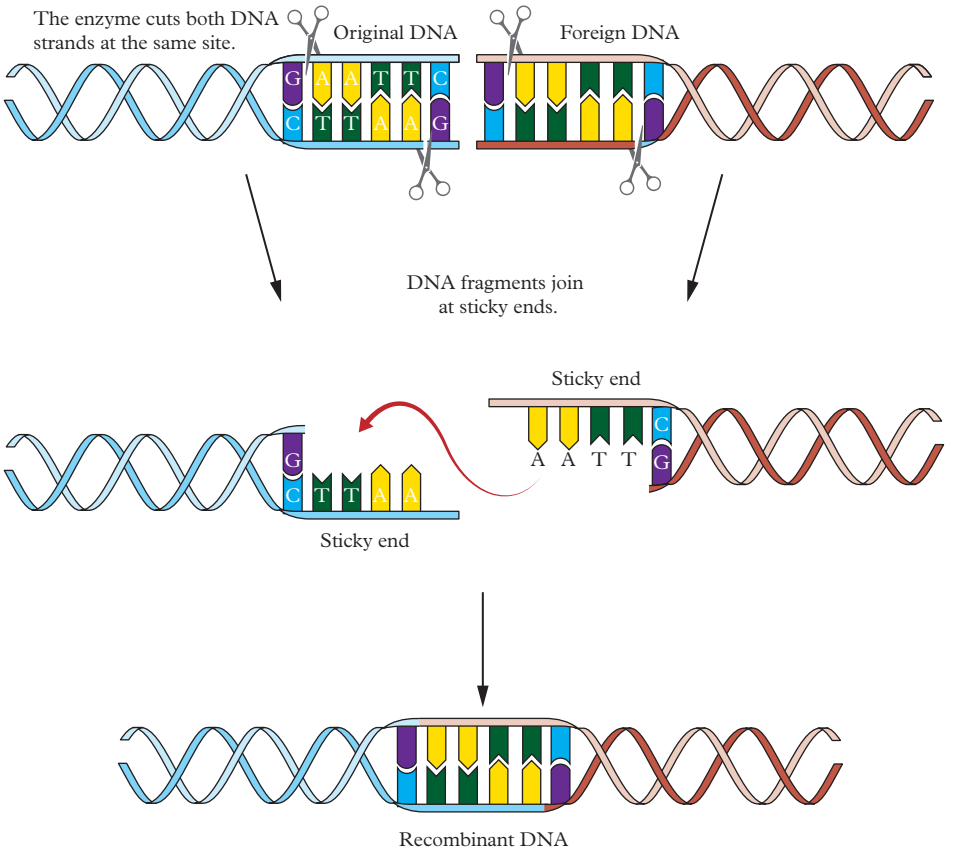


FIGURE 2 Restriction enzyme action of EcoRI used to produce sticky ends on two different DNA molecules

When it finds the specific recognition sequences, EcoRI will cut the DNA between the base guanine (G) and the base adenine (A) of both strands of DNA, leaving the nitrogenous bases of four nucleotides unattached to their complementary base. These 'free' bases have a high affinity to bond with their complementary base and will attach to any surrounding free nitrogenous bases. Due to this high affinity to bond with free nitrogenous bases, the end is often referred to as a **sticky end**.

sticky end
the overhanging nucleotides that occur when a strand of DNA is cut by some endonucleases; these ends are more likely to rejoin

blunt end
the double-stranded end of the nucleotide chain that is generated when some endonucleases cut strands of DNA

Some enzymes (like SmaI) cut the DNA in the centre of the sequence, thereby generating a **blunt end** with no free nitrogenous bases exposed.

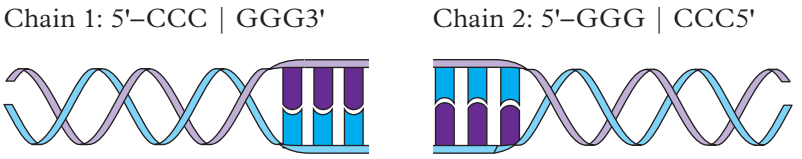


FIGURE 3 The SmaI enzyme cuts DNA to leave blunt ends.

TABLE 1 Some endonucleases and their sources

Enzyme	Source of restriction enzyme	Target sequence (cut at *) 5' → 3'
AvaI	*Anabaena variabilis*	G*C/TCGA/GG
BamHI	*Bacillus amyloliquefaciens*	G*GATCC
BglII	*Bacillus globigii*	A*GATCT
EcoRI	*Escherichia coli* RY13	G*AATTC
EcoRII	*Escherichia coli* R245	*CCA/TGG
HaeIII	*Haemophilus aegyptius*	GG*CC
HhaI	*Haemophilus haemolyticus*	GCG*C
HindIII	*Haemophilus influenzae* RD	A*AGCTT
HpaI	*Haemophilus parainfluenzae*	GTT*AAC
KpnI	*Klebsiella pneumoniae*	GGTAC*C
MboI	*Moraxella bovis*	*GATC
PstI	*Providencia stuartii*	CTGCA*G
SmaI	*Serratia marcescens*	CCC*GGG
SstI	*Streptomyces stanford*	GAGCT*C
SalI	*Streptomyces albus* G	G*TCGAC
TaqI	*Thermus aquaticus*	T*CGA
XmaI	*Xanthomonas malvacearum*	C*CGGG

Reverse transcriptase

reverse transcriptase
an enzyme used to produce complementary DNA from mature mRNA

Another enzyme that is often used by molecular scientists is **reverse transcriptase**. This enzyme is used to create DNA from eukaryotic mRNA so that it can then be inserted into bacterial DNA.

There is one important difference between the functional genes in prokaryotes (bacteria and archaea) and those of eukaryotes. Prokaryotes do not have introns, small sections of DNA that are not translated into the final protein. Because they do not have introns, prokaryotes will transcribe and translate the whole inserted eukaryotic gene. If the DNA of a eukaryote is inserted into the DNA of a prokaryote, the resulting protein will be much longer than normal. This is because both the introns and exons in the eukaryotic DNA will have been transcribed and translated. As a result, the protein produced by the bacteria will be non-functional.

Instead, molecular scientists use the mature messenger RNA from a eukaryote's cell that has already had the introns spliced out. Reverse transcriptase is then used to make a complementary DNA (cDNA) version of the mature mRNA that contains no introns. DNA polymerase is used to make the cDNA double-stranded so that it can be inserted back into the bacterial DNA and can then be translated and transcribed to create a functional protein by the bacterial cells.

CASE STUDY 3.1

Green fluorescent protein

Green fluorescent protein (GFP) is a 238 amino acid protein produced by the *Aequorea victoria* jellyfish. The jellyfish uses chemical reactions to produce a bioluminescence that the GFP absorbs and emits as a low-energy green light. This protein is one of a group of proteins that are used as markers by molecular biologists. If the GFP is attached to a virus, then the molecular biologist can observe the green glowing virus spreading throughout the host organism. If the marker is attached to a cancer cell, scientists can watch as the cell spreads through the organism. The pGLO gene that holds the GFP can be inserted into organisms and used to identify whether an organism has an underdeveloped zygote (cell containing sperm and an egg).

The GFP was first isolated from *Aequorea victoria* in the 1960s and 1970s by Osamu Shimomura. The GFP has a barrel structure of eleven beta pleated sheets (ß-pleated sheets). It has an alpha helix (α-helix) running through its centre and five smaller helices forming caps at the end of the structure.

Because the GFP gene was isolated from the eukaryotic *Aequorea victoria*, it could not be inserted directly into a bacterial cell. In 1992, the GFP gene was reverse transcribed (from mature mRNA to cDNA) and DNA polymerase was used to make the single-stranded cDNA into double-stranded DNA. Restriction enzymes were used to produce sticky ends so that the GFP gene could be inserted into a different DNA strand's complementary sticky ends.

In 2008, Osamu Shimomura won a Nobel Prize for his work, together with Martin Chalfie (who first used the GFP as a luminous genetic tag) and Roger Y Tsien (who produced a variety of similar proteins with different colours).

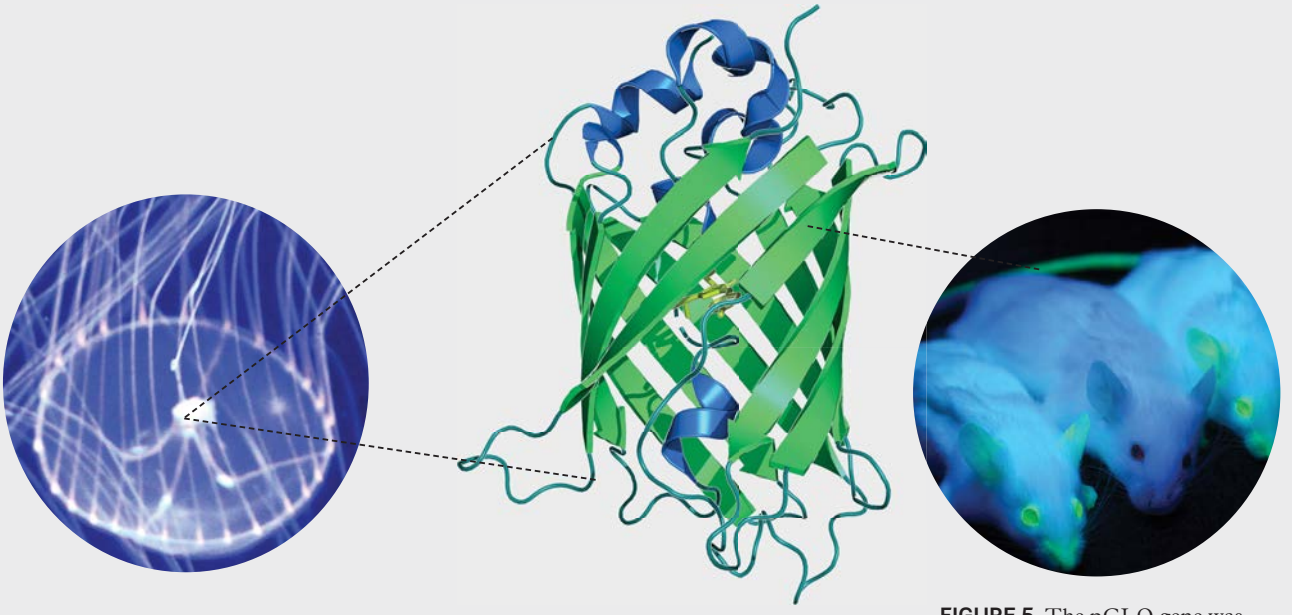


FIGURE 4 Green fluorescent protein is sourced from *Aequorea victoria*, a jellyfish. The barrel-shaped protein can be inserted into an organism and used as a marker.

FIGURE 5 The pGLO gene was added to these mice when they were undeveloped zygotes.

CHECK YOUR LEARNING 3.1

Describe and explain

1 What is an enzyme?

2 Why are restriction enzymes often referred to as scissors?

3 Explain the function of the following enzymes in the natural environment in which each is found.

- **a** DNA polymerase
- **b** DNA ligase
- **c** Endonuclease
- **d** Reverse transcriptase

4 If a section of circular DNA was cut twice by an endonuclease, how many fragments of DNA would be produced?

Apply, analyse and compare

5 Which of the following is a recognition sequence for an endonuclease? Justify your answer. **Hint:** Use Table 1 on page 80 to help you answer this question.

GATTC

GAATC

GAATTC

CATAC

6 What is the difference between the 'blunt end' and the 'sticky end' produced by an endonuclease?

7 Consider Case study 3.1 on page 81. Apply your knowledge of reverse transcription to explain why the green fluorescent protein could not be inserted directly into a bacterial host cell.

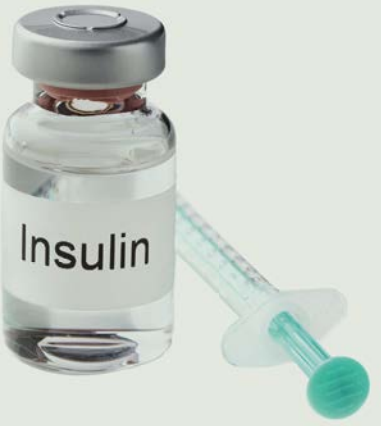


FIGURE 6 An insulin ampoule

Design and discuss

8 The endonucleases EcoRI and BamHI produce the sticky ends AATT and GATC, respectively. Would a gene cut with EcoRI be able to join with a gene cut with BamHI? Justify your answer.

9 A student claimed that the human gene for insulin can be grown in a bacterial cell.

- **a** State whether you agree or disagree with the claim.
- **b** Justify your decision with evidence from this chapter. You may need to do further research of your own to answer this question.

10 Research other applications where the green fluorescent protein has been used as a marker gene.

3.2 CRISPR-Cas9

KEY IDEAS

In this topic, you will learn that:

- CRISPR-Cas9 is a naturally occurring enzyme system used to protect prokaryotes from viruses
- molecular biologists use CRISPR to control where the Cas9 cuts DNA.

Viruses are non-living parasites that infect all organisms, including bacteria. Bacterial viruses bind to a molecule on the surface of the bacterial cell and inject nucleic acid into the cell (Figure 1).

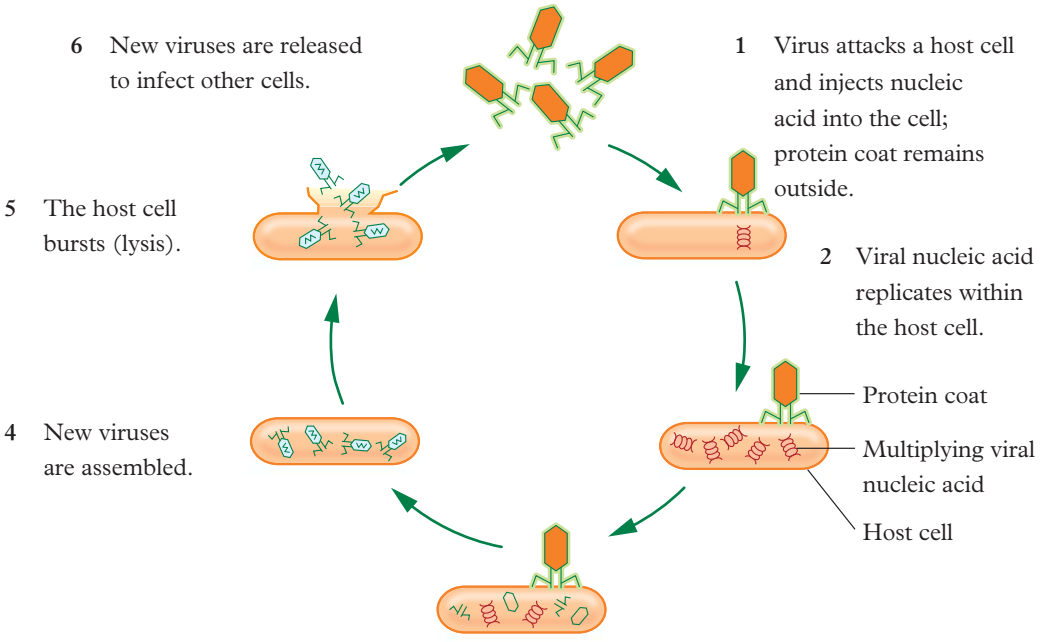


FIGURE 1 Replication phases of a bacteriophage – a virus that attacks bacteria

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
a short section of naturally occurring viral DNA that is inserted into a bacterial chromosome and used to prevent future infections

Cas9
a specific enzyme complex that is able to cut the nucleotides of viral (and other) DNA

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

Over millions of years, bacterial cells have evolved a way of defending themselves against viruses by cutting up viral DNA using endonucleases and then storing the small pieces in the bacterial genome. These short sections of viral DNA are often clustered together and arranged in repeated segments known as clustered regularly interspaced short palindromic repeats (**CRISPR**).

The next time the bacterial cell is exposed to a nucleic acid that has been injected from a virus, it makes an RNA copy of the stored viral DNA. This complementary RNA copy of the viral gene binds with **Cas9**, an enzyme that digests nucleotides.

The RNA segment attaches to its complementary section of the injected DNA and the Cas9 enzyme cuts the viral DNA so that it cannot produce new viral particles. This prevents the virus from spreading and allows the bacterial cell to protect itself from infection.

Video
CRISPR-Cas9

Using CRISPR to manipulate DNA

Molecular biologists can control the CRISPR-Cas9 complex to cut any sequence of DNA. Instead of relying on old sections of viral DNA to guide the Cas9 process, scientists can generate their own short sequence of RNA. The guide RNA is used to target specific genes in cells, allowing the Cas9 endonuclease enzyme to cut out the defective section of DNA and replace it with the correct DNA gene sequence. The main aim of CRISPR-Cas9 is to form the basis of a genome editing system that is able to permanently modify DNA by editing and repairing genes.

Figure 2 shows how CRISPR-Cas9 can be used to recognise and locate the damaged sections in an embryonic cell using specially prepared RNA segments to cut it out. The cell's repair mechanism then steps in to repair the DNA. Using this technique, it is possible to replace a defective gene.

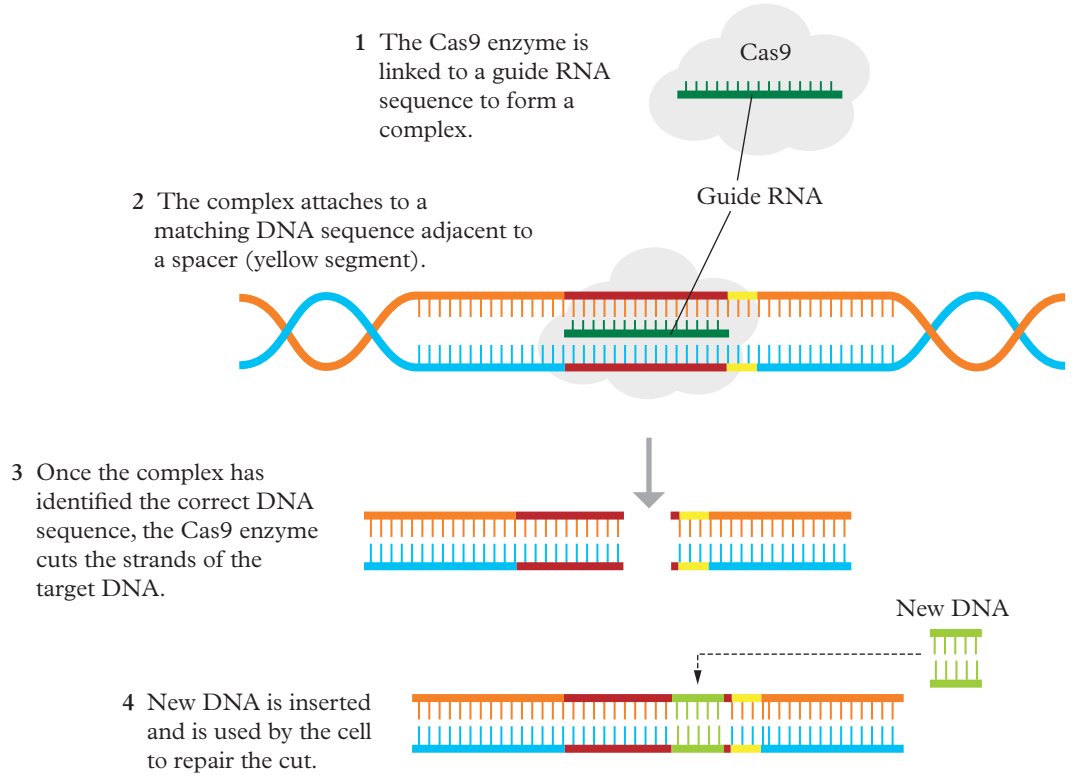


FIGURE 2 How CRISPR-Cas9 works

The CRISPR-Cas9 process has the potential to treat or even cure a variety of genetic diseases.

CASE STUDY 3.2

Using CRISPR to fight cancer

Cancer are normal body cells that have grown abnormally. From the cell surface it is often difficult to tell the difference between a normal body cell and a cancer cell, even for the body's own immune system (such as T-cells).

One of the most recent methods scientists are using to find and destroy cancer cells is to use CRISPR to introduce a gene into important immune cells. The CRISPR complex is able to select a specific place in the genetic material of the immune (T) cell, cutting the DNA and inserting the new gene. This gene is able to transcribe and translate a special protein receptor (chimeric antigen receptor) in the immune cell. When this new receptor reaches the membrane of the T-cell, the receptor will bind to a specific molecule on the cancer cell. The immune cell is now able to recognise the cancer cell and kill it.

Molecular scientists are able to grow large numbers of the chimeric antigen receptor T-cells (CAR T-cells) in the laboratory and infuse them into a cancer patient (Figure 3). You will lean more about immune cells in Chapter 9.

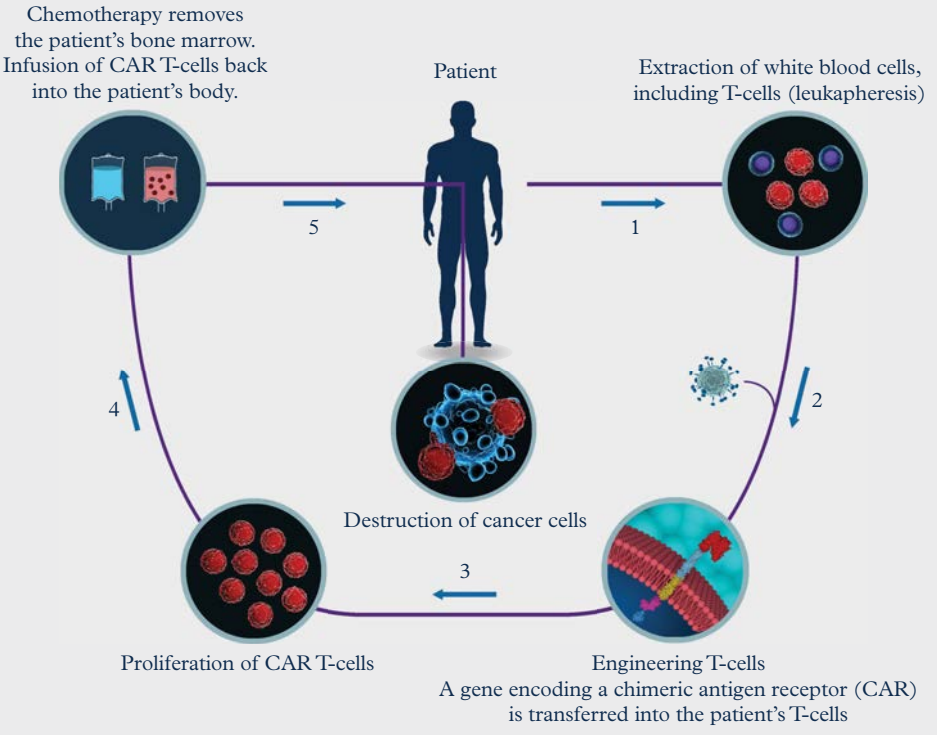


FIGURE 3 CAR T-cell therapy uses CRISPR to insert a gene into immune cells.

CHECK YOUR LEARNING 3.2

Describe and explain

1 What do the letters CRISPR represent?

2 In what type of cell was CRISPR-Cas9 first discovered?

Apply, analyse and compare

3 CRISPR-Cas9 is described as a complex molecule. What does this mean?

4 Compare the similarities and differences between an endonuclease and the CRISPR-Cas9 complex.

Design and discuss

5 CRISPR-Cas9 is able to cut DNA that is complementary to the short section of RNA in the complex. What would happen if the viral DNA were to change (mutate) before it infected the bacterial cell a second time?

6 It was recently determined that CRISPR-Cas9 did not always need to have a perfect match with the complementary viral DNA. What advantage does this give the bacterial cell in preventing viral infections?

3.3 Polymerase chain reaction

KEY IDEAS

In this topic, you will learn that:

- DNA can be amplified using a polymerase chain reaction
- gel electrophoresis can be used to separate DNA fragments according to size
- gel electrophoresis can be used to generate a DNA profile.

Even though the chemical structure of DNA is identical in every organism, there are differences in the sequence or order of the nitrogenous bases adenine, thymine, guanine and cytosine. Before scientists can compare the DNA of different organisms, they must be able to produce multiple copies – or amplify the DNA. This is done through a process called **polymerase chain reaction (PCR)**. This reaction uses a naturally occurring DNA polymerase found in a species of bacteria (*Thermus aquaticus*) that lives in high-temperature geothermal hot springs. The enzyme from this bacteria is called *Taq* DNA polymerase and it is used because of its ability to tolerate the high temperatures used in a polymerase chain reaction.

polymerase chain reaction (PCR) a process that amplifies the amount of a sample of DNA

FIGURE 1 The Champagne Pool in New Zealand is a geothermal hot spring. These kinds of environments are home to thermophiles (organisms that can live in temperatures >40°C) such as *Thermus aquaticus*.

Steps in the polymerase chain reaction

There are four steps in a polymerase chain reaction.

1. **Denaturation:** The two strands of DNA are heated to 90–95°C. This causes the hydrogen bonds that hold the two DNA strands together to separate into single strands.
2. **Annealing:** The mixture is cooled to 52–55°C. This allows short single strands of nucleotides called primers to bind to the DNA. The primers are chosen carefully so that they are complementary to the starting point of DNA replication.

3 **Elongation:** The mixture is heated to 72–75°C so that the *Taq* polymerase is able to generate a complementary strand of DNA from the primer site. This results in a double helix that contains an original copy of the DNA and a new complementary strand.

4 **Repeat:** The whole process is repeated so that the chain reaction allows many billions of copies of DNA to be formed.

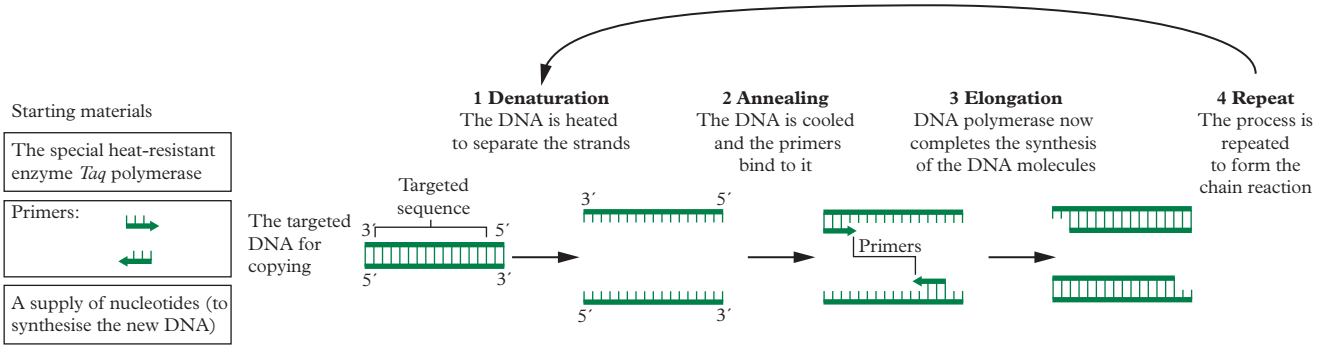


FIGURE 2 The polymerase chain reaction (PCR)

Each automated cycle of rapid heating and cooling can take between 1 and 3 minutes, depending on the length of the DNA being copied. Each time the cycle is repeated, the amount of DNA will double.

PCR applications

Although the cells of many organisms have several metres of DNA, it can be difficult to locate enough DNA to be used in testing procedures. Sometimes only a minute amount of DNA from a single cell or virus can be detected. For this reason, the amplification of the DNA through PCR is essential to allow for the detection of low concentrations of DNA.

Gel electrophoresis

The short segments of DNA that have been duplicated by PCR can be used to identify differences between individual organisms through a process called **gel electrophoresis**. Although the DNA is structurally and chemically identical, there can be small differences in the non-coding regions (introns) of a gene. These differences are usually inherited from an individual's parents and can be used to identify relatedness between individuals or families. Half of your genetic material comes from each parent: that is, half your DNA will match half of your mother's DNA, and the remaining half of your DNA will match half of your father's DNA.

gel electrophoresis a process that separates negatively charged fragments of DNA according to size

When the DNA of two unrelated individuals is cut with the same endonuclease (also called a restriction enzyme), the DNA of the two individuals will have different length fragments. The differences in lengths are called **restriction fragment length polymorphisms** (*poly* = many, *morph* = gradual change). Half of your restriction fragment length polymorphisms (RFLP) will match half of your mother's RFLP, and the other half will match half the RFLP from your father.

restriction fragment length polymorphisms (RFLP) the difference in the length of DNA fragments that results from cutting DNA with endonucleases

The different length fragments can then be loaded into a thin sheet of gelatinous material (called a gel). Each gel is made up of many tangled fibres of agarose. When an electric current is run through the gel, the negatively charged DNA will start moving towards the positive end. Small pieces of DNA can travel more easily through the gel and will move further down

Video
Gel electrophoresis

the gel than the larger DNA strands. With staining, the DNA of two individuals can be compared. If the gel has been warmed before being turned on, or if a higher current is used, the DNA will move faster through the gel. Often a DNA ladder (a series of DNA fragments, the lengths of which are known) is used to determine the length of the unknown strands.

FIGURE 3 A gel electrophoresis box separates strands of DNA based on size. The negatively charged DNA travels towards the positive end of the gel.

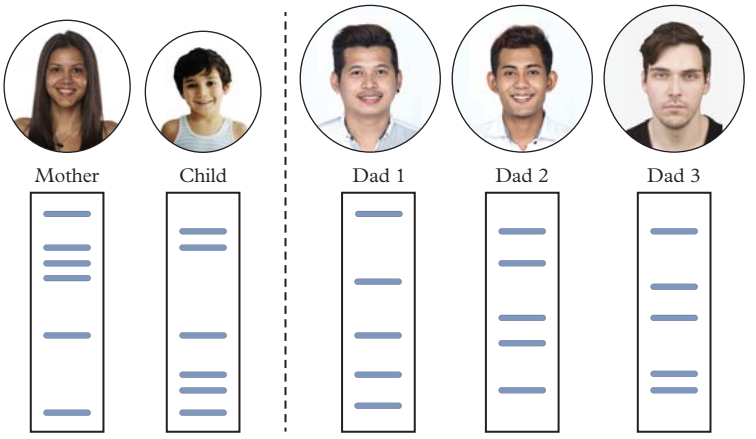


FIGURE 4 Visual representation of fragments of DNA following gel electrophoresis. Each individual inherits half of their chromosomes from their mother and half from their father. In this example, the third dad has provided half the genetic material for the child.

DNA probes

A probe is a small section of DNA that is complementary to a DNA sequence and which has a dye attached at one end (either fluorescent or radioactive). When a probe is combined with the single-stranded DNA (i.e. it is hybridised), it can identify the important section of DNA or gene.

DNA profiling

DNA profiling (fingerprinting) when common nucleotide sequences are used to identify key characteristics such as hair or eye colour

PCR and gel electrophoresis are important components of forensic DNA typing, commonly called **DNA profiling (fingerprinting)**. It involves taking a DNA sample from a crime scene and using PCR to increase the amount of DNA in the sample so that it can be used for gel electrophoresis. The DNA that is produced is cut with restriction enzymes to produce fragments. Every person has a unique arrangement of fragments due to their different RFLP. The crime scene sample of DNA is then compared to that of a suspect. If all the RFLP match, then it can be used as evidence that the suspect has visited the crime location. It does not identify the day or time of the visit. Some DNA probes can be used to identify sections of DNA that contain the code for brown or blue eyes, or blonde or red hair. This allows forensic scientists to build a possible physical profile of a person.

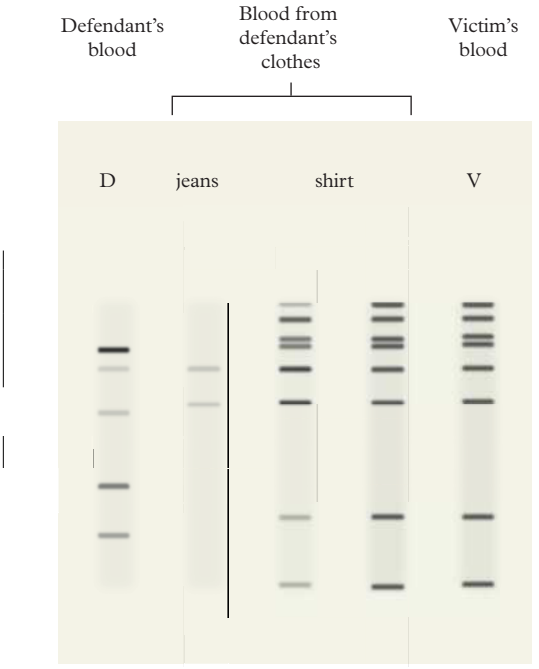


FIGURE 5 A DNA profile output. This DNA profile (fingerprint) shows that the blood on the defendant's clothes is that of the victim (V) and not the defendant (D).

FIGURE 6 Forensics can test the blood at a crime scene and run a DNA profile on it to assess a match.

Study tip

Always use a ruler to measure from the bottom of a gel to the DNA band.

CHALLENGE 3.3

DNA gels

The same endonuclease (EcoRI) was used to cut sections of DNA from two individuals. The lengths of the segments are shown in Figure 7.

Sample A	325 bp	161 bp	502 bp
Sample B	486 bp	502 bp	

FIGURE 7 Two cut DNA sections from two different individuals

Both DNA samples were loaded onto a gel with a standard DNA ladder and an electric current applied. The gel was run.

1 Using the data from Figure 7, draw the resultant DNA bands that could be expected on the gel in Figure 8. Use the standard DNA ladder as your guide.

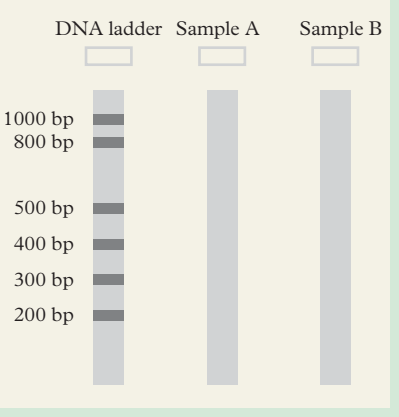


FIGURE 8 The PCR gel with just the DNA ladder present

CASE STUDY 3.3

Family tree forensics

In 2018, a small genealogy website – used by biological families of adoptees to upload sections of DNA usually related to health data – was used to identify the 'Golden State Killer' in America. The murderer had been unidentified for over 30 years. The DNA was extracted from the crime scene, amplified through PCR and uploaded onto the genealogy website. There were twelve matched relatives from fifth to third cousins. When grouped together, the cousins represented four different family trees. The forensic geneticist worked backwards to determine the ancestor of each family tree.

Once identified, census records, newspaper archives, school yearbooks and social media were used to identify every descendant of the four family trees. Eventually the family trees joined together, and the geneticist was able to identify two brothers who lived in the area, of which one was the murderer. The police were able to obtain a piece of rubbish that contained DNA from one of the brothers. The use of PCR, endonuclease and gel electrophoresis enabled a positive identification of the suspect. He was eventually sentenced to 80 years in prison.

FIGURE 9 PCR is used by police as an analytical technique in investigations.

CHECK YOUR LEARNING 3.3

Describe and explain

1 What do the letters 'PCR' represent?
2 Describe the four steps involved in PCR.
3 What are restriction fragment length polymorphisms?

Apply, analyse and compare

4 Genetically identical twins have exactly the same RFLPs. How would this impact their DNA profile?
5 What causes DNA to move through an agarose gel in gel electrophoresis?

Design and discuss

6 Why should DNA that will be compared in gel electrophoresis be cut with the same endonuclease?
7 Consider Case study 3.3.
   a Discuss the advantages and disadvantages of sending genetic samples to an ancestry website. Would you send your DNA to an ancestry company? Why or why not?
   b Discuss the ethics of someone using your uploaded DNA in an ancestry website to find someone.

3.4 Transformation of bacterial cells

KEY IDEAS

In this topic, you will learn that:

- recombinant plasmids can be used as a vector to transform bacterial cells
- human insulin can be produced by transformed bacterial cells.

The genetic code is considered universal, meaning the same DNA triplet in one organism will produce the same mRNA codon and connect the same amino acid in any other organism on the planet. For example, AAA will be transcribed into UUU and translated into phenylalanine in every organism from bacteria to humans. This implies that a gene from one organism can be transferred into another organism and the same protein will be produced.

Biological vectors

biological vector
a molecule or virus that is capable of carrying genetic material into another cell

DNA is unable to move through the nuclear or plasma membrane of cells. Instead, a **biological vector** (i.e. a DNA molecule capable of carrying genetic material into another cell) must be used. There are many types of vectors that can be used to introduce new genes into organisms, such as plasmids, viruses or gene guns. Gene guns are able to 'shoot' DNA-coated pellets at high pressure through the cell wall and membrane of plant cells.

Viruses are able to inject their DNA into a cell (which may include a gene from another organism). The cell is then forced to use the viral DNA to produce the viral proteins. The most common vectors used by molecular biologists, however, are plasmids.

Study tip

Prokaryotes do not have a post-transcriptional modification process that removes introns. Eukaryotic genes must be prepared through reverse transcription before they can be transferred into prokaryotic (bacterial) cells.

FIGURE 1 DNA gene gun

Plasmids

plasmid
small circular section of DNA found in prokaryotes that is not part of their chromosome

Plasmids are small circular sections of DNA found in prokaryotes, but which are not part of their chromosome. Instead, they are additional sections of circular DNA that carry genes that may be useful to the bacterial cell but are not essential for life. For example, many plasmids carry genes that enable the bacterial cell to be resistant to a particular antibiotic. These plasmids can be transferred between bacterial cells (horizontal or lateral gene transfer), passing on the resistance to the antibiotic to other bacterial cells of the same species. More information on horizontal and lateral gene transfer is discussed in Chapter 11.

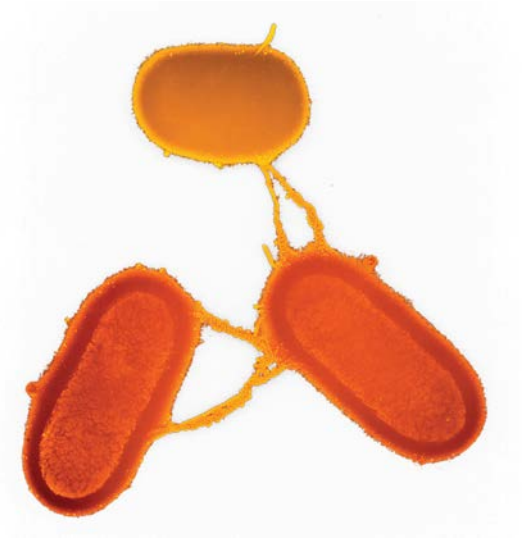


FIGURE 2 Bacterial cells undergo conjugation in order to share their plasmids with neighbouring bacteria.

Plasmids can be used by molecular scientists to transfer genes into bacterial cells. They do this by creating a **recombinant** plasmid.

recombinant
containing DNA from two different sources are joined together

- The first step involves cutting both the gene of interest and the plasmid with the same endonuclease. This ensures that the same sticky ends are produced in the plasmid and the gene.
- When the plasmid and cut gene are combined, the overhanging nitrogenous bases form hydrogen bonds between the plasmid and the gene. These are joined (annealed) together using DNA ligase.
- The recombined plasmid can then be inserted into a bacterial cell. When the bacterial cell grows and reproduces, the plasmid will also be reproduced.

This process is called **gene cloning**, because the gene contained within the plasmid is constantly cloned by the bacterial cell.

gene cloning
a process in which a gene is inserted into a plasmid, placed into a host cell, and the cell reproduced in a medium

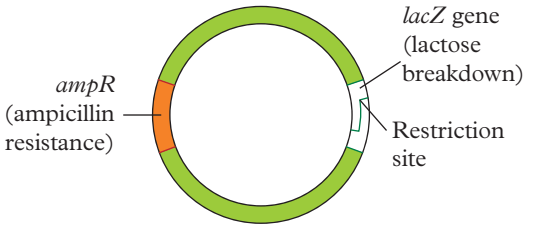
Identifying transformed cells

The process of transforming a bacterial cell is not always successful. Sometimes the DNA ligase is unable to join the plasmid. Other times, the plasmid is unable to get inside the bacterial cell. It is also impossible to see through a microscope exactly which bacterial cell has the plasmid. Instead, scientists use other genes in the plasmid to determine its presence. Sometimes, the plasmid will have an ampicillin resistance gene combined with the desired gene to be cloned.

If the plasmid carrying both genes is present in the cell, then it will be able to survive and reproduce on agar containing ampicillin. Cells without the plasmid will be killed by the antibiotic.

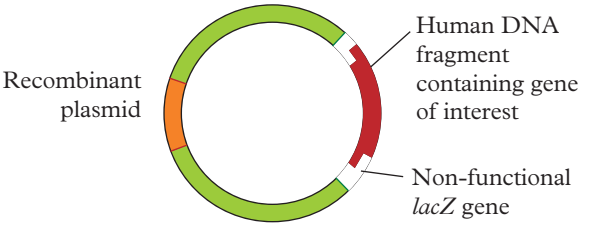
FIGURE 3 To identify a transformed cell, biologists can grow the cells on an agar plate. Some plasmids have a resistance to antibiotics, so the ones with the resistance will continue to grow in the presence of an antibiotic.

1 Bacterial plasmid before the addition of the foreign gene



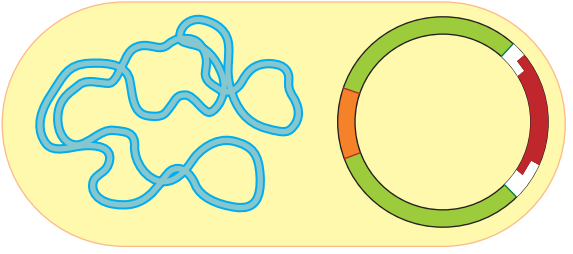
The site at which the restriction enzyme cuts is in the middle of the *lacZ* gene; the gene for ampicillin resistance is on the opposite side of the plasmid.

2 Plasmids are cut with restriction enzymes, then mixed with foreign DNA (e.g. human genes).



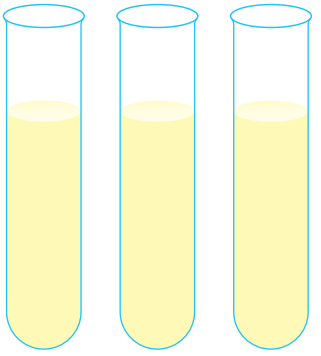
The human DNA has been inserted into the middle of the plasmid, making the *lacZ* gene non-functional.

3 Recombinant plasmid is transformed into bacterial cell.



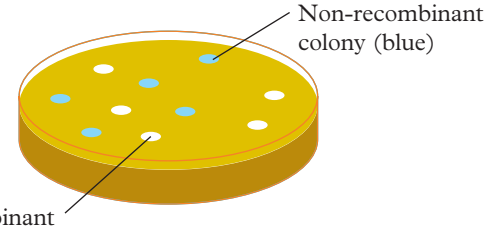
The recombinant plasmids are transformed into the bacteria. Some plasmids will not be recombinant and in other cases, no plasmids will transform into the bacterial cells.

4 Cells are cloned.



The bacterial cells are grown in tubes with a nutrient broth to produce many identical cells (clones).

5 Cells are grown and recombinant cells are identified.



The cells are plated onto a growth medium containing ampicillin and X-gal; the clones of cells containing recombinant plasmids are identified by their white colour. An intact *lacZ* gene produces a blue pigment.

6 Recombinant DNA clones are grown to produce the required product (e.g. insulin).

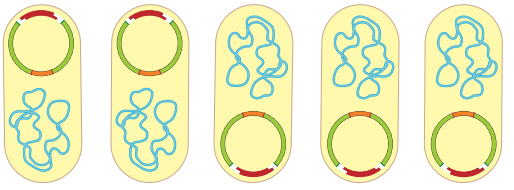


FIGURE 4 Identification of recombinant plasmids in bacteria

Video
Transforming bacterial cells

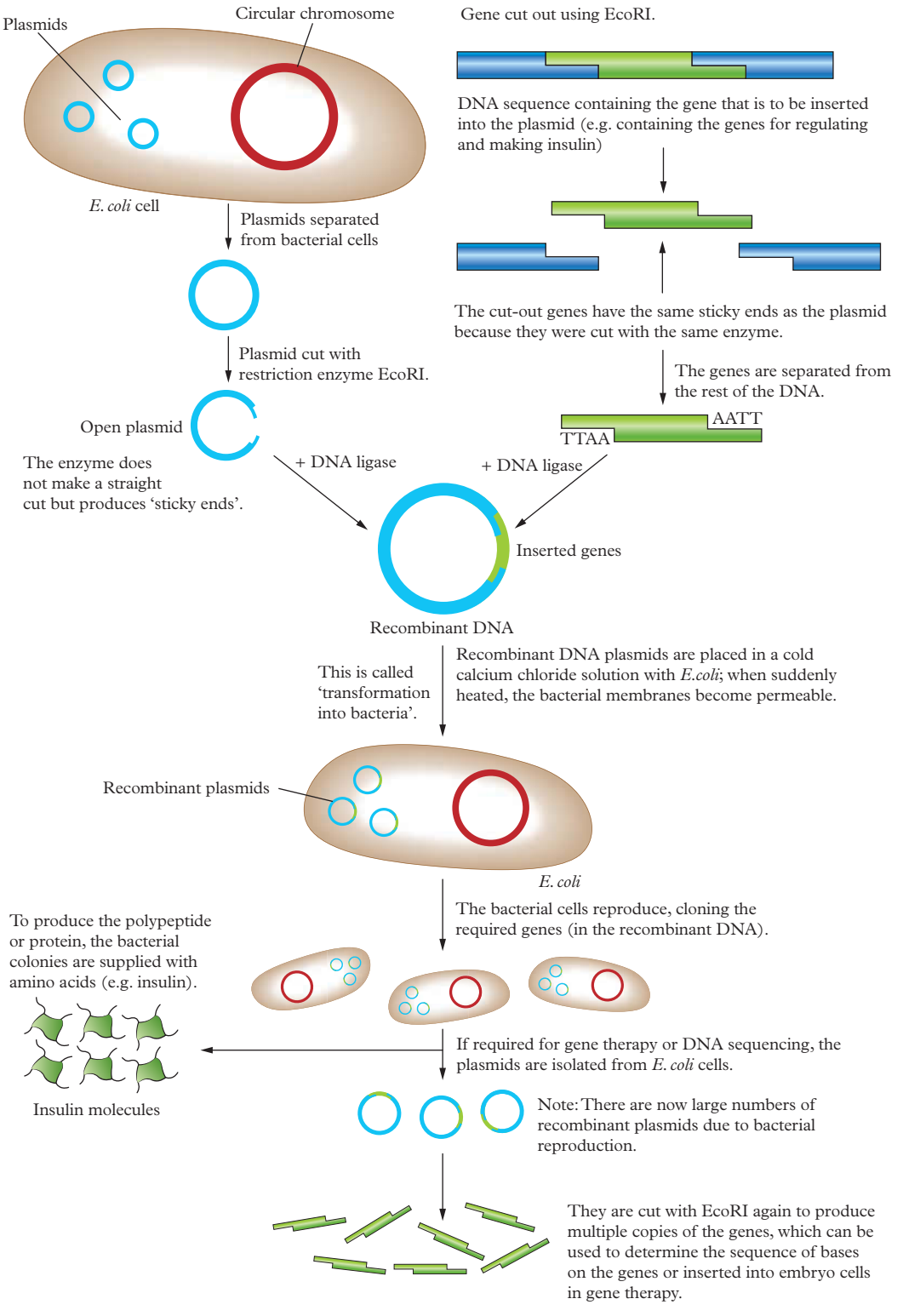


FIGURE 5 Recombinant DNA technology

Using transformed bacteria

There are many uses for bacteria that carry human genes. One of the most common forms of insulin used by people with diabetes is Humulin™. This insulin is produced by double-stranded cDNA sourced from human mRNA. The new form of human insulin gene has been inserted into a plasmid, before being taken up by bacterial cells. The bacterial cells are grown in large vats, continually producing the insulin protein required to treat diabetes.

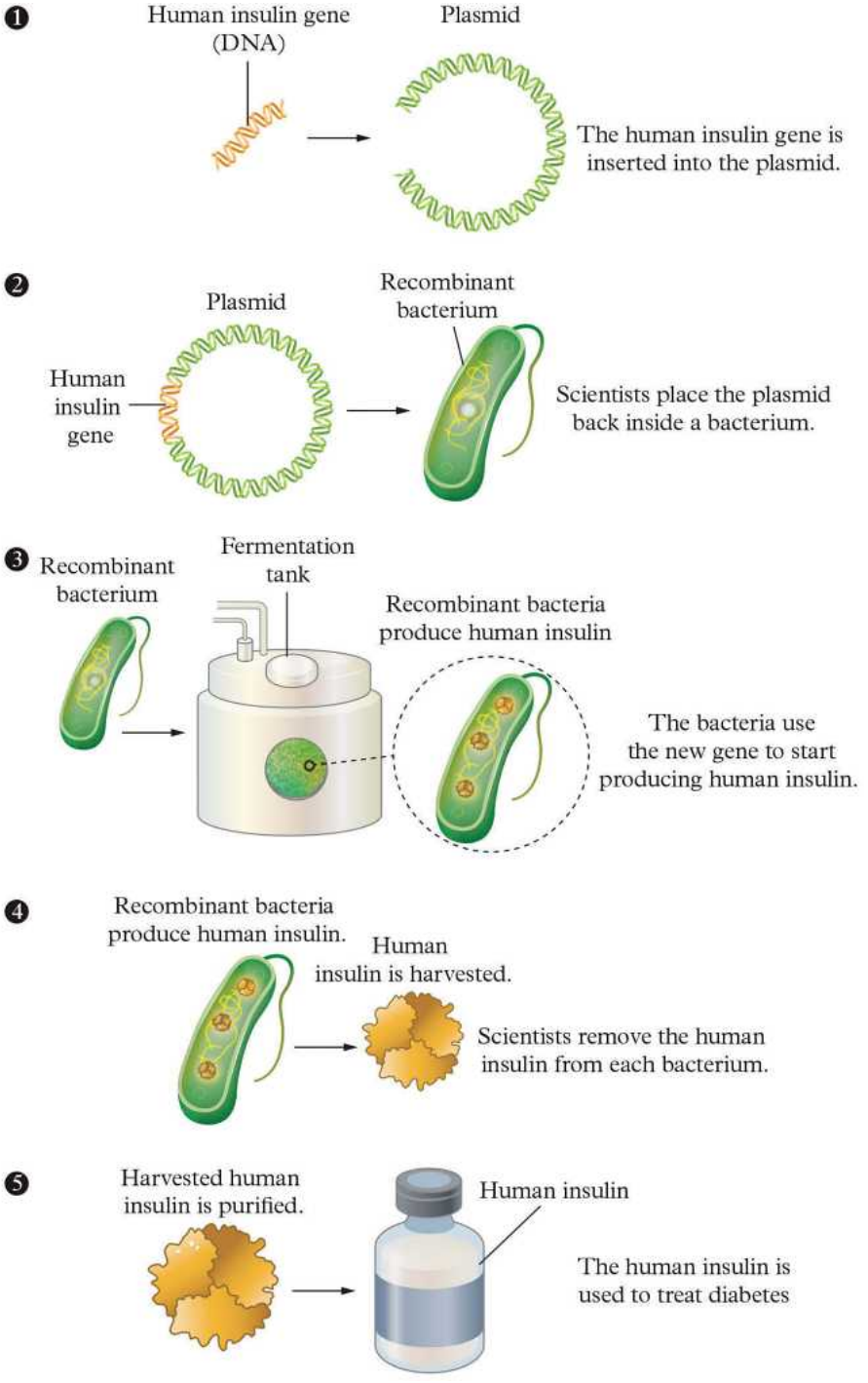


FIGURE 6 The process involved in producing Humulin insulin for patients suffering from diabetes

CHALLENGE 3.4

A new plasmid

A plasmid was produced for research purposes. It contained two genes: one for resistance to the antibiotic ampicillin (called *ampR*), while the other produced the lactose digesting enzyme – galactosidase (called *lacZ*). The *lacZ* gene produces an enzyme that can digest the sugar X-gal to produce a blue protein.

Any bacteria that is transformed with this plasmid will be able to survive and reproduce on agar containing ampicillin and can be identified by the blue protein.

In the middle of the *lacZ* gene, there is a restriction site specific to a particular endonuclease. If the endonuclease is used to cut the plasmid, a new gene can be inserted into this site.

1 Describe how this will affect the production of the blue protein.

2 Describe how a molecular biologist can use this property to identify which bacterial cells contain the new gene.

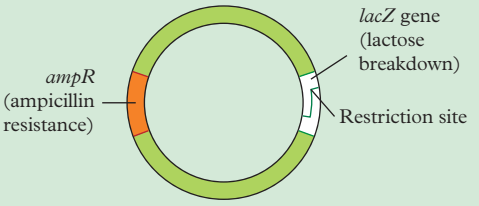


FIGURE 7 A gene being inserted into a plasmid

CHECK YOUR LEARNING 3.4

Describe and explain

1 What is meant by the phrase 'DNA is universal'?

2 Describe three types of vector that can introduce new genes into organisms.

3 What is a plasmid?

4 Explain how a plasmid can be used as a vector to transform bacterial cells.

Apply, analyse and compare

5 Compare the process of gene cloning and recombinant technology to produce human insulin.

6 Identify and describe the function of each of the enzymes used to produce a recombinant plasmid.

Design and discuss

7 Before insulin was produced using recombinant technology, purified pig insulin was used by people with diabetes. Describe the advantages and disadvantages of both methods of insulin production.

FIGURE 8 Purified pig insulin has been previously used by people with diabetes.

3.5 Genetically modified and transgenic organisms

KEY IDEAS

In this topic, you will learn that:

- genetically modified organisms (GMOs) have had their genome intentionally changed by humans
- transgenic organisms have had DNA from another organism transferred into their genome
- genetically modified organisms are used in agriculture to increase crop productivity and resistance to disease
- scientists must consider the ethical issues involved in genetically modifying organisms.

Humans have been modifying organisms for thousands of years. Initially, this was by choosing the most docile (tame) wolves that ate food left around early human camps. This allowed humans to domesticate wolves into what we now know as dogs. By the nineteenth century, breeders would choose dogs that were the most effective hunters or that had the most desired features. Domesticating dogs allowed humans to use them for hunting, pulling sleds, protection, search and rescue, and so on.

In the twenty-first century, scientists are now deliberately modifying the genome of organisms to achieve the desired characteristics.

FIGURE 1 The Saarlooswolfhond is a cross between a German shepherd and Eurasian grey wolf. It is its own dog breed and one of the closest to the original wild wolves.

Genetically modified organisms

genetically modified organism (GMO) an organism that has had its genome intentionally changed by humans

Genetically modified organisms (GMOs) are organisms that have had their genetic material intentionally changed by humans. The process for this may take many forms. Genes may be corrected or removed, mutations may deliberately be introduced or genes from different organisms may be added. There are different ways of genetically modifying organisms and some have been included below.

Induced mutagenesis

mutation a permanent change in the genetic sequence of an organism

Induced mutagenesis is the deliberate exposure of a plant or seed to DNA-altering chemicals or ionising radiation, to induce (cause) a permanent change in a DNA sequence. This permanent change is called a **mutation**. Plant breeders can adjust the dose of chemicals used or radiation exposure so that random changes occur in any of the genes in the organism. Some of these mutations can be fatal for the organism. Other changes may result in a new trait that is useful to the breeder. Examples of plants that have been produced by induced mutagenesis include durum wheat, Ruby Red grapefruit and drought-tolerant rice.

FIGURE 2 Ruby Red grapefruit

Transgenic organisms

transgenic organism
an organism that has had DNA from another organism inserted into its genome

Transgenic organisms are genetically modified to have DNA from another organism transferred into their genome. These genes may be from an unrelated organism of the same species or from a different species. One reason this might be done is to make the modified organism resistant to a particular disease.

Increased crop productivity

Rice is a staple food that feeds our ever growing global population, yet it requires a lot of water to grow. As temperatures increase and the amount of dry land increases worldwide, the need for drought-resistant plantations has grown. Plants can adapt to withstand drought conditions, but it takes a significant time to acquire this adaptation. Scientists at the RIKEN Center for Sustainable Resource Science in Japan have created a genetically modified strain of rice that is resistant to drought. The team inserted the drought-resistant *Arabidopsis GolS2* gene into Brazilian and African rice. After several attempts, they were successful in growing these plants in many arid locations.

Resisting disease

Some tomato farmers spray copper-based pesticides multiple times a year in an attempt to kill bacterial spot, an infection caused by a proteobacterium, *Xanthomonas perforans*. A related member of the tomato family, sweet pepper (*Capsicum chacoense*) has the *Bs2* gene, which gives it resistance to *Xanthomonas perforans*. Transferring this gene into the tomato plants prevents them from becoming infected and increases both the plant's and farmer's productivity.

FIGURE 3 Bacterial spot on tomatoes, caused by *Xanthomonas perforans*

CHALLENGE 3.5

Golden rice

Golden rice was created by introducing two genes (one from daffodils and one from a common soil bacterium) to increase the amount of vitamin A in the central edible section of the rice grain. The goal was to produce a food source to combat the vitamin A deficiency experienced in many parts of the world.

Many ethical questions have been raised as part of this process. Consider the ethical implications when responding to the following questions.

1. Could Golden Rice crops impact the environment and its biodiversity?
2. Could the new proteins cause allergies in humans?
3. Is it fair that the rice is primarily designed for people in low-income countries who may not have economic choice?
4. Do the risks of this genetic engineering outweigh the benefits?

FIGURE 4 Daffodil genes were used in the creation of golden rice.

CASE STUDY 3.5

GMO ban in Tasmania

Tasmania is one of the few states in Australia to have a total ban on any genetically modified organisms (GMO). Prior to 2001, genetically altered canola plants were grown at a secret test site. However, some of the GMO crop spread into the surrounding area. As a result, the state initiated a moratorium (a temporary ban) on any GM organisms being allowed in the state while a thorough review was conducted. The final report was released in 2019, suggesting that the state did not need to use GMOs to maintain high production levels, and that a GMO-free status might be an important part of the state's brand. Honey producers were especially happy with the ban being maintained because overseas markets such as Europe and Japan bought Tasmanian honey because it was GMO-free.

FIGURE 5 Tasmania is currently one of the few state or territory in Australia to ban GMO.

There is one exception to the ban. Site-directed nuclease (SDN1) changes involve using genetic modification techniques to deliberately change a single nucleotide in a cell. This is indistinguishable from a natural mutation. When the cell proliferates, the modification that inactivates the gene is inherited. This organism is not regulated as a GMO because it cannot be distinguished from natural genetic changes in an organism.

Not all farmers are happy with the ban. They suggest that the inability to grow omega-3 canola or other fortified seed crops will be disadvantageous for them compared with the farmers on the mainland who use GM crops to gain higher yields.

CHECK YOUR LEARNING 3.5

Describe and explain

1 Why do you think ethics is involved in discussions about genetically modified food?

2 Define the term 'transgenic organism' and provide an example of one.

3 Identify another crop that has been genetically modified to withstand difficult conditions.

Apply, analyse and compare

4 Other than increasing crop productivity and disease resistance, what other benefits are provided by genetically modified foods?

Design and discuss

5 Design your own transgenic organism.
- **a** What organism would you choose?
- **b** What gene would you introduce? Name the gene and its origin.
- **c** What ethical issues would you have to consider?

6 In small groups, consider the ethical standpoint of Tasmania's view on genetically modified food in Case study 3.5.
- **a** Discuss the state's decision to ban GMOs, and evaluate the pros and cons.
- **b** Decide together whether you would ban or support genetically modified food.

CHAPTER 3

Review

Chapter summary

3.1

- DNA can be altered with the help of enzymes.
- DNA polymerase produces new DNA polymers, DNA ligase repairs DNA and endonuclease cuts DNA at specific locations.

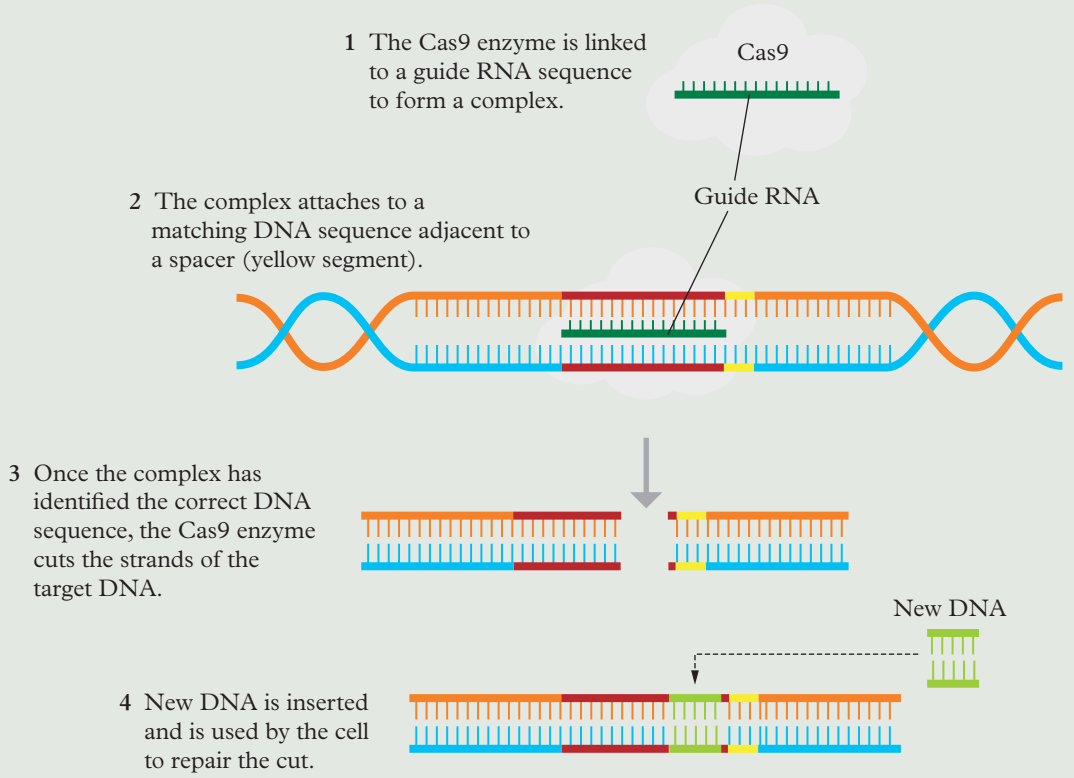


FIGURE 1 The process of CRISPR

3.2

- CRISPR-Cas9 is sourced from prokaryotes and helps protect them from viruses.
- Molecular biologists can use the CRISPR-Cas9 system to cut DNA.

3.3

- PCR is a method of multiplying sections of DNA through heating and cooling steps.
- Gel electrophoresis can be used to separate DNA sections according to size and build a DNA profile.

3.4

- Pieces of DNA, called vectors, can be removed from one organism and inserted into another.
- Human insulin can be produced through transformed bacterial cells.

3.5

- Organisms can be genetically modified by altering the genome.
- Organisms are considered transgenic organisms when DNA is transferred from one organism into another.
- Scientists must consider the ethical issues involved in genetically modifying organisms.

Revision questions

Multiple choice

1 Which of the following enzymes is able to make DNA?

A Endonuclease
B DNA ligase
C DNA polymerase
D Reverse transcriptase

2 Which of the following enzymes is able to cut DNA at a specific recognition sequence?

A Endonuclease
B DNA ligase
C DNA polymerase
D Reverse transcriptase

3 A plasmid is:

A a circular section of DNA.
B a circular section of DNA from a prokaryote that is not part of the chromosome.
C a circular section of DNA from a eukaryote.
D a circular section of DNA from a prokaryotic chromosome.

4 The correct order of the stages of PCR is:

A denaturation, elongation, annealing, repeat.
B elongation, denaturation, annealing, repeat.
C annealing, denaturation, elongation, repeat.
D denaturation, annealing, elongation, repeat.

5 In DNA fingerprinting, gel electrophoresis is used to:

A form restriction fragment length polymorphisms.
B match a gene with its function.
C separate fragments of DNA.
D match a gene with its function.

6 Examine the results from gel electrophoresis shown in Figure 2. Who are the parents of the child?

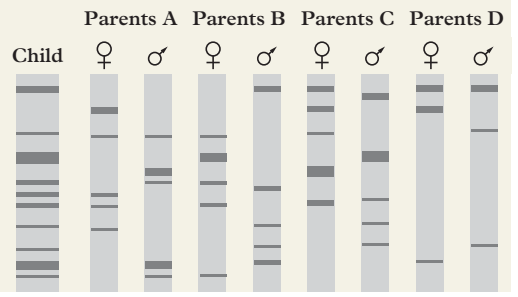


FIGURE 2 DNA results

A Parents A
B Parents B
C Parents C
D Parents D

7 Recombinant DNA is:

A DNA joined together by DNA ligase.
B DNA from two different organisms.
C DNA from two different cells.
D DNA recombined to form a double helix.

8 Genetically modified organisms:

A are always dangerous.
B are always bacteria.
C may be beneficial.
D occur naturally.

9 Which of the following base pairs is likely to be an endonuclease recognition site?

A $\frac{ACTG}{TGAC}$

B $\frac{ATTT}{TAAA}$

C $\frac{TACG}{ATGC}$

D $\frac{GTTAAC}{CAATTG}$

10 A particular gene was used in the PCR process. After ten cycles, how many copies of this gene were produced?

A 200
B 800
C 1024
D 2048

Short answer

Describe and explain

11 Describe the difference between endonuclease and CRISPR technology.

12 Describe the difference between DNA ligase and DNA polymerase.

13 Explain two situations where gel electrophoresis could be used to identify an individual.

14 Describe how an enzyme such as DNA polymerase can be used to manipulate DNA.

15 Explain why endonucleases are referred to as 'DNA scissors' and how they are used to manipulate DNA.

16 Explain the process of polymerase chain reaction and identify its purpose.

Apply, analyse and compare

17 A group of researchers wanted to evaluate GMO crop yields versus non-GMO crop yields. To do this, they compared yields of corn from European Union countries (where GMO crops are banned) and GMO corn crops from the US.

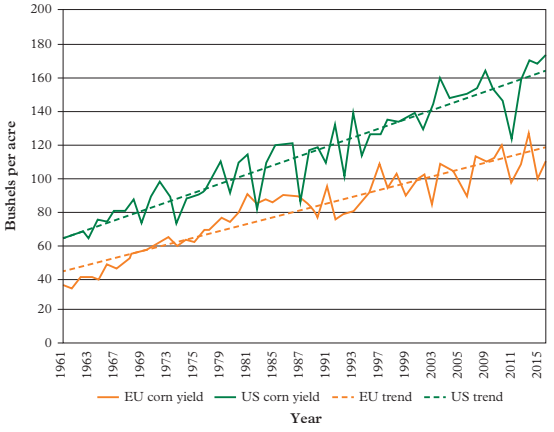


FIGURE 3 US and EU corn yields, 1961–2016

a Examine the graph and describe the difference in crop yields between US GMO crops and European non-GMO crops.

b Use the data to draw a conclusion about the effect of genetic modification on crop yields.

c Discuss the validity of the data collected and presented in the graph.

18 Draw a labelled diagram of what happens to DNA at each step of the polymerase chain reaction.

19 Humulin (human insulin) is produced using recombinant bacteria. Describe how the human gene for insulin was produced and then placed in the bacterial cell.

20 Apply your knowledge of DNA probes to describe how they could be used during profiling for the identification of an individual.

21 In 1976, *Taq* polymerase – the enzyme that replicates DNA – was isolated from the thermophilic bacterium *Thermus aquaticus*. Describe and compare the characteristics of *Taq* polymerase in relation to human polymerase. Use Figure 4 to help your response.

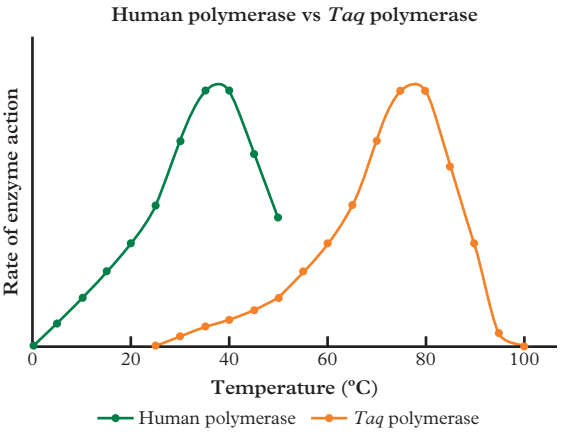


FIGURE 4 Comparison of human and *Taq* polymerase

Design and discuss

22 The CSIRO have genetically modified the cotton plant to grow coloured cotton. This breakthrough will decrease the need for large

quantities of dyes to be used to colour cotton fabrics. The process of dying the natural fibres of cotton makes the cotton industry less environmentally friendly.

a Describe a possible advantage of this breakthrough.

b Describe why these new cotton plants may not be grown in Tasmania.

23 The local supermarket has set out two trays of tomatoes. The tomatoes on one tray are covered in small dark spots, while the other tomatoes are bright red and have a sign above identifying them as genetically modified. Which tomato would you buy? Explain your choice.

24 The CSIRO have generated canola plants that are capable of producing high quantity oils rich in omega-3 DHA (a nutrient currently only found in ocean-based algae and fish). Draw a flowchart that shows a possible process that could be used to produce these plants.

FIGURE 5 Canola plants

25 There are calls for a moratorium (pause) in the clinical use of CRISPR technology in editing human sperm, eggs or embryos to produce babies.

a Suggest why this moratorium has been called for.

b Do you agree or disagree with the call for a moratorium? Provide reasoning to support your view.

26 Retroviruses contain RNA as their genetic material instead of DNA.

a Explain why PCR cannot be used to identify a retrovirus.

b Describe how the viral RNA could be copied into DNA before the PCR procedure.

27 What is the difference between genetically modified organisms and transgenic organisms?

28 *Bt* cotton has a bacterial gene inserted that allows the plant to produce a molecule that is toxic to the lining of an insect's intestine.

a Describe a possible advantage of this genetic modification to the farmer.

b Describe a possible disadvantage of this genetic modification.

29 Wheat is widely cultivated across the world as a cereal grain. As the climate becomes less predictable, scientists have been challenged to improve the ability of wheat to survive drought conditions. One group of genes have been found to produce transcription factors that protect the plant from dessication. Suggest how a scientist could modify the genes of wheat to improve its ability to survive in a dry climate.

Exam essentials

Responding to questions

In your exam, you may be expected to format your response according to the task word in the question. Refer to Topic 1.7 in the Chapter 1 Biology toolkit for more information about the different styles of questions.

Respond to the task word

You first need to identify the task word (also called the directional or command word). This task word will give you direction on how to respond to the question.

Memory task words include the following: define, describe, list, outline, name, state. Your responses to these key words should present your knowledge on the topic. Dot points are usually a great way to answer these types of questions.

Analysis task words include the following: explain, justify, compare, discuss. Your responses to these key words should demonstrate your analytical skills and application of knowledge.

The following question is taken from the 2014 VCE Biology Examination. Read the question carefully, then consider whether the responses are suitably formatted to the task word in the question.

QUESTION 11a (2014 Biology Written Examination)

In 1991, the body of a man was found frozen beneath a glacier in Italy. Researchers named him Ötzi. It was determined that Ötzi died 5300 years ago and that his body is the oldest mummified human body ever found. Scientists have successfully extracted DNA from the nucleus of his frozen cells.

a Describe the process scientists would use on a small sample of Ötzi's DNA to obtain larger quantities of identical DNA. 3 marks

Source: 2014 Biology Written Examination Question 11a, Short answer, reproduced by permission © VCAA

Response 1

This process is polymerase chain reaction. The process is as follows.

- Denaturation: Heating the DNA 90–90°C to separate the strands.
- Annealing: Cooling to 50–55°C to attach primers.
- Elongation: Heating to 72°C for *Taq* polymerase to bind and form complementary strands.

This process is repeated several times to obtain many copies of the DNA sample.

Dot points are a suitable format for the directional word 'describe'.

When describing PCR, do not forget to include this statement.

This response focuses on the directional word 'describe'. The process of PCR is described in succinct detail with reference to the conditions of each stage. This response would receive full marks.

Response 2

The process is known as polymerase chain reaction, where the sample is subjected to different temperatures in order to obtain larger quantities to work with. There are several steps in the process: denaturation, annealing and elongation. This process is repeated many times so that large quantities of the DNA can be obtained.

This statement is vague and not descriptive.

Stages are named but not described.

This answer names the process and lists the steps, but it does not have any description. This response demonstrates the need to focus on the directional word to maximise marks on the exam.

Think like an examiner

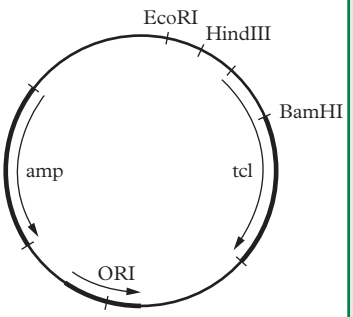
To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

Question 9b (2017 Biology Written Examination)

A particular bacterial plasmid contains recognition sites for the restriction enzymes EcoRI, HindIII and BamHI, along with two antibiotic-resistant genes, ampicillin resistance (amp) and tetracycline resistance (tcl), and an origin of replication (ORI). The diagram below shows the positions of these recognition sites and antibiotic-resistant genes as well as the position of the origin of replication within this plasmid.



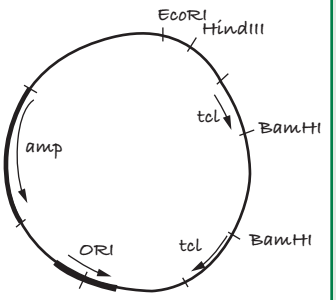
One purpose of using recombinant bacterial plasmids is to produce bacteria capable of synthesising human protein.

b The restriction enzyme BamHI was used to help insert a gene coding for a human protein into this plasmid.

i Describe how restriction enzymes such as BamHI are used to help insert a gene coding for a human protein into this plasmid. 2 marks

They help to insert the gene into the plasmid at the recognition site.

ii Draw and label a diagram in the space below to show the position of the human gene in this plasmid when BamHI is used. Include the position of the recognition sites for the restriction enzymes EcoRI, HindIII and BamHI on the plasmid. 1 mark



Source: 2017 Biology Written Examination Question 9b, Short answer, reproduced by permission © VCAA

Marking guide

Question 9 b i	- 1 mark for identifying the restriction enzyme cuts both the plasmid and desired site. - 1 mark for stating the gene can then be inserted into the plasmid because the recognition sequences are complementary.
Question 9 b ii	- 1 mark for drawing a *labelled* plasmid with the human gene inserted between BamHI sites.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

CHAPTER

4

Biochemical pathway regulation

Cellular metabolism is the sum of all the chemical reactions that occur within the cell. These reactions include all the reactants and products, and the actions of enzymes and coenzymes. The biochemical pathways discussed in this chapter – photosynthesis and cellular respiration – involve high-energy molecules used in a multitude of chemical processes throughout the cell. The regulation of these pathways occurs when the factors that affect the enzyme action change, such as temperature, pH, concentration and inhibition.

KEY KNOWLEDGE

- → the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- → the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration
- → 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

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

FIGURE 1 The Dandenong Ranges in Victoria. Life on the Earth relies on many factors for its existence. To function and survive, the cells that make up all living things rely on energy transformation reactions for their energy requirements.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

4A In the chemical reaction shown below, identify:

- **i** The reactants
- **ii** The products
- **iii** The number of hydrogen and oxygen atoms on the left side of the equation
- **iv** The physical states of each molecule

$$2H_2(g) + O_2(g) \rightarrow 2H_2O(l)$$

4A Groundwork resource
Balancing chemical equations

4B What does the term 'pH' refer to?

4B Groundwork resource
The pH scale

PRACTICALS

PRACTICAL	**4.2** Enzymes in washing detergent

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

4.1 Structure of biochemical pathways

KEY IDEAS

In this topic you will learn that:

- energy transformation reactions (photosynthesis and cellular respiration) have complex biochemical pathways

biochemical pathway
a series of linked enzymatic reactions

A **biochemical pathway** describes a series of linked reactions occurring within a cell. Most often the product of one reaction is the reactant for the next, and each reaction is catalysed by a specific enzyme as shown by the figure below.

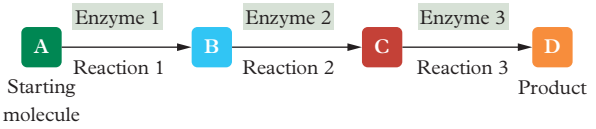


FIGURE 1 The basic structure of biochemical pathways

By-products and waste products are also produced in biochemical pathways. These are either used by the cell or excreted as waste.

Biochemical pathways occur in different locations within the cell, depending on the type of reactions. For example, glycolysis (first stage of cellular respiration) occurs in the cytosol, whereas the electron transport chain (third stage of cellular respiration) takes place on the mitochondrial membrane. The final product of a biochemical pathway may be used by the cell straight away, stored for later use, or to initiate another biochemical pathway. A simplified version of the glycolysis biochemical pathway is shown in Figure 3.

FIGURE 2 Trees gain a large amount of energy from the Sun through photosynthesis.

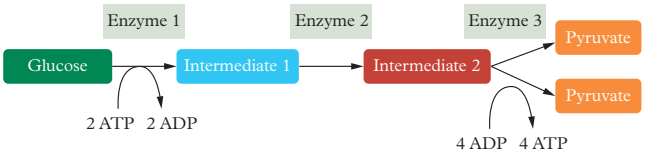


FIGURE 3 Glycolysis biochemical pathway

Both photosynthesis and cellular respiration are a series of very complex biochemical pathways that are described in more detail in Chapters 5 & 6. You do not need to remember every step of the biochemical pathway, but you do need to learn the initial reactants and final products for both photosynthesis and cellular respiration, as shown in Table 1.

TABLE 1 The initial reactants and final products of photosynthesis and cellular respiration

	Initial reactants	Final products
Photosynthesis	Sunlight Carbon dioxide Water (12 molecules)	Glucose Oxygen (by-product) Water (6 molecules)
Cellular respiration	Glucose Oxygen	Carbon dioxide (by-product) Water ATP

CHECK YOUR LEARNING 4.1

Describe and explain

1 Identify the reactants and products of photosynthesis.

2 Identify the reactants and products of cellular respiration.

3 Define the term 'biochemical pathway' in your own words.

4 Explain what happens to the products of a biochemical pathway.

Apply, analyse and compare

5 Carbon dioxide is produced during cellular respiration but then not used to produce the final product. It is therefore considered a by-product. Propose where this carbon dioxide would be after cellular respiration has taken place.

6 Analyse the biochemical pathway in figure 1. If a pathway had five reactions, how many enzymes would you expect to be involved?

Design and discuss

7 Phenylalanine is an amino acid that we obtain from our diet. It is needed for our cells to produce proteins and other important molecules. Review the phenylalanine biochemical pathway below and answer the following questions.

a Identify the initial reactant in the biochemical pathway.

b Determine which enzyme catalyses the reaction of tyrosine to L-DOPA.

c Discuss what would happen to the level of Dopamine if we had a reduced amount of phenylalanine in our diet.

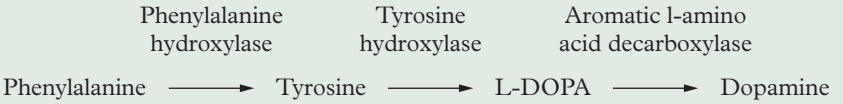


FIGURE 4 Phenylalanine biochemical pathway

4.2 The role of enzymes and coenzymes

KEY IDEAS

In this topic you will learn that:

- enzymes have certain properties to enable them to catalyse metabolic reactions
- enzymes and coenzymes facilitate steps in biochemical pathways
- cycling of coenzymes is necessary in energy transformation reactions.

Enzymes catalyse biochemical reactions

Biochemical reactions within a cell are regulated by enzymes. Each enzyme controls only one (or one type of) reaction. This enzyme selectivity occurs because the active site of any enzyme can only accommodate a certain shape of substrate molecule. Therefore, many different enzymes may be required in a multi-step reaction (such as in cellular respiration or photosynthesis). The total set of biochemical reactions that occurs within the living cells of an organism is known as its **metabolism**.

metabolism the total set of biochemical reactions occurring within a cell

Properties of enzymes

Enzymes have the following properties.

- They reduce the activation energy needed for a chemical reaction to take place.
- They speed up the rate of reaction without being used up.
- A small amount of enzyme catalyses a large amount of **substrate**.
- They contain an **active site**, where the substrate can attach to the enzyme.
- They are specific in action and generally catalyse one specific reaction or part of a reaction.

substrate the substance on which an enzyme acts

active site the portion of an enzyme in contact with the substrate; this site has a specific shape that corresponds to the shape of at least a portion of the substrate molecule

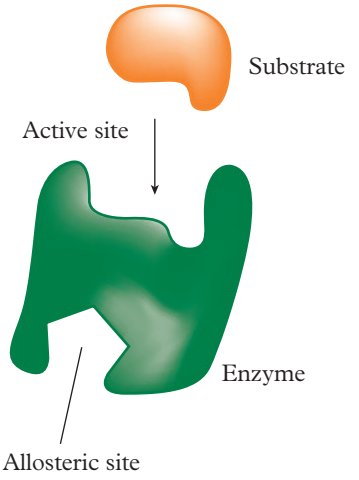


FIGURE 1 The substrate binds to the active site of the enzyme when uninhibited.

Activation energy

All chemical reactions require a certain amount of energy before they can occur. This starting energy is called the activation energy. An enzyme provides a surface for reactants to meet at the correct angle, or for a reactant to be placed under stress so that it can break apart. Without the enzyme, the reactants would need more energy to start the reaction. This means that the presence of enzymes lowers the activation energy.

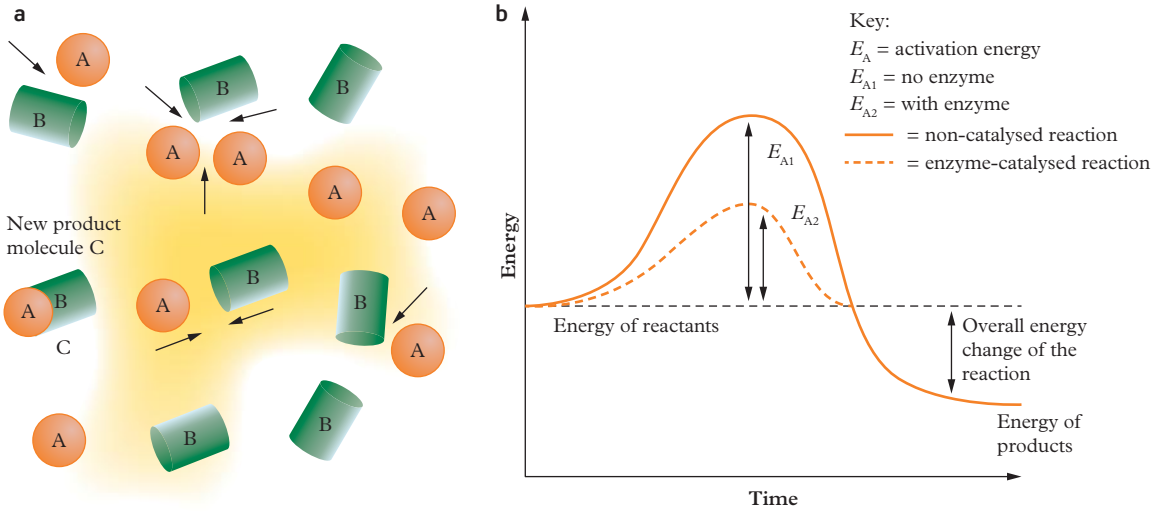


FIGURE 2 Enzymes as catalysts: **a** moving molecules must collide with enough energy and at the correct angle before they react; **b** energy diagrams show that the presence of an enzyme lowers the activation energy for the reaction

Biochemical reactions can be either anabolic or catabolic:

- Anabolic reactions combine two or more substrate molecules to produce a larger product. Anabolic reactions take in energy from the surroundings to form molecules with higher energy levels.

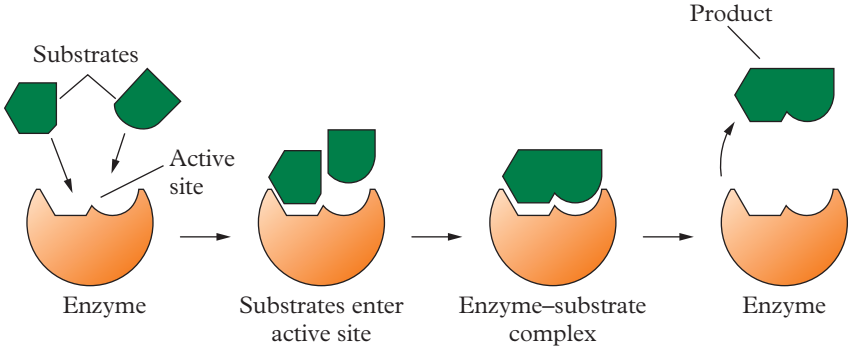


FIGURE 3 Enzyme catalysing an anabolic reaction

- Catabolic reactions break down one substrate molecule to produce one or more products. Catabolic reactions release energy when they break apart a high-energy molecule into lower-energy molecules.

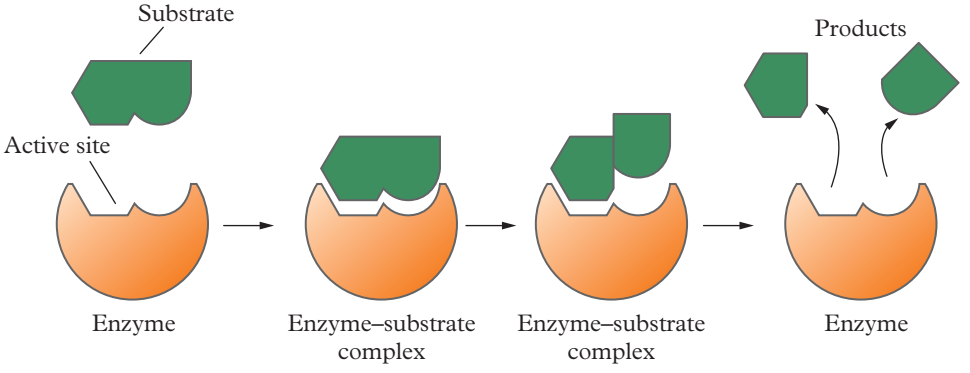


FIGURE 4 Enzyme catalysing a catabolic reaction

Enzymes catalyse these reactions by speeding up the rate at which they occur. Without enzymes, these reactions would occur too slowly for adequate cell functioning. Enzymes do not form part of the end product of biochemical reactions, so they can be used many times over to catalyse a specific reaction.

A general biochemical pathway

Each step in a biochemical pathway uses a different enzyme. In a two-step pathway, like the one shown in the equation above, enzyme A would control the amount of intermediate substance formed from the substrate. This intermediate substance would then be converted to the final product by enzyme B.

If just one enzyme in a biochemical pathway is absent or faulty, the correct sequence of chemical reactions cannot occur. For example, if enzyme B was faulty, an excess of the intermediate substance would form. Too much of this substance may be dangerous to the cell's health and metabolism.

CASE STUDY 4.2

Bioluminescence in the Gippsland Lakes

Bioluminescence is a common occurrence in the living world, with 76% of ocean animals found to emit light created by chemical reactions within their body.

With the help of the enzyme luciferase, a molecule called luciferin reacts with oxygen in an exergonic reaction. The energy released from this reaction is in the form of cool light rather than heat, and can be seen in organisms such as insects, fungi, fish and algae. In some cases, marine animals take bioluminescent bacteria into their body cavities to help them glow.

Every few years, the shores of the Gippsland Lakes in East Gippsland light up from the exergonic reactions of bioluminescent plankton called *Noctiluca scintillans*. When disturbed, such as by crashing waves on the shore or from someone walking through the water, the plankton glow bright blue. This is thought to be a defensive strategy so that, if they are eaten by a predator like a crustacean, the crustacean then would also glow blue, making it more likely to be seen by predators along the dark shoreline.

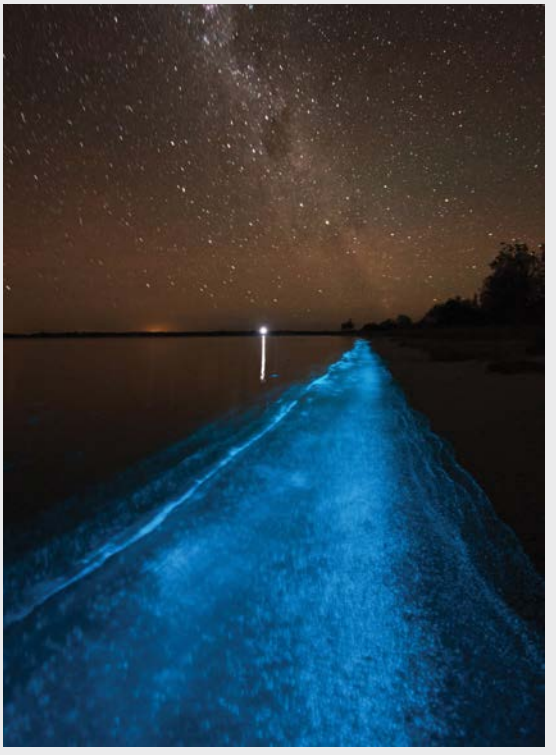


FIGURE 5 Bioluminescence of the Gippsland Lakes, Victoria, Australia

Cofactors and coenzymes

A cofactor is any non-protein chemical that is needed for an enzyme reaction to proceed. Cofactors can be inorganic ions, or organic molecules called **coenzymes**. An apoenzyme is a protein enzyme without its cofactor; it is inactive in this form. Once it has bound to its cofactor, the apoenzyme becomes an active holoenzyme.

coenzyme
an organic, non-protein compound needed for an enzyme to function

There are many types of coenzymes, most of which are needed for the energy transformation reactions of photosynthesis and cellular respiration. They include adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide phosphate (NADPH).

Study tip

You don't need to remember the full names of these coenzymes, only the abbreviated terms for them: ATP, NADH, NADPH and $FADH_2$.

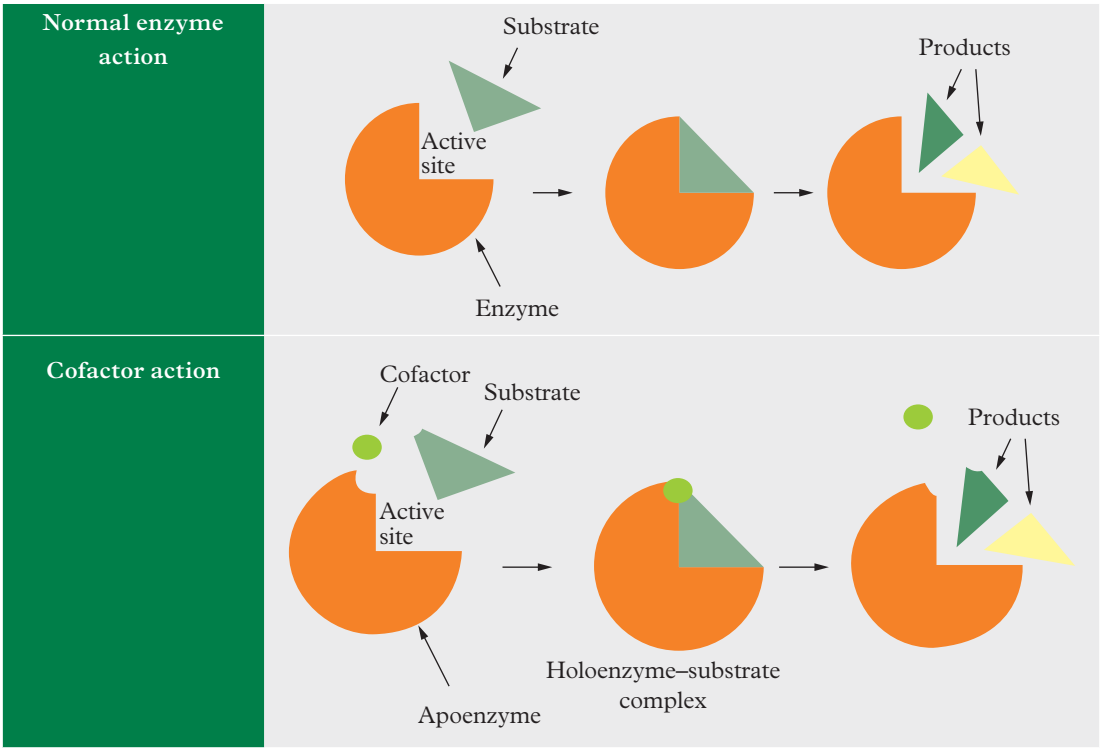


FIGURE 6 Enzyme reactions with and without cofactor action

Many vitamins are cofactors of biochemical processes. The B group vitamins (B1, B2, B3, B5, B6, B7, B9, B12) are all cofactors in cell metabolism. Vitamin C is a coenzyme used in the repair of bones, blood vessels and skin. Interestingly, there are a few groups of animals that can't synthesise their own vitamin C and must obtain it from their diet: these are humans, bats and guinea pigs.

The cycling of coenzymes

The coenzymes involved in photosynthesis and cellular respiration (ATP, NADH, $FADH_2$ and NADPH) can carry chemical groups, protons or electrons between different reactions. This is necessary for the enzymes in those reactions to function. Energy is stored in the coenzyme and released when the coenzyme releases the chemical group, proton or electron. These coenzymes are recycled continuously between carrying and releasing chemical groups, protons and function (being loaded or unloaded). This is known as the cycling of coenzymes.

TABLE 1 The loaded and unloaded versions of coenzymes used in energy transformation reactions

Loaded	Unloaded
ATP	ADP
NADH	NAD^+
NADPH	$NADP^+$
$FADH_2$	FAD^+

ATP and ADP

ATP (adenosine triphosphate) a compound consisting of an adenosine molecule bonded to three phosphate groups; a high-energy molecule

Adenosine triphosphate (ATP) contains three phosphate molecules ('tri' meaning three) as seen in Figure 7. When ATP passes one of its three phosphates to other molecules, it becomes adenosine diphosphate (ADP). This causes energy to be released, powering other chemical reactions including enzyme reactions.

ADP is the unloaded form of ATP with two phosphate molecules ('di' meaning two). A phosphate can be added to ADP via a process called **phosphorylation**, and then the coenzyme returns to its loaded form of ATP.

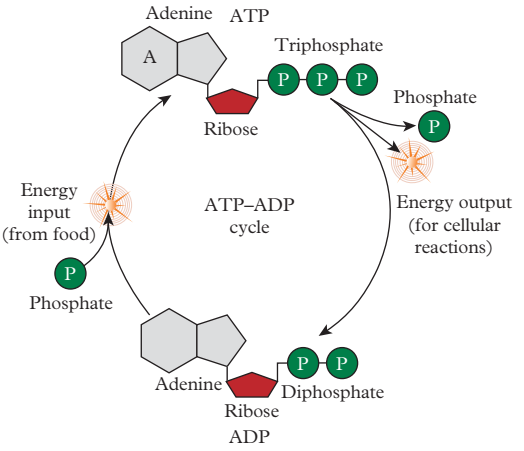


FIGURE 7 The cycling of coenzyme ATP and ADP

phosphorylation the process of adding a phosphate to an organic compound

ATP is needed for both photosynthesis and cellular respiration. During cellular respiration, many ADP molecules are phosphorylated to become ATP. These are then used by cells for different metabolic reactions.

Study tip

To remember that NADPH is the coenzyme for photosynthesis and NADH is the coenzyme of cellular respiration, remember 'P' for photosynthesis as NAD'P'H.

NADH, NADPH and $FADH_2$

NADH is the loaded coenzyme of NAD^+ and is involved in the transfer of electrons during cellular respiration. NADH contains an additional hydrogen ion compared to its unloaded form, NAD^+, and this means it is also carrying an additional electron. Like NADH, $FADH_2$ also carries hydrogen ions and electrons around the cell in cellular respiration.

NADPH is the loaded form of $NADP^+$, carrying the additional hydrogen ion as well. However, this coenzyme is needed during photosynthesis. It is loaded during the light-dependent reaction and used in the light-independent reaction. It cycles in the same way as NADH/NAD^+ but contains an additional phosphate.

CHECK YOUR LEARNING 4.2

Describe and explain

1 Why are enzymes described as catalysts?
2 What is the difference between ATP and ADP?
3 Define the term 'active site'.
4 Which coenzymes are necessary for cellular respiration?
5 Are all enzymes proteins? Explain.
6 Draw a diagram to show the action of a cofactor.

Apply, analyse and compare

7 Compare the following pairs of terms.
  a Cofactor and coenzyme
  b Enzyme and coenzyme
  c Loaded and unloaded coenzyme
8 How does an enzyme control only one type of reaction rather than many different types of reactions?
9 Why are multiple enzymes needed for a biochemical pathway?
10 **a** Use Case study 4.2 to explain how enzymes can be used by other organisms as a defence mechanism.
  b Apply this knowledge to research another example of an organism using an enzyme to protect itself.

Design and discuss

11 How many enzymes could be involved in the following simple biochemical pathway?
Substrate → intermediate product 1 → intermediate product 2 → product
12 Draw a diagram to represent the cycling of coenzyme NADH.

4.3 Factors affecting enzyme function

KEY IDEAS

In this topic, you will learn that:

- temperature, pH, enzyme concentration and substrate concentration all affect enzyme action in photosynthesis and cellular respiration
- inhibitors such as competitive inhibitors and non-competitive inhibitors slow and/or stop enzyme action.

There are many enzymes that catalyse reactions in both photosynthesis and cellular respiration. Like all proteins, enzymes have an optimal range of conditions in which they function. The following factors affect enzyme action:

- temperature
- pH
- enzyme concentration
- substrate concentration
- competitive and non-competitive inhibitors.

Temperature

At low temperatures, substrates and enzymes move slowly. This reduces the likelihood that enzymes and substrates will collide, thereby decreasing the rate of reaction. As the temperature increases, the molecules move faster, increasing the number of collisions between the enzyme and the substrate and in turn the rate of reaction.

Optimum temperature

All enzymes have an optimum temperature at which they operate. Once the temperature increases above the optimum temperature, the bonds that hold together the tertiary structure of the protein start to break. This changes the shape of the enzyme's active site (affecting the three-dimensional tertiary structure), preventing the substrate from binding and reducing the rate of the reaction. This process is called **denaturation** and is irreversible.

denaturation the destruction of a protein (including enzymes) caused by high temperature, pH changes and other factors

In photosynthesis, the optimum temperature needed by the photosynthesising enzymes will be different for each plant. For example, plants in desert regions have a higher optimum temperature range than plants in arctic regions. Below the optimum temperature, the movement of the enzyme and substrates will be slow and therefore the rate of reaction will be slow. Above the optimum temperature range, the enzymes will denature and the rate of reaction will cease and photosynthesis will not occur.

FIGURE 1 Greenhouse thermometer measuring temperature of plants so that they remain at the optimum temperature to maximise the rate of photosynthesis

In cellular respiration, maintaining the optimum temperature will allow the rate of reactions to stay high. This temperature will vary depending on the species. In humans, the ideal body temperature is around 37°C and is therefore the optimum temperature for cellular respiration in humans. Above this temperature (e.g. 41°C in humans) the enzymes will denature and cellular respiration will slow down. Below the optimum temperature, cellular respiration will occur too slowly to maintain life.

FIGURE 2 Humans increase their internal body temperature during exercise, and to recover they need to cool down so the enzymes involved in cellular respiration can work optimally.

pH

pH is a measure of hydrogen ion concentration in moles per litre. Acidic substances have a lot of free hydrogen ions available that can interfere with the hydrogen bonds, ionic bonds, and hydrophilic and hydrophobic bonds that hold together the tertiary structure of enzymes. Basic substances tend to attract hydrogen ions, potentially degrading the same bonds.

Optimum pH

All enzymes have an optimum pH at which they maximise the rate of reaction. If the conditions are too acidic or basic, the enzyme will be denatured, resulting in a reduction in the rate of the reaction.

In photosynthesis, ribulose-1, 5-biphosphate carboxylase oxygenase (or Rubisco) is one of the key enzymes involved in the light-dependent stage. This enzyme has an optimum pH of 8, where the rate of photosynthesis is at its highest. When the pH drops below a pH of 6, or above a pH of 10, Rubisco will begin to denature and photosynthesis will cease. Other than Rubisco, there are many other enzymes involved in photosynthesis that can be be affected by pH in a similar way.

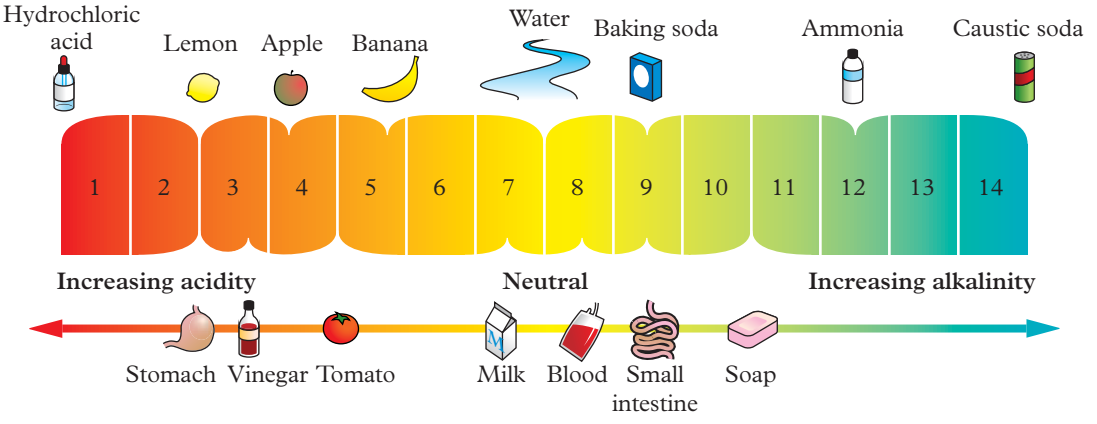


FIGURE 3 The pH scale has a wide range, but enzymes have sweet spots in which they work optimally.

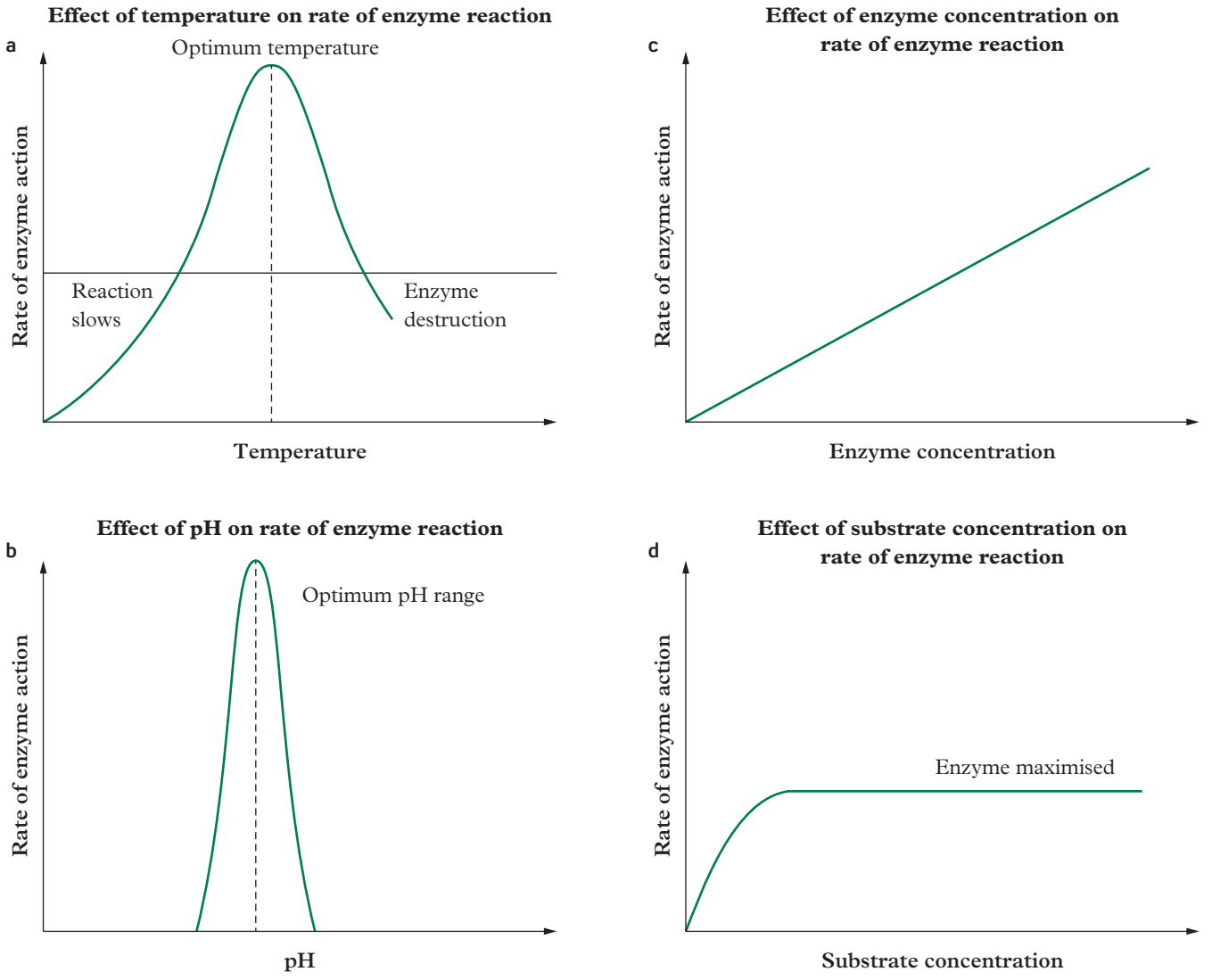


FIGURE 4 Enzyme activity is affected by **a** temperature, **b** pH, **c** enzyme concentration and **d** substrate concentration.

In cellular respiration, the optimum pH range will be different for various enzymes across different species. In humans, most of the body maintains a pH range of 7.35–7.45, so most enzymes involved in cellular respiration will have an optimum pH within this range.

Study tip

Learn the graphs in Figure 4 since you may be asked to interpret or draw a graph to represent a factor affecting the rate of an energy transformation reaction.

Enzyme concentration

Increasing the concentration of an enzyme means there are more enzyme molecules available to bond with the substrates. As the product leaves the active site, more substrate is available to bind and react. This increases the rate of the reaction, assuming there are no other limiting factors, such as substrate concentration. An increase in enzyme concentration with no other limiting factors will continually increase the rate of photosynthesis and cellular respiration.

Substrate concentration

As the concentration of substrate increases, more substrate molecules are available to bind to the enzyme. This increases the likelihood that an enzyme and substrate will collide, increasing the rate of the reaction. Eventually all the enzymes will have formed an enzyme–substrate complex, causing the rate of the reaction to level off or plateau. This occurs for both photosynthesis and cellular respiration, where an increase in substrate concentration increases the rate of the reaction until the amount of enzyme becomes a limiting factor. In photosynthesis, the key limiting substrates are carbon dioxide (CO_2) and water (H_2O), and in cellular respiration they are glucose ($C_6H_{12}O_6$) and oxygen (O_2).

Inhibitors

Inhibitors are molecules that bind to an enzyme and slow or even stop their action. Inhibitors act for both photosynthesis and cellular respiration to control the rate of the reaction. Many poisons, such as weedkillers, are enzyme inhibitors. There are two types of inhibitors: **competitive inhibitors** and **non-competitive inhibitors**.

competitive inhibitor
a substance that occupies the active site of an enzyme and prevents the normal substrate from binding

Competitive inhibitors

Competitive inhibitors compete with the substrate to bind to the active site. They may be a similar shape to the substrate and block the active site completely. This means the active site is not available for the substrate to bind, and can no longer catalyse that reaction. In some cases, they may only bind to part of the active site, also preventing the substrate from binding.

non-competitive inhibitor
a substance that occupies the allosteric site of an enzyme, changing the shape of the active site, preventing the normal substrate from binding

Non-competitive inhibitors

Non-competitive inhibitors do not bind to the active site, and instead bind to another location away from the active site. The site where inhibitors bind away from the active site is called an **allosteric site**. This binding causes the structure of the enzyme to change shape, which affects the shape of the active site. If the enzyme's active site is distorted, the substrate cannot bind, thus slowing the rate of reaction.

allosteric site
a site on an enzyme that allows an effector molecule to bind in a location away from the active site

FIGURE 5 Weedkiller contains an enzyme inhibitor that stops the weed enzymes from acting.

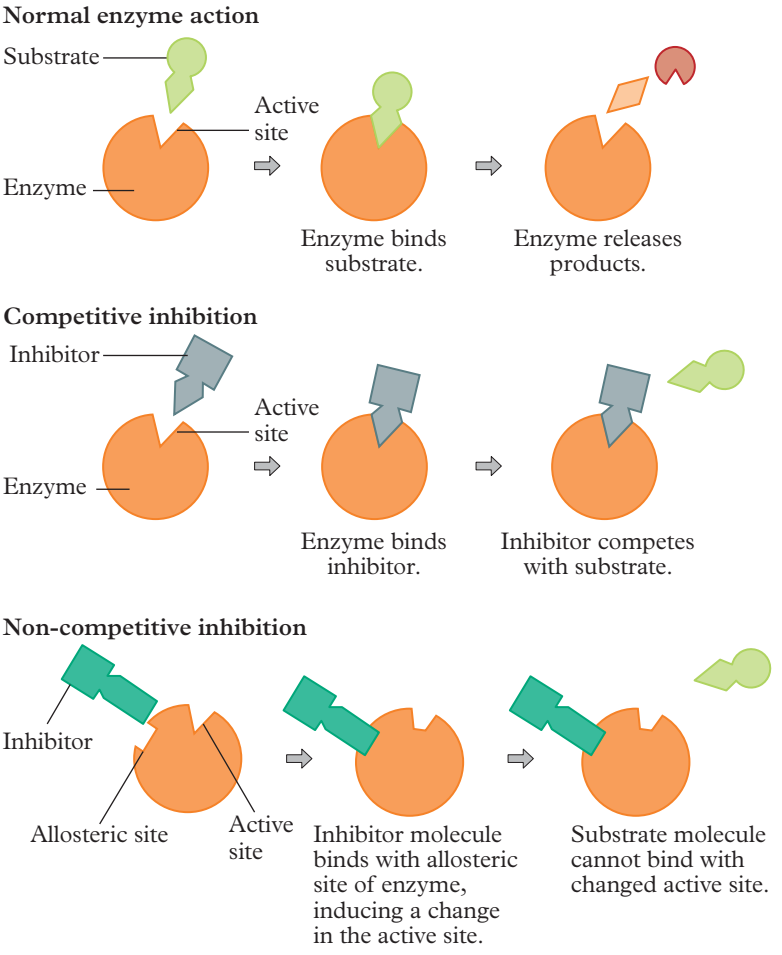


FIGURE 6 A comparison of normal enzyme action with competitive and non-competitive inhibition

CHECK YOUR LEARNING 4.3

Describe and explain

1 What factors affect enzyme function?
2 Why do some enzymes react poorly at low temperatures?
3 Define the term 'denaturation'.
4 How do competitive and non-competitive inhibitors affect enzyme action rates?

Apply, analyse and compare

5 **a** What would a graph of enzyme action look like if enzyme concentration was low and substrate concentration was high?
b If the concentration of enzyme was increased, how would this affect the graph of enzyme action?
6 Contrast the effects of temperature and pH on enzyme activity.

CHAPTER 4

Review

Chapter summary

4.1

- An enzyme can increase the rate of a reaction, including a metabolic reaction.
- Enzymes and coenzymes facilitate the steps in biochemical pathways such as photosynthesis and cellular respiration. An example of this is the cycling of ATP and ADP.
- Cycling of coenzymes is necessary in energy transformation reactions.

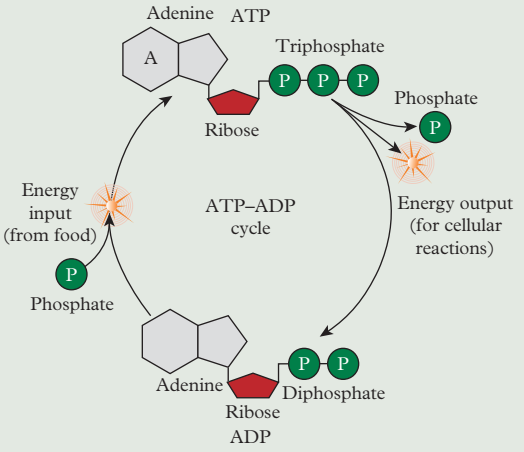


FIGURE 1 The process of ATP and ADP production

4.2

- Temperature, pH, enzyme concentration and substrate concentration all affect enzyme action.
- Enzymes work best when they have optimum conditions. If they fall below or above these conditions, they may slow down function or even stop.
- Inhibitors such as competitive inhibitors and non-competitive inhibitors also slow and stop enzyme action.

Key formulas

Simple enzyme reaction	$\text{Substrate} \xrightarrow{\text{Enzyme A}} \text{intermediate substance} \xrightarrow{\text{Enzyme B}} \text{product}$
Cycling of coenzyme ATP and ADP	$\text{ATP} \underset{\text{Enzyme}}{\rightleftharpoons} \text{ADP} + \sim\text{P} + \text{Energy}$

Revision questions

Multiple choice

1 Which of the following statements is true?

A Activation energy decreases with the addition of an enzyme.

B Activation energy decreases as temperature decreases.

C Activation energy is high in dilute solutions.

D Activation energy is used to describe the rate of reaction.

The graph in Figure 2 shows the energy changes during a reaction with and without an enzyme present. Use this graph to answer questions 2 and 3.

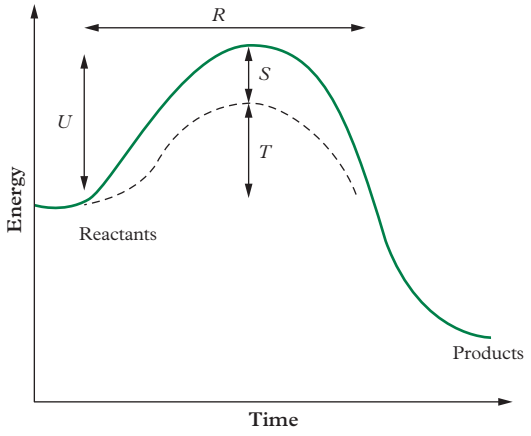


FIGURE 2 Energy changes during a reaction

2 The activation energy without an enzyme is represented by:

A R.

B S.

C T.

D U.

3 Select the correct letter for the following statement: 'There is a decrease in activation energy with an enzyme present compared to without an enzyme.'

A R

B S

C T

D U

4 A substrate is:

A a substance that catalyses a reaction.

B a substance that is catalysed by an enzyme.

C a substance that binds to an enzyme permanently.

D a substance that denatures enzymes.

5 Select the substrate for the enzyme in Figure 3.

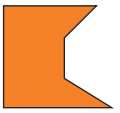


FIGURE 3 An enzyme

A

B

C

D

6 Urease breaks down urea, a waste product of protein metabolism, into carbon dioxide and ammonia. Nickel ions must be present for urease to work. In this example:

A urease is the enzyme, ammonia is the substrate, nickel ions are the cofactor.

B urease is the enzyme, urease is the substrate, nickel ions are the product.

C urease is the enzyme, urea is the substrate, nickel ions are the cofactor.

D urease is the enzyme, urea is the substrate, ammonia is the cofactor.

7 An organic, non-protein molecule needed for an enzyme to function is known as a:

A coenzyme.

B competitive inhibitor.

C phosphate group.

D chemical group.

8 What does Figure 4 most likely represent?

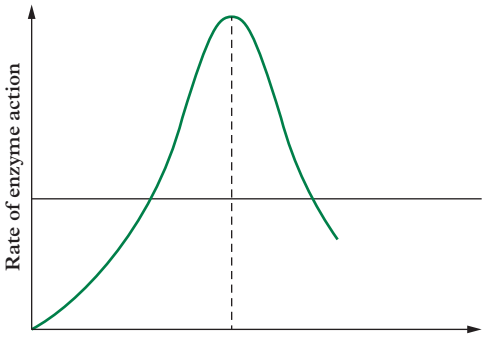


FIGURE 4 Rate of enzyme action

A Rate of enzyme action versus pH
B Rate of enzyme action versus temperature
C Rate of enzyme action versus substrate concentration
D Rate of enzyme action versus enzyme concentration

9 A non-competitive inhibitor binds to:
A the enzyme's active site.
B the substrate.
C the enzyme's allosteric site.
D part of the enzyme's active site.

10 Which factor would increase the rate of enzyme activity, assuming there are no other limiting factors?
A Temperature
B pH
C Enzyme concentration
D Coenzymes

11 What happens to an enzyme outside of its optimum pH range?
A If the enzyme is below its optimum pH range, then it is less efficient.
B At a high pH, the enzyme works more efficiently but the rate of enzyme action plateaus due to other limiting factors.
C When the pH is below the optimum range, the enzyme denatures and when the pH is above the optimum range the enzyme works more efficiently.
D When the pH is outside of the optimum range, the enzyme's tertiary structure degrades and the substrate can no longer bind.

12 Which of the following factors does not affect enzyme function?
A pH
B Temperature
C Substrate concentration
D Other enzymes

Short answer

Describe and explain

13 Enzymes are protein molecules with an active site.
a What is the active site of an enzyme?
b How does this site enable the enzyme to have specificity?

14 Explain why the coenzymes involved in photosynthesis and cellular respiration have both loaded and unloaded forms.

15 Draw an image to represent the cycling of NADH and NAD^+.
Note: You do not need to draw the chemical structures.

16 What is the difference between a cofactor and a coenzyme?

17 Describe the action of a competitive inhibitor.

18 Define the term 'allosteric site'.

19 Identify the initial products and final reactants of both cellular respiration and photosynthesis.

Apply, analyse and compare

20 Substrate concentration is a limiting factor for enzyme action, as seen in Figure 5.

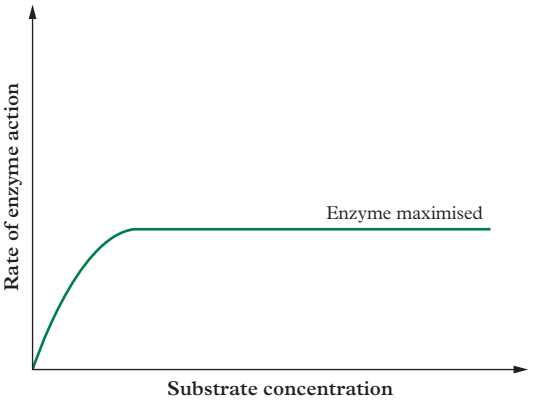


FIGURE 5 The substrate concentration of an enzyme in a reaction

a Explain why the rate of enzyme action initially increases as substrate concentration increases.

b The graph of substrate concentration versus rate of enzyme action plateaus at a certain point. Explain why.

21 A particular enzyme is found in a plant that can function at temperatures as low as 1°C and up to 42°C, with an optimum temperature of 27°C.

a Draw a graph to represent the enzyme's rate of action from temperatures of 0°C to 45°C.

b Referring to your graph, justify the enzyme's rate of reaction at:

i 0°C

ii 27°C

iii 40°C.

22 Describe what happens to the structure of an enzyme when it denatures.

23 Apply your knowledge of enzyme actions to explain why pH and temperature show peaks in their graphs and why substrate and enzyme concentration graphs do not. What do these peaks represent?

24 Figure 6 shows the activity of Enzyme A and Enzyme B.

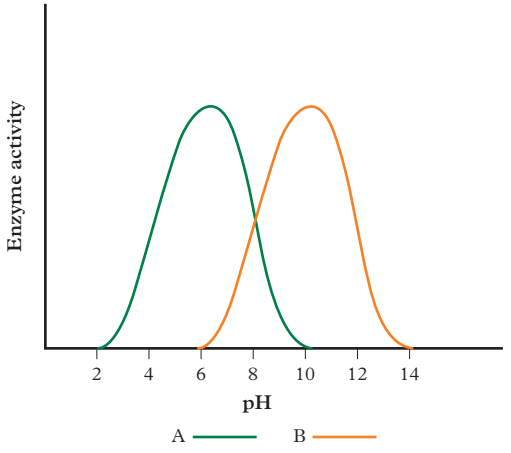


FIGURE 6 Enzyme A and Enzyme B

a Identify the optimum pH for Enzyme A.

b Identify the optimum pH for Enzyme B.

c Explain which enzyme would have higher enzyme activity at pH 12.

Design and discuss

25 Consider Case study 4.2 on page 112. Discuss what would happen to the bioluminescence at the Gippsland Lakes if the temperature of the water was to decrease.

26 Design an experiment to test the optimum temperature range of photosynthetic enzymes in a mung bean plant. Identify the following:

- Independent variable
- Dependent variable
- Controlled variables
- Sample size
- Expected results.

FIGURE 7 The mung bean

Exam essentials

Responding to questions

In your exam, you may be expected to design an experiment. Almost all past VCAA Biology examinations include a question relating to experimental method or the design of an experiment. It is a good reminder that key science skills, found in Topic 1.2 of Chapter 1, are examinable.

Describe the variables when designing experiments

For experiment design questions, you will most likely have to explicitly describe the independent and dependent variables.

The following question is taken from the 2016 VCE Biology Examination. Read the question carefully, then consider whether the responses have directly described the variables.

QUESTION 2c (2016 Biology Written Examination)

Plant materials containing cellulose and other polysaccharides are reacted with acids to break them down to produce glucose. This glucose is then used by yeast cells for fermentation.

A by-product of the acid treatment of plant materials is a group of chemical compounds called furans. It has been observed that as the concentration of furans increases, the rate of fermentation decreases. The enzyme alcohol dehydrogenase is required for the process of fermentation.

c Design an experiment to test the hypothesis that one of the furans, called furfural, is an inhibitor of the enzyme alcohol dehydrogenase. Assume that the experiment will be repeated many times and that environmental factors are kept constant. 4 marks

Source: 2016 Biology Written Examination Question 2c, Short answer, reproduced by permission © VCAA

Response 1

- Independent variable would be the presence and absence of furfural.
- Set up four conical flasks with the same amount of glucose and alcohol dehydrogenase in each. These are both controlled variables.
- In the first flask add 1 mL of furfural, in the second flask add 2 mL, in the third flask add 3 mL, and in the fourth flask include no furfural. The fourth flask is the control.
- The dependent variable would be to measure the amount of carbon dioxide produced after a set amount of time.

Clearly states the independent variable.

Clearly states the dependent variable.

This response is set out logically and each of the variables is clearly described. It is easy to follow the experimental design and it includes the key controlled variables. The use of dot points is a suitable format for answering these types of questions and it also helps to lay out the question according to the marking scheme (i.e. four dot points for 4 marks).

Response 2

This experiment is testing the effect of furfural on the rate of fermentation. Set up two flasks, one with furfural and one without. Add glucose and alcohol dehydrogenase to these flasks and record the amount of carbon dioxide produced.

The aim is not necessary for this response, unless the question specifically asks for one.

The student needed to state the independent variable rather than just describe it.

Again, the student needed to state the dependent variable rather than just describe it.

This response is too brief and does not state all the variables. Although the description of the independent variable is included, it is not clear that this is what the student was describing. There is also no reference to controlled variables.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

Question 8a (2018 Biology Written Examination)

Methotrexate is a drug used in the treatment of some cancers and an autoimmune disease, psoriasis, which affects the skin and sometimes the joints. In both of these diseases, cells grow rapidly. Methotrexate is structurally very similar to folic acid. Methotrexate works by inhibiting an enzyme that catalyses the change of an inactive form of folic acid into an active form. The active form of folic acid is needed for DNA production.

a Based on the information given, explain how methotrexate could be acting as a competitive inhibitor of the enzyme. 2 marks

Methotrexate would bind to the enzyme to inhibit the binding of the inactive form of folic acid.

Source: 2018 Biology Written Examination Question 8a, Short answer, reproduced by permission © VCAA

Marking guide

Question 8 a	- 1 mark for identifying that methotrexate would have a similar structure to folic acid and therefore may attach to the active site of the enzyme. - 1 mark for identifying that methotrexate binding to the active site of the enzyme would reduce the binding of the inactive form of folic acid.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

Check your Student obook pro for these digital resources and more: pro

Video tutorial
Describe the variables when designing experiments

Weblink
Past examinations and examiners' reports

CHAPTER 5

Photosynthesis

All plants carry out photosynthesis by converting light energy to chemical energy. Photosynthesis occurs in the chloroplasts of plant cells in two general reactions. The first is known as the light-dependent reaction, which uses light energy to split water into hydrogen ions and oxygen gas. The hydrogen ions are then combined with carbon dioxide in the second reaction, which is known as the light-independent reaction. In the second step, glucose is produced and used as an energy source for cellular respiration and other metabolic reactions.

Rubisco is an important enzyme necessary for the Calvin cycle of the light-independent stage. It is involved in carbon fixation, incorporating the carbon from carbon dioxide into an organic molecule. C_3, C_4 and CAM plants are classified according to their different photosynthetic pathways. These different pathways have provided plants with adaptations for particular environmental conditions in order to maximise the efficiency of photosynthesis.

The rate of photosynthesis is affected by several factors including temperature, light availability and intensity, water availability and carbon dioxide concentration.

KEY KNOWLEDGE

- → 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)
- → the role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis
- → the factors that affect the rate of photosynthesis: light availability, water availability, temperature and carbon dioxide concentration

FIGURE 1 Leaves are a major site for photosynthesis since they absorb sunlight with the help of chlorophyll found in chloroplasts and also take in carbon dioxide (a main reactant in photosynthesis).

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

5A What structural features are present only in plant cells?

5B How does water move through a plant from the roots to the leaves?

5A Groundwork resource
Plant cell

5B Groundwork resource
Water transport in plants

PRACTICALS

PRACTICAL	**5.1** Testing for photosynthesis using alginate balls with *Chlorella*

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

5.1 Photosynthesis

KEY IDEAS

In this topic, you will learn that:

- photosynthesis is a biochemical pathway that occurs in chloroplasts
- photosynthesis has inputs and outputs at each stage.

Video
The process of photosynthesis

Heterotrophs obtain glucose for cellular respiration from the food they eat. Autotrophs such as plants need to produce that glucose, and they do this through a process called photosynthesis. During photosynthesis, light energy is used to convert carbon dioxide and water to glucose and oxygen. Besides cellular respiration, many other metabolic pathways use these products.

Photosynthesis is often represented by the following **simplified equation**:

$$6CO_2(g) + 6H_2O(l) \xrightarrow[\text{Chlorophyll + enzymes}]{\text{Sunlight}} C_6H_{12}O_6(s) + 6O_2(g)$$

chlorophyll
a pigment in photosynthetic organisms that facilitates photosynthesis

chloroplast
a small photosynthetic organelle in green plants, containing chlorophyll in membranous grana and enzymes in liquid stroma

stroma
the fluid matrix of the chloroplast, containing enzymes for the light-independent reaction of photosynthesis

thylakoid
the membranous, flattened, disc-shaped sac in chloroplasts on which the light-dependent reaction of photosynthesis occurs

granum
stacked portions of membranes in chloroplasts; site of the light-dependent reaction for photosynthesis

As with cellular respiration, photosynthesis is more complicated than this simple equation would suggest. Photosynthetic organisms possess a pigment called **chlorophyll** that, along with energy from the Sun, catalyses the reaction. In eukaryotes, the process of photosynthesis occurs in the **chloroplast**.

Chloroplasts

The chloroplast is a double-membraned organelle. This means there are two separate layers of the phospholipid bilayers that make up the membrane. The fluid that is enclosed by these membranes is called the **stroma**. Small flattened disc-shaped membrane sacs (**thylakoids**) lie one on top of another like a stack of coins. Each stack is called a **granum** (plural grana). Grana are usually connected to each other. The pigment chlorophyll is found within the thylakoid sacs.

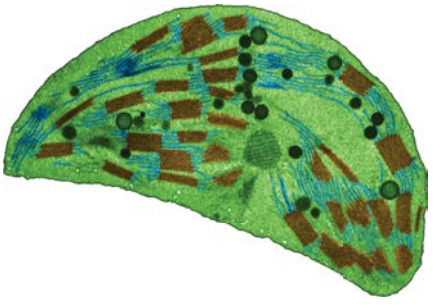


FIGURE 1 A colour-enhanced transmission electron micrograph of a chloroplast

Photosynthesis

Photosynthesis involves processes that take place in many small steps, each of which is controlled by a specific enzyme. This can be shown in an **improved version of the photosynthesis equation**:

$$6CO_2(g) + 12H_2O(l) \xrightarrow[\text{Chlorophyll + enzymes}]{\text{Sunlight}} C_6H_{12}O_6(s) + 6O_2(g) + 6H_2O(l)$$

This reaction has water on both sides of the equation. This is due to twelve water molecules being needed to start the reaction, and six water molecules being produced at the

end of the photosynthesis pathway. As a result, there is a net six water molecules used to produce each glucose molecule.

Photosynthesis occurs in two distinct steps, the **light-dependent reaction** and the **light-independent reaction**.

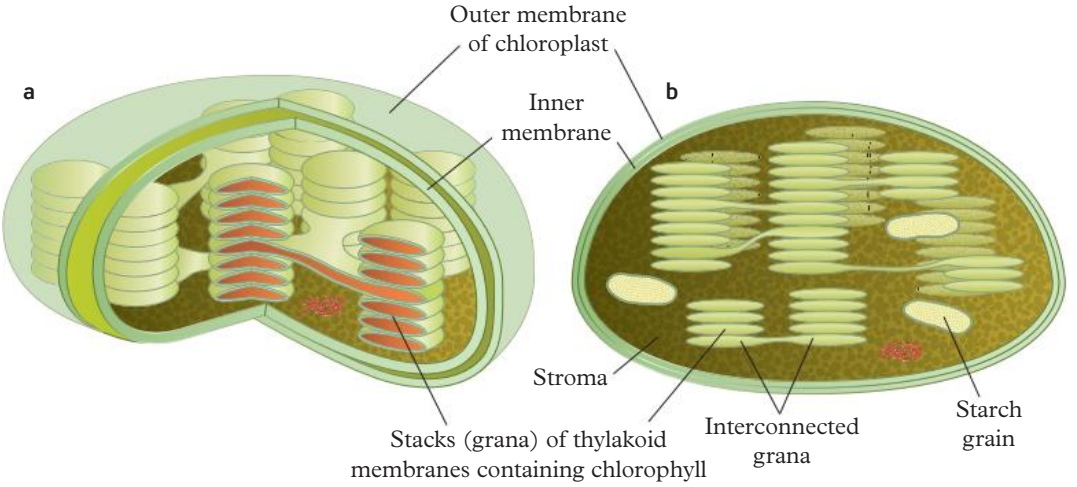


FIGURE 2 The internal structure of a chloroplast: **a** cut-away view; **b** cross-section

light-dependent reaction
a chemical reaction in the chloroplast involving the splitting of water in the presence of sunlight

light-independent reaction
the second stage of photosynthesis, in which carbon dioxide is reduced to form glucose; does do not need the presence of light

Study tip

Spelling can be important in the exam. Remember that *stroma* is a fluid-filled space in the chloroplast while the *stoma* (no 'r') are the small holes in a leaf where oxygen moves in and out.

The light-dependent reaction

The first step of photosynthesis involves the light-dependent reaction. This takes place within the thylakoid sacs of the chloroplast. In this process:

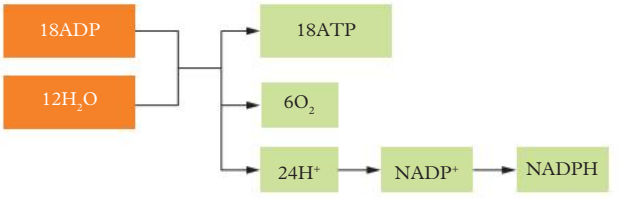


FIGURE 3 Light-dependent reaction

- The chlorophyll pigments absorb light energy, predominantly from the blue and red bands of the visible light spectrum.
- Light energy is used:
  - to split 12 water molecules ($12H_2O$) into 24 hydrogen ions ($24H^+$) and 6 oxygen molecules ($6O_2$)
  - in the formation of 18 ATP molecules.
- The hydrogen ions are taken up by $NADP^+$ (a coenzyme) and the oxygen is released from the plant as a waste product.

TABLE 1 Location, inputs and outputs of the light-dependent stage of photosynthesis

Process	Location	Inputs	Outputs
Light-dependent reaction	Thylakoid membrane of chloroplast	$12H_2O$ 18ADP $24NADP^+$	$6O_2$ 18ATP 24NADPH

Study tip

NADH (nicotinamide adenine dinucleotide) is used in cellular respiration and NADPH (nicotinamide adenine dinucleotide phosphate) is used in photosynthesis. It's important to recognise the difference between the two, but remember that NADH and NADPH are both found in plant and animal cells.

The light-independent reaction (Calvin cycle)

The second step is called the light-independent reaction because it does not require light energy. The light-independent reaction occurs in the stroma of the chloroplast. In this process:

- The hydrogen (carried by NADPH from the light-dependent reaction) combines with carbon dioxide.
- ADP is formed as all the ATP from the light-dependent reaction (and more) are used.
- Glucose ($C_6H_{12}O_6$) and six water molecules ($6H_2O$) are formed.

Calvin cycle
the cyclic reactions of the light-independent phase of photosynthesis

Study tip

In this chapter, you are learning about photosynthesis in plants, which occurs in the chloroplasts. However, prokaryotes (bacteria) do not contain organelles. Photosynthesis can still occur in the cytoplasm.

This complex series of reactions is also known as the **Calvin cycle,** or the Calvin-Benson cycle. Plants obtain carbon dioxide (CO_2) via open stomata in the leaves and this carbon dioxide is used in the first stage of the Calvin cycle. The first stage is known as carbon fixation, where the carbon from CO_2 is fixed into an intermediate organic molecule. NADPH and ATP are then used to convert this organic molecule in a complex biochemical pathway in order to produce the final products of glucose and water.

TABLE 2 Location, inputs and outputs of the light-independent stage of photosynthesis

Process	Location	Inputs	Outputs
Light-independent reaction	Stroma of chloroplast	$6CO_2$ >18ATP 24NADPH	$C_6H_{12}O_6$ $6H_2O$ >18ADP $24NADP^+$

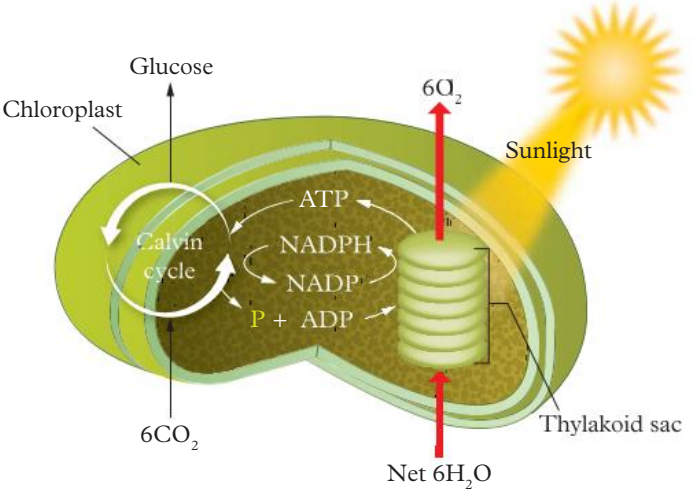


FIGURE 4 The overall process of photosynthesis

CHECK YOUR LEARNING 5.1

Describe and explain

1 What is the purpose of photosynthesis?
2 Which organelle in a plant cell is responsible for photosynthesis?
3 Photosynthesis takes place in two distinct steps: the light-dependent and light-independent reactions. Where in the chloroplast does each step occur?
4 What is the role of chlorophyll in the light-dependent reaction?

Apply, analyse and compare

5 Which coenzymes are necessary for photosynthesis and what is their role in the light-dependent stage of photosynthesis?
6 Use Table 2 to write a chemical equation to represent the light-independent reaction of photosynthesis.

Design and discuss

7 Draw a diagram to illustrate how the light-dependent and light-independent reactions are linked together.

5.2 Rubisco and plant adaptations

KEY IDEAS

In this topic, you will learn that:

- Rubisco is an enzyme required for photosynthesis
- C_3, C_4 and CAM plants are classified according to their different photosynthetic pathways and are adapted for different environmental conditions.

Rubisco
the key enzyme needed for carbon fixation in the Calvin cycle

Rubisco (RuBP oxygenase-carboxylase) is a key enzyme in photosynthesis. It is responsible for fixing the gaseous carbon dioxide molecule to ribulose bisphosphate (RuBP) to form a molecule with three carbon atoms (3-phosphoglyceric acid). This is the first stage of the Calvin cycle, in the light-dependent stage of photosynthesis. It is also the beginning of the inert carbon dioxide becoming a carbon-based organic molecule. Optimum conditions for Rubisco are high carbon dioxide levels and low environmental temperature.

Rubisco has two substrates

Like all enzymes, Rubisco has an active site that can link carbon dioxide to RuBP. This active site is also able to bind oxygen as a substrate instead of carbon dioxide. When oxygen binds to the RuBP, it prevents the Calvin cycle and ultimately prevents the formation of glucose. Without glucose, the plant will not be able to store and use the energy in the high-energy molecule. This process of Rubisco using oxygen instead of carbon dioxide is called **photorespiration**.

photorespiration
the wasteful pathway where Rubisco incorporates oxygen with RuBP, reducing photosynthesis efficiency

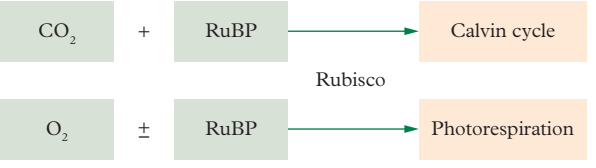


FIGURE 1 Rubisco can act on CO_2 or O_2, which brings about different reactions in the cell.

Study tip

Many of the organic molecules in the Calvin cycle are complex. Scientists often refer to the number of carbon atoms to describe their complexity.

When a plant's stomata are open, CO_2 diffuses into the leaves and O_2 diffuses out. When this occurs, the concentration of CO_2 in the leaves increases, and the concentration of O_2 decreases. This favours Rubisco acting on CO_2 to initiate the Calvin cycle. However, when plants close their stomata – for example, to reduce water loss via evaporation – the concentration of O_2 can build up in the leaves. When this occurs, the rate of photorespiration increases and the rate of photosynthesis decreases.

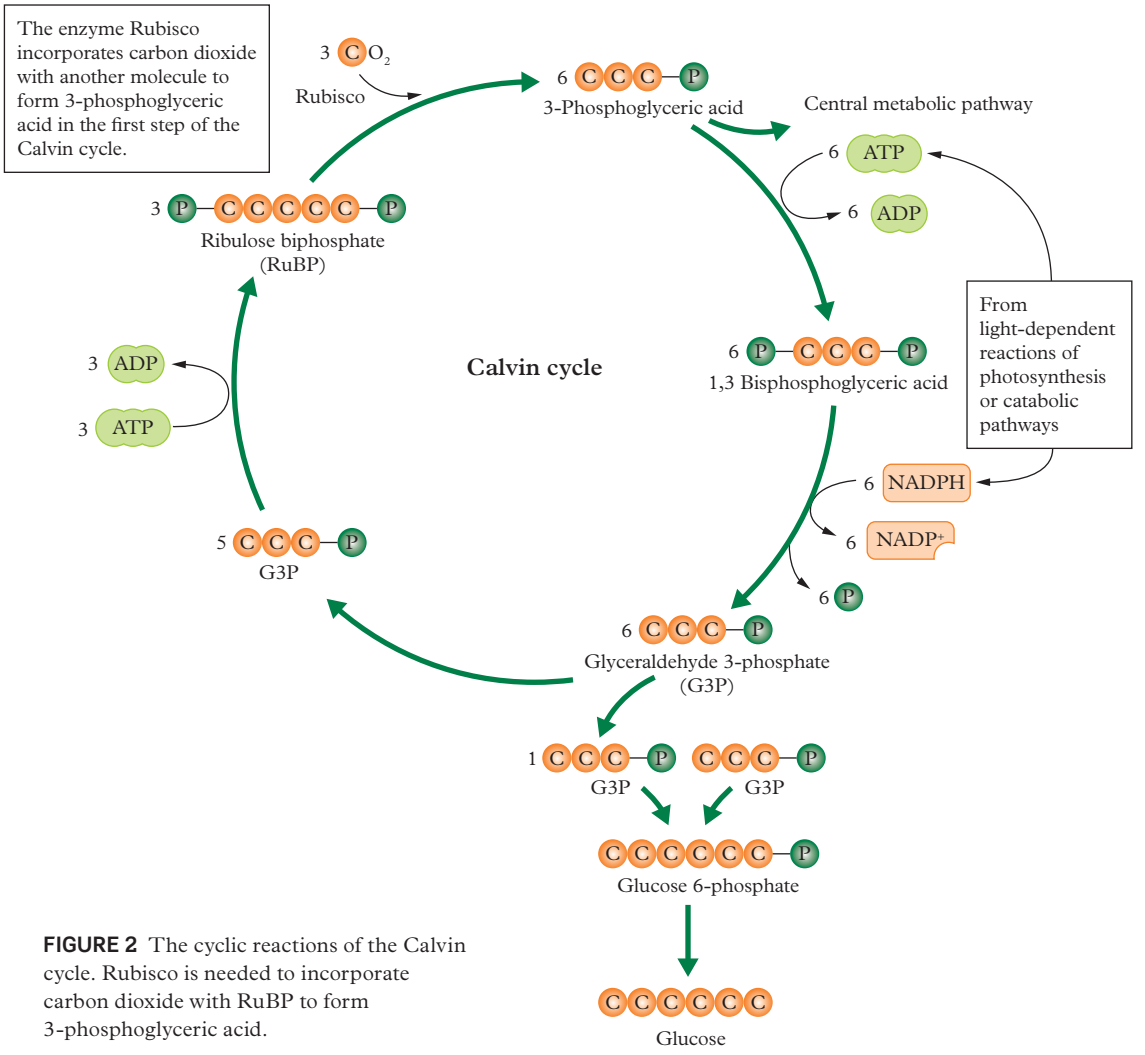


FIGURE 2 The cyclic reactions of the Calvin cycle. Rubisco is needed to incorporate carbon dioxide with RuBP to form 3-phosphoglyceric acid.

Adaptations of C_3, C_4 and CAM plants

In dry or hot conditions, plants require adaptations to minimise Rubisco acting on O_2. There are three types of plants, classified according to their photosynthetic pathways: C_3 plants, C_4 plants and CAM plants. C_3 plants are best in wet and cool conditions when stomata are able to open and let oxygen and carbon dioxide move through freely. In hot conditions, the stomata may need to close to prevent water loss. C_4 plants have a biochemical adaptation that allows them to survive in hot conditions with a lot of sunlight, while CAM plants have adapted to survive in dry and hot conditions.

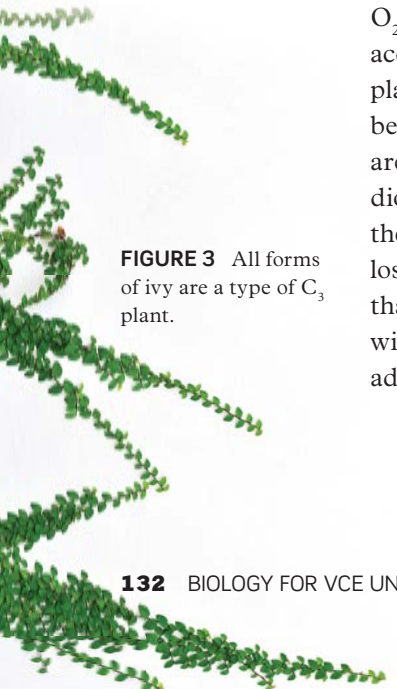


FIGURE 3 All forms of ivy are a type of C_3 plant.

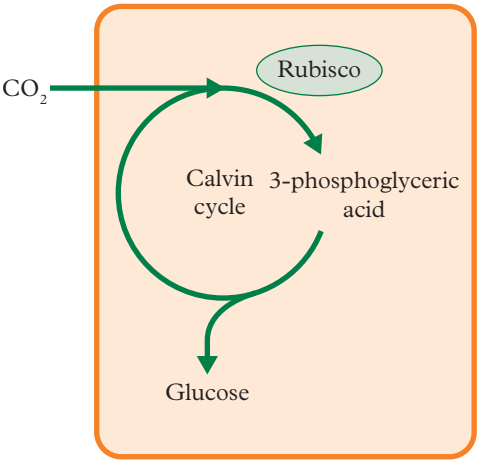


FIGURE 4 C_3 plants use Rubisco for carbon fixation in the first stage of the Calvin cycle.

C_3 plants

In **C_3 plants**, the first step of the Calvin cycle is the fixation of carbon dioxide into a 3-carbon organic molecule that is catalysed by Rubisco. They are the most common type of plant, making up approximately 85% of all plant species. C_3 plants do not need an alternative pathway to catalyse the binding of carbon dioxide to Rubisco instead of oxygen. The Calvin cycle in these plants occurs in the mesophyll cells of the leaves.

C_3 plants are best adapted for wet and cool conditions. These conditions maximise the opening of the stomata to increase CO_2 concentration in the leaves, and a lower temperature limits Rubisco binding with O_2.

C_3 plant
a plant that uses the carbon fixation pathway as the only mechanism to convert carbon dioxide into an organic molecule, 3-phosphoglyceric acid

C_4 plants

About 3% of land plants are known as **C_4 plants** because they use the C_4 pathway for photosynthesis. The light-dependent and light-independent stages of photosynthesis are physically separated since they occur in different cells of C_4 plants. The light-dependent stage still occurs in the mesophyll cells of the leaf; however, the Calvin cycle occurs in cells that surround the vein of the leaf, known as bundle-sheath cells.

These plants use a different enzyme in the mesophyll cells, known as **PEP carboxylase**, which fixes CO_2 into a 4-carbon compound (oxaloacetate). PEP carboxylase doesn't bind with O_2 like Rubisco, so C_4 plants have a higher rate of photosynthesis. The oxaloacetate converts into another intermediate carbon compound (malate) and then back into CO_2. The CO_2 is pumped into the bundle-sheath cells, using energy, which increases the concentration of available CO_2, enabling Rubisco to initiate the Calvin cycle. It costs more energy (ATP) than the C_3 photosynthetic pathway, but minimises the photorespiration pathway and this outweighs the cost in energy. This process is shown in Figure 5.

This adaptation enables C_4 plants to withstand higher temperatures, and therefore C_4 plants are more common in hotter climates. They thrive on long-lasting hot seasons with a lot of access to sunlight.

C_4 plant
a plant that has another pathway in a different cell, before the Calvin cycle initiates, to maximise the efficiency of photosynthesis

PEP carboxylase
an enzyme found in C_4 and CAM plants that incorporates carbon from carbon dioxide into oxaloacetate

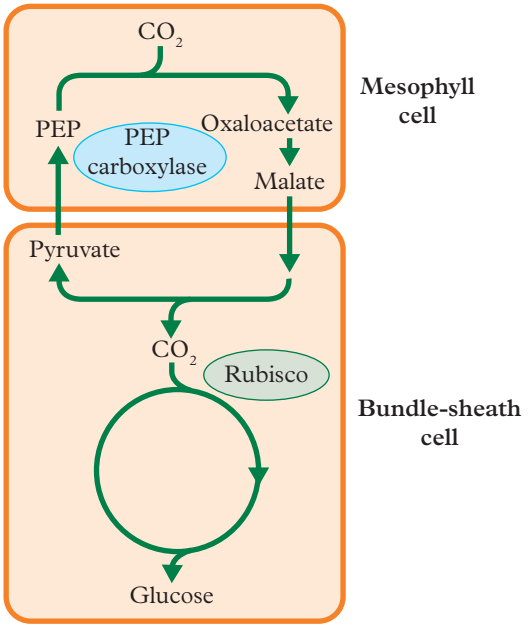


FIGURE 5 C_4 plants have two cells involved in photosynthesis – mesophyll cells and bundle-sheath cells – which increase the efficiency of photosynthesis.

FIGURE 6 *Pennisetum setaceum* is a C_4 plant that is native to open grasslands of East Africa, Asia and the Middle East.

CAM plants

CAM plant
a plant that has another pathway in the dark, before the Calvin cycle initiates during daylight hours, to maximise the efficiency of photosynthesis

Instead of physical separation, crassulacean acid metabolism plants (**CAM plants**) separate the stages of photosynthesis by time. CAM plants open their stomata at night, allowing CO_2 to enter the leaves and fix into oxaloacetate by PEP carboxylase. This is the same process as with C_4 plants. Oxaloacetate is converted into malate and is stored in vacuoles until daylight hours. When exposed to sunlight, CAM plants release the malate from the vacuoles and convert it back into CO_2 before entering the Calvin cycle. This release of CO_2 during the day means there is a high concentration of CO_2 to bind to the Rubisco in the leaves.

The CAM pathway, like the C_4 pathway, requires more energy than the C_3 photosynthetic pathway. However, these plants minimise the photorespiration pathway and are adapted for dry conditions since their stomata are only open at night. This ultimately reduces water loss while maximising the availability of CO_2 in the leaves.

CAM plants include pineapples, cacti, most succulents and orchids, among other plants. They are adapted for hot and dry conditions.

FIGURE 7 The pineapple plant is a CAM plant that is adapted for dry conditions.

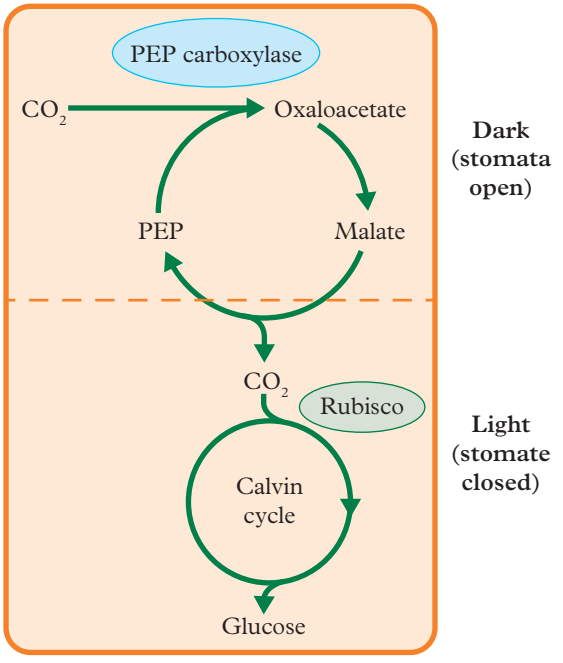


FIGURE 8 The CAM pathway occurs in two stages, separated by time.

CHECK YOUR LEARNING 5.2

Describe and explain

1 What is the role of Rubisco in carbon fixation?
2 Explain how oxygen reduces the rate of photosynthesis.
3 When a plant's stomata are open, how does the concentration of carbon dioxide increase in the leaf and the concentration of oxygen decrease?

Apply, analyse and compare

4 Compare the different photosynthetic pathways of C_4 and CAM plants.
5 Analyse why C_3 plants are most efficient in wet and cool conditions.

Design and discuss

6 Due to climate change, increasing global temperatures and a rise in atmospheric carbon dioxide, which plant type will have a survival advantage? Justify your choice by referring to all three plant types.

5.3 Factors that affect the rate of photosynthesis

KEY KNOWLEDGE

In this topic, you will learn that:

+ Factors that affect the rate of photosynthesis include light availability and intensity, water availability, temperature and carbon dioxide concentration.

There are many factors that can affect the rate of photosynthesis. As discussed in Chapter 4, the rate of photosynthesis can be affected by factors that affect enzyme action.

The key factors affecting the rate of photosynthesis include the following:

- light availability
- water availability
- temperature
- carbon dioxide concentration

Light availability

One of the most important factors controlling the rate of photosynthesis is the availability and intensity of light. The amount or intensity of light can affect the light-dependent reaction. If less light energy is available, then chlorophyll will not have enough energy to split water into hydrogen and oxygen.

The rate of photosynthesis increases with increasing light intensity as long as there is enough chlorophyll to capture the light. Once all the chlorophyll is working at a maximum rate, the rate of photosynthesis will stabilise or plateau (Figure 1).

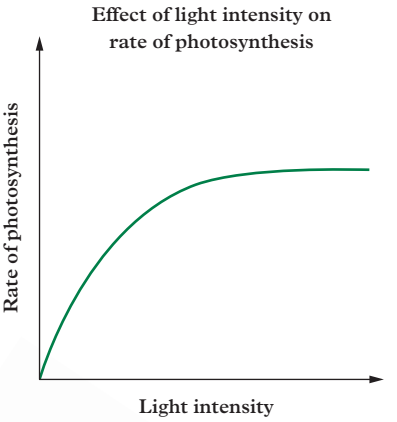


FIGURE 1 The effect of light intensity on the rate of photosynthesis

Water availability

The availability of water can also limit the rate of photosynthesis. Water is a reactant in the light-dependent reaction and splits into hydrogen ions and oxygen gas. If water is not available, then the light-dependent reaction will stop and photosynthesis will not occur.

Desert plants have difficulty producing glucose through photosynthesis due to the lack of water in their environment. This means the plant may not have access to the chemical energy that glucose provides. As a result, plants grow more slowly or die.

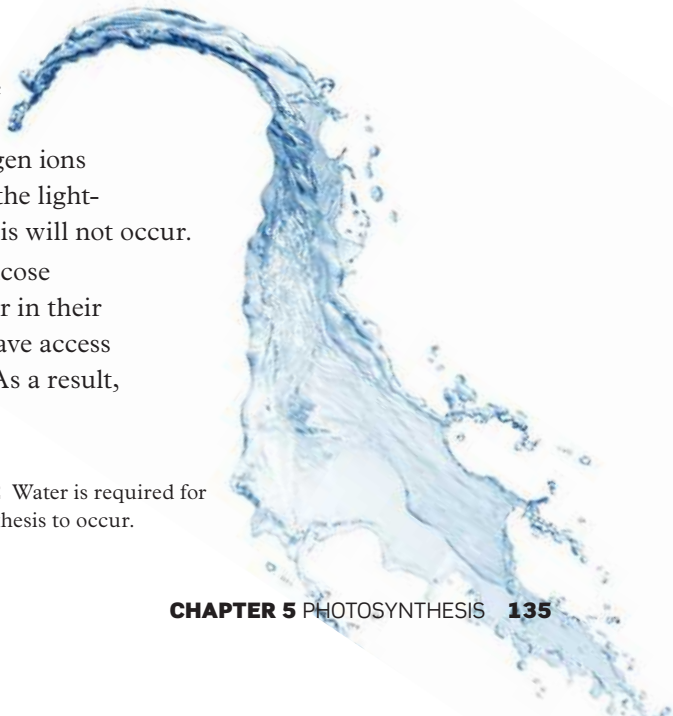


FIGURE 2 Water is required for photosynthesis to occur.

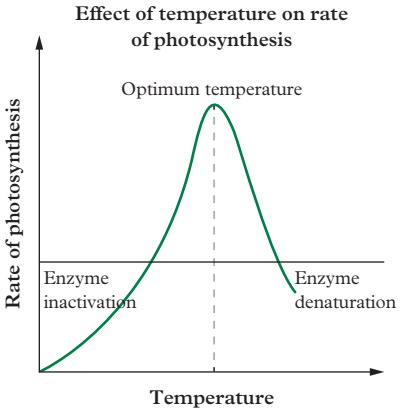


FIGURE 3 The effect of temperature on the rate of photosynthesis

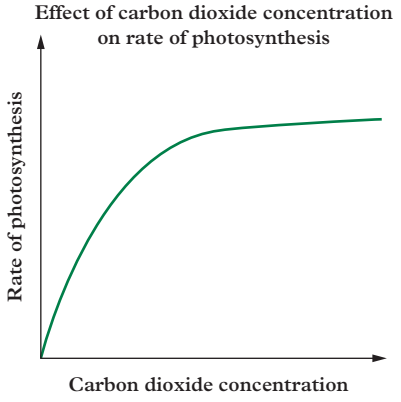


FIGURE 4 The effect of carbon dioxide concentration on the rate of photosynthesis

Temperature

As discussed in Chapter 4, an optimum temperature is required for photosynthesis to occur, since enzymes are temperature sensitive. At low temperatures, the energy of the molecules is low and the collisions between enzymes and substrates are decreased. As the temperature increases, the action of the enzymes also increases. At the optimum temperature, the rate of photosynthesis is also at an optimum. Above the optimum temperature, enzymes begin to denature and the rate of photosynthesis will decrease and eventually stop.

Carbon dioxide concentration

Carbon dioxide is a gas that is readily available in all environments. The concentration of carbon dioxide can vary in different environments and this affects the rate of photosynthesis. Like water, carbon dioxide is also a substrate in the light-independent reaction. This means it is important in the formation of high-energy glucose. When the amount of carbon dioxide is limited, the rate of photosynthesis decreases. As the concentration of carbon dioxide increases, the enzymes in the Calvin cycle can increase the rate of reaction. When these enzymes are all maximised, the rate of photosynthesis is also maximised. This causes the plateau seen in Figure 4.

CASE STUDY 5.3

CO_2 fertilisation effect

With increasing carbon emissions across the globe, the levels of carbon dioxide (CO_2) in the atmosphere have increased, driving climate change.

Claims have been made about the benefits of rising CO_2 levels, such as increased CO_2 concentrations benefitting plants as rates of photosynthesis increase. With increased photosynthesis, plants will have increased growth and thus greater volume of food production. This is known as the CO_2 fertilisation effect. There is an element of truth to this concept, although there are many other factors affecting the rate of photosynthesis.

FIGURE 5 Crops that have been grown with increased CO_2 could have decreased nutrient levels.

Climate change brings about other effects that reduce plant growth, such as drought (limited water availability) and heat stress (denaturing enzymes), which have negative consequences for the rate of photosynthesis.

Increasing CO_2 levels could also have an effect on human health. Researchers at Harvard University found that if crops are exposed to elevated CO_2 levels, they lose significant amounts of iron and zinc, and grains lose protein. The loss of key nutrients from food crops could cause nutrient deficiencies and other issues for consumers.

CHALLENGE 5.3

Deep sea photosynthesis

The ocean is a major source of photosynthetic life on Earth. Algae is the dominant plant life that supports the process of photosynthesis.

1 Using your knowledge of factors that affect the rate of photosynthesis and Figure 6, discuss what you think will happen to the rate of photosynthesis as you go deeper into the ocean.

2 As you dive deeper into the ocean, temperature starts to decrease and dissolved carbon dioxide starts to increase. Consider how this additional information would change your answer to Question 1.

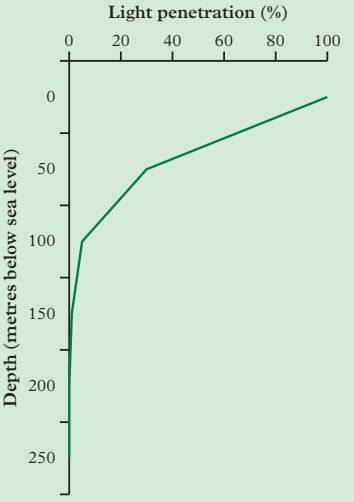


FIGURE 6 Light penetration (%) with depth below sea level

CHECK YOUR LEARNING 5.3

Describe and explain

1 Explain how temperature affects the rate of photosynthesis.

2 Light is often described as the most important factor controlling the rate of photosynthesis. Explain why.

Apply, analyse and compare

3 Referring to Case study 5.3, why are increased CO_2 levels not necessarily going to be beneficial to food crops?

4 Despite high light intensity, many desert plants have a limited rate of photosynthesis. Explain why.

5 The graph in Figure 7 shows rate of photosynthesis versus carbon dioxide concentration. Use the graph to answer the following questions.

a Analyse what is occurring at points A, B and C.

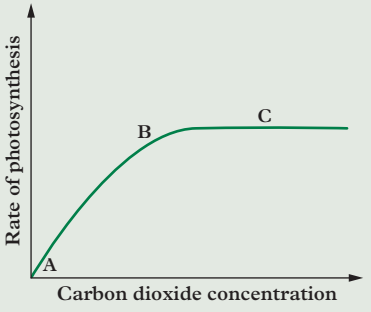


FIGURE 7 Rate of photosynthesis versus carbon dioxide concentration

b The graph plateaus at point C. What is likely to be the limiting factor at this point?

Design and discuss

6 The following data represent the rate of photosynthesis in a plant at different temperatures.

Temperature (°C)	Rate of photosynthesis
0	0.2
5	2
7	4
10	10
13	15
17	20
20	26
23	37
28	45
31	25
34	15

a Produce a graph to demonstrate the data. On the graph, predict the curve that will occur beyond 34°C.

b Use the structure of substrates and enzymes to explain what is occurring:

- below 10°C
- at 28°C
- above 34°C.

CHAPTER 5

Review

Chapter summary

5.1

- Photosynthesis involves the breakdown of water and carbon dioxide to produce oxygen and glucose, which occurs in the chloroplast in eukaryotes.
- The light-dependent reaction breaks apart the water molecule and converts ADP to ATP.
- The light-independent reaction requires no sunlight, combining hydrogen and carbon dioxide to produce glucose.

5.2

- Rubisco is an important enzyme in the Calvin cycle of photosynthesis.
- C_3 (approximately 85% of plants), C_4 (approximately 3% of plants) and CAM (approximately 7% of plants) plants are grouped according to their different photosynthetic pathways and are adapted for different environmental conditions.
- C_3 plants are not adapted to prevent photorespiration.
- C_4 plants reduce photorespiration by physically separating CO_2 fixation and the Calvin cycle (occuring in different cells).
- CAM plants reduce photorespiration by separating the light-dependent and light-independent stages by time (between night and day). Their stomata are only open at night, also reducing water loss.

5.3

- Factors that affect the rate of photosynthesis include light availability and intensity, water availability, temperature and carbon dioxide concentration.

Key formulas

Photosynthesis (simplified)	$6CO_2(g) + 6H_2O(l) \xrightarrow[\text{Chlorophyll + enzymes}]{\text{Sunlight}} C_6H_{12}O_6(s) + 6O_2(g)$
Photosynthesis (improved)	$6CO_2(g) + 12H_2O(l) \xrightarrow[\text{Chlorophyll + enzymes}]{\text{Sunlight}} C_6H_{12}O_6(s) + 6O_2(g) + 6H_2O(l)$

Revision questions

Multiple choice

1 The first stage of photosynthesis occurs in membranous sacs of the chloroplast called:
- **A** grana.
- **B** thylakoid.
- **C** stroma.
- **D** stoma.

2 Colours of light most suitable for photosynthesis are:
- **A** green and blue.
- **B** red and blue.
- **C** green and red.
- **D** violet and yellow.

3 During which stage of photosynthesis is oxygen gas produced?
- **A** Light-independent reaction
- **B** Calvin cycle
- **C** Light-dependent reaction
- **D** Krebs cycle

4 The chlorophyll pigment that absorbs light for photosynthesis in eukaryotes is located:
- **A** within the thylakoid sacs.
- **B** in the cytoplasm.
- **C** in the stroma of the chloroplast.
- **D** in all of the above places.

5 Which of the following will increase the rate of photosynthesis in a cold-climate plant?
- **A** Decreasing the temperature from 35°C to 5°C
- **B** Increasing the temperature from 35°C to 50°C
- **C** Decreasing the amount of carbon dioxide
- **D** Increasing the intensity of the light

6 In which stage of photosynthesis do ATP and NADPH become unloaded and become ADP and $NADP^+$?
- **A** Light-dependent reaction
- **B** Light-independent reaction
- **C** Both light-dependent and light-independent reactions
- **D** After photosynthesis occurs

7 Which of the following is correct?
- **A** ATP is produced in photosynthesis.
- **B** ATP is produced in the light-dependent reaction.
- **C** ATP is produced in the light-independent reaction.
- **D** ATP is produced in the Calvin cycle.

8 What is the location of the light-independent stage of photosynthesis in C_3 plants?
- **A** Thylakoid sacs
- **B** Stroma
- **C** Cytoplasm
- **D** Stoma

9 Which of the following is not an output of the light-independent reaction?
- **A** Water
- **B** ADP
- **C** NADPH
- **D** Glucose

10 Which is the most common plant type?
- **A** C_3 plants
- **B** C_4 plants
- **C** CAM plants
- **D** C_4 and CAM plants combined

11 How are C_4 plants different to C_3 and CAM plants?
- **A** C_4 plants physically separate the different stages of photosynthesis within the mesophyll cells.
- **B** C_4 plants have two different cells that undergo photosynthesis: the mesophyll and bundle-sheath cells.
- **C** C_4 plants separate the stages of photosynthesis by time, where during the night the light-dependent stage occurs and during the day the Calvin cycle occurs.
- **D** C_4 plants fix CO_2 with Rubisco, but unlike C_3 plants they do not require PEP carboxylase to convert CO_2 into an intermediate molecule.

12 Which plants are best adapted for dry conditions?

A CAM plants

B C_4 plants

C C_3 plants

D Both C_4 and C_3 plants

Short answer

Describe and explain

13 What is the chemical equation for photosynthesis?

14 In Figure 1, which steps of photosynthesis occur at sites A and B?

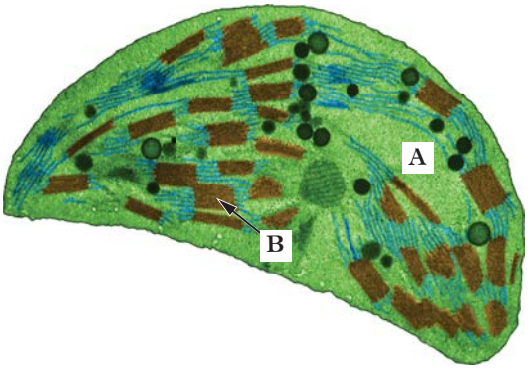


FIGURE 1 A colour-enhanced transmission electron micrograph of a chloroplast

15 What are the inputs, outputs and locations of the light-dependent stage of photosynthesis?

16 What is the role of light energy in the light-dependent stage of photosynthesis in C_3 plants?

17 Why are CAM plants more efficient in dry conditions?

18 Draw a graph to represent the effect of light intensity on the rate of photosynthesis.

19 Compare the light-dependent and light-independent reactions across all of the different types of plants (C_4, C_3 and CAM).

Apply, analyse and compare

20 Periwinkle (*Vinca major*) is a creeping plant that is considered a weed in southern Australia, forming a dense mat that shades out native plants. It has dark green leaves and blue five-petalled flowers. A variegated form of periwinkle with white and green leaves exists that is not as aggressive as the wild type. Explain why this could be.

FIGURE 2 Variegated periwinkle (*Vinca major*)

21 Does photosynthesis occur in the dark? Justify your response.

22 Compare C_3, C_4 and CAM plants by their photosynthetic pathway and explain how they are adapted for different environmental conditions.

23 Consider the three different types of plants you have learned about in this chapter (C_3, C_4 and CAM). Can you identify three plants (or plant foods) that you come across every day in your garden, at school or at the supermarket that fit each of these plant types?

Design and discuss

24 Biology students investigated the effect of carbon dioxide concentration on the rate of photosynthesis. They used spinach discs that were treated to remove all of the gases in the leaf so that the discs would sink when placed in solution. The students placed 10 spinach discs each in five different concentrations of sodium carbonate, which supply the leaf with carbon dioxide. The five beakers were supplied with constant UV light. They measured how long it took for five of the 10 spinach discs to float to

the surface of the beaker. They obtained the results in Table 1.

TABLE 1 Time taken for five spinach discs to rise in various concentrations of sodium carbonate

Sodium carbonate concentration (%)	Time taken for five discs to rise (seconds)
0	No result
2.5	190
5	100
7.5	40
10	20

FIGURE 3 A syringe can be used to force the gases from the spinach discs in preparation for this experiment.

a What is the independent variable in this investigation?

b What variables would need to be controlled? Describe three of these variables.

c Draw a graph to represent the data in Table 1.

d How does the time taken for five discs to rise give an indication of the rate of photosynthesis?

e Explain the 'no result' at 0% sodium carbonate concentration.

f Write a conclusion based on the data recorded.

25 a Research a plant that has been modified by humans to increase its rate of photosynthesis.

b Using what you learned in Chapter 3 about genetically modified plants, consider the ethics behind the plant you have selected. Do you agree or disagree with this choice of modification? Support your answer with evidence.

26 A large glass-stoppered flask containing nasturtium leaves and air was exposed to bright sunlight for 3 hours. Figure 4 shows the apparatus used.

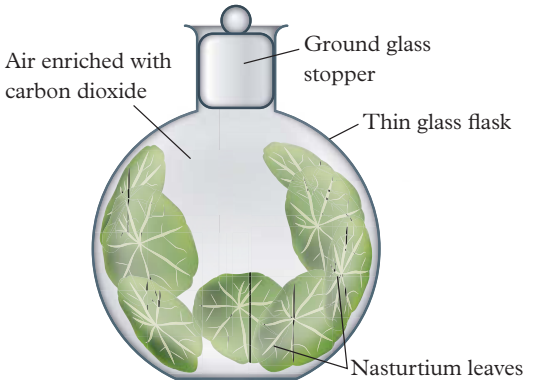


FIGURE 4 Glass containing nasturtium leaves and air

Two samples of gas were withdrawn from the apparatus. The first (sample A) was withdrawn at the beginning of the experiment and the second (sample B) was withdrawn at the end of the 3 hours. The table below shows the results of the experiment.

TABLE 2 Results of the CO_2 experiment

	Carbon dioxide concentration
Sample A	6%
Sample B	4%

Discuss the difference in carbon dioxide concentration between the two samples.

Exam essentials

Responding to questions

In your exam, you may be expected to answer a question that requires you to use key terms to receive full marks.

Focus on key terms

When responding to short-answer questions, it is important to think about which key terms the examiner is looking for in your response. Consider the following:

- First, read the question to identify the topic and key terms.
- Plan your response around the key terms so that you can provide the required information.

Key terms could include the names of processes, biomacromolecules, concepts or other scientific terminology. Consider the key terms in the following question taken from the 2020 VCE Biology Examination.

QUESTION 3a (2020 Biology Written Examination)

Greenhouses have been used to generate higher crop yields than open-field agriculture. To encourage plant growth in greenhouses, the conditions required for photosynthesis are controlled. Commercial greenhouses, like the ones shown below, often use a lot of energy for heating, ventilation, lighting and water.

a Consider the reactions of photosynthesis. Why would it be important to maintain the temperature within narrow limits in a commercial greenhouse? Justify your answer. 2 marks

Response 1

Enzymes in photosynthesis, such as rubisco, have an optimum temperature, and therefore it is best to keep the greenhouse within that optimal range. Those enzymes will denature at high temperatures and lose activity at low temperatures. Therefore, for optimal plant growth theenzymes need to have a high rate of reaction.

This key term was not necessary to receive full marks; however, it shows that you know the enzymes involved in photosynthesis.

This is the justification that the question has asked for.

This response would receive 2 marks because it clearly justifies why the temperature in a greenhouse needs to be maintained within narrow limits. The key terms that are required include enzymes, optimal temperature, denature and rate of reaction. If these terms were not included, then it is unlikely the student would have received full marks for the response.

Response 2

Photosynthesis works best at approximately 20°C. Below this temperature plants do not grow very well and above this temperature plants may die.

This is a vague statement.

This response would not receive any marks since it does not make the link that enzymes are involved in photosynthesis. Some key terms were used, but not enough to respond to the question and provide the required justification.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

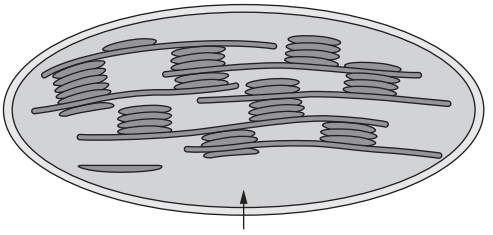
QUESTION 2 (2019 Biology Written Examination)

a A chloroplast is surrounded by a double membrane.

i Name two molecules, as inputs for photosynthesis, that would need to diffuse from the cytosol of the plant cell across the chloroplast membranes and into the chloroplast. 1 mark

Water and oxygen

ii Under high magnification, the internal structure of a chloroplast is visible. The diagram below shows part of this structure.



Region R

A higher concentration of oxygen is found in Region R when a plant is photosynthesising compared to when it is not photosynthesising.

Account for the differences in oxygen concentrations found in this region. 2 marks

Oxygen is produced when water is split. Oxygen concentration will vary depending on the rate of this reaction.

Source: 2019 Biology Written Examination Question 2, Short answer, reproduced by permission © VCAA

Marking guide

Question 2 a i	- 1 mark for identifying carbon dioxide and water.
Question 2 a ii	- 1 mark for identifying that oxygen is produced during the light-dependent stage. - 1 mark for determining that the concentration of oxygen will vary depending on the availability of light.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

CHAPTER 6

Cellular respiration

Cellular respiration is critical for the survival of living organisms since it breaks down glucose and makes energy available for cellular processes through the short-term carrier adenosine triphosphate (ATP). Cellular respiration can occur in the presence of oxygen (aerobic respiration) or absence of oxygen (anaerobic fermentation). Glycolysis is the first stage of cellular respiration and occurs in the cytoplasm of cells. If oxygen is present, the Krebs cycle and electron transport chain occur to produce many ATP molecules in the mitochondria. If oxygen is absent, fermentation can occur and produces different products depending on the organism.

The rate of cellular respiration is affected by factors that influence enzyme action. These factors include temperature, glucose availability and oxygen concentration.

KEY KNOWLEDGE

- → 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)
- → the location, inputs and the difference in outputs of anaerobic fermentation in animals and yeasts
- → the factors that affect the rate of cellular respiration: temperature, glucose availability and oxygen concentration

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FIGURE 1 Fermentation is a process that releases energy from a carbon compound in the absence of oxygen. In soil, deep underground, no oxygen is present and bacteria are capable of respiring anaerobically.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

6A Animals cannot convert light energy to chemical energy. How do they obtain chemical energy?

6A Groundwork resource
Ecosystem energy transfer

6B What is the role of an enzyme in a biochemical reaction?

6B Groundwork resource
Enzymes

6C What is the plasma membrane?

6C Groundwork resource
The plasma membrane

PRACTICALS

PRACTICAL **6.3** Factors that affect the rate of aerobic cellular respiration

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

6.1 Cellular respiration

KEY IDEAS

In this topic, you will learn that:

- mitochondria are the site of cellular respiration in eukaryotic cells
- there are three stages of aerobic respiration: glycolysis, the Krebs cycle and the electron transport chain.

Video
Aerobic cellular respiration

Plant cells, protists and some bacteria convert light energy into chemical energy. Other organisms, such as humans, consume food in order to access the chemical energy they need to survive. This energy needs to be converted into a form that can be used by the cell. The chemical energy that is used by the cell is stored in high-energy molecules called ATP (adenosine triphosphate).

Cellular respiration is usually represented by the chemical equation:

$$C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l) + 36 \text{ or } 38 \text{ ATP}$$

While this looks like a straightforward reaction, in reality there are multiple steps involved. Some steps are endergonic (requiring a supply of energy to proceed); several are exergonic and store the extra energy in the form of ATP.

There are three steps involved in aerobic cellular respiration: glycolysis, the Krebs cycle and the electron transport chain. The last two of these steps occur in the mitochondria.

mitochondrion
plural mitochondria; a membrane-bound cellular organelle with a fluid matrix and inner membrane forming folds (cristae) on which are enzymes for aerobic respiration

cristae
the folds formed by the inner membrane of a mitochondrion

matrix
the internal space of a mitochondrion enclosed by the inner membrane

aerobic cellular respiration
the complete conversion of glucose to carbon dioxide and water in the presence of oxygen, resulting in a large release of energy (net gain of 36 ATP)

Mitochondria

Mitochondria are the site of cellular respiration within a eukaryotic cell. A **mitochondrion** (plural, mitochondria) is a double-membraned cellular organelle. Its inner membrane is deeply folded to form a series of **cristae** (as seen in Figure 1) that increase the surface area of this membrane.

In the structure of mitochondria:

- The outer membrane controls the passage of materials into and out of the mitochondrion.
- The inner membrane contains enzymes responsible for ATP production.
- The **matrix** within the mitochondrion contains ribosomes, circular mitochondrial DNA and the enzymes required for many of the steps in aerobic cellular respiration.

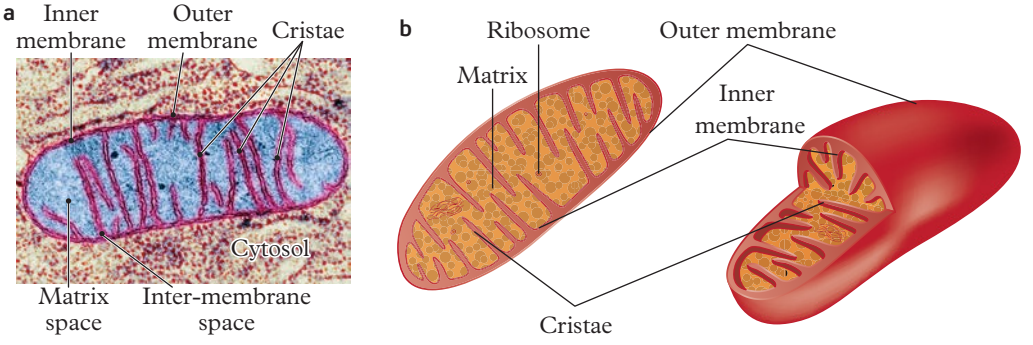


FIGURE 1 **a** Electron micrograph and **b** diagram of a mitochondrion

Aerobic cellular respiration stages

Each step in cellular respiration is controlled by a specific enzyme. Cellular respiration may occur either in the presence of oxygen (**aerobic cellular respiration**) or in the absence of oxygen (**anaerobic fermentation**). Aerobic cellular respiration occurs in three stages in eukaryotic cells: **glycolysis**, **pyruvate conversion** and the **Krebs cycle**, and the **electron transport chain**.

Stage 1: Glycolysis

Glycolysis occurs in the cytoplasm of the cell. Glycolysis does not require oxygen, so it can be referred to as an anaerobic process. The reaction starts with a glucose ($C_6H_{12}O_6$) molecule reacting with two ATP molecules, which produces two pyruvate molecules. In this process, four ATP molecules are formed. This means there is a net gain of two ATP molecules (two ATP were used, but four were produced). The coenzyme NAD also collects the extra hydrogen ions and their high energy electrons to become its loaded form, NADH.

If there are oxygen molecules available, the pyruvate is free to move into the mitochondria. If there is no oxygen present, then anaerobic fermentation will occur. This will be discussed in Topic 6.2.

TABLE 1 Location, inputs and outputs of glycolysis

Process	Location	Inputs	Outputs
Glycolysis	Cytoplasm	2ATP Glucose ($C_6H_{12}O_6$) 2ADP $2NAD^+$	2 pyruvate 4ATP (net gain of 2ATP) 2NADH

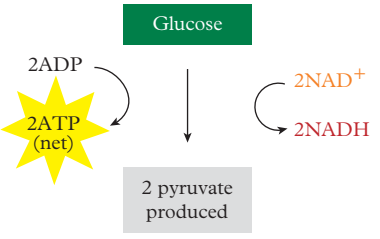


FIGURE 2 Glycolysis

Stage 2: Krebs cycle

If oxygen is present, the two pyruvate molecules enter the mitochondrion by passing through both the outer and inner membranes. The next step takes place in the matrix of the mitochondrion.

In order for pyruvate to enter the Krebs cycle it must undergo a molecular change. The two pyruvate molecules are transformed with an acetyl group via a carrier molecule, **coenzyme A**. The resulting molecules are known as **acetyl-CoA**. This reaction also results in the production of two molecules of carbon dioxide and two NADH.

The acetyl-CoA enters the Krebs cycle, which is a series of reactions that produce CO_2, NADH, $FADH_2$ and ATP (see Figure 3 on the next page). The cycle occurs twice since there are two acetyl-CoA molecules.

anaerobic fermentation
cellular respiration in the absence of oxygen, resulting in a net gain of 2 ATP and lactic acid (in animals), ethyl alcohol and carbon dioxide (in plants and fungi) or other products (in bacteria)

glycolysis
the first stage in cellular respiration; occurs in the cytoplasm and results in the net gain of 2 ATP and 2 pyruvate molecules

pyruvate conversion
a reaction that occurs before the Krebs cycle in the mitochondrial matrix where pyruvate is converted into acetyl-CoA

Krebs cycle
the cyclic series of chemical reactions in the mitochondria of cells in which 2 ATP are formed and coenzymes NAD^+ and FAD^+ become loaded

electron transport chain
a series of enzymes embedded in the inner membrane of the mitochondria that enable the transfer of electrons from coenzymes; this is the final stage in aerobic cellular respiration in which most ATP molecules are formed

coenzyme A
a coenzyme used in biochemical reactions inside the cell (including during the Krebs cycle in cellular respiration)

acetyl-CoA
the substrate that enters the Krebs cycle

TABLE 2 Location, inputs and outputs of the Krebs cycle for two pyruvate molecules

Process	Location	Inputs	Outputs
Pyruvate conversion	Mitochondrial matrix	2 pyruvate 2 coenzyme A $2NAD^+$	2 acetyl-CoA $2CO_2$ 2NADH
Krebs cycle	Mitochondrial matrix	2 acetyl-CoA $6NAD^+$ $2FAD^+$ 2ADP	2 coenzyme A $4CO_2$ 6NADH $2FADH_2$ 2ATP

The outputs from the two pyruvate molecules at this stage include:

- six carbon dioxide molecules ($6CO_2$), which leave the mitochondrion and are removed from the cell as metabolic waste
- two ATP molecules
- hydrogen ions with high-energy electrons bound within coenzymes NADH and $FADH_2$.

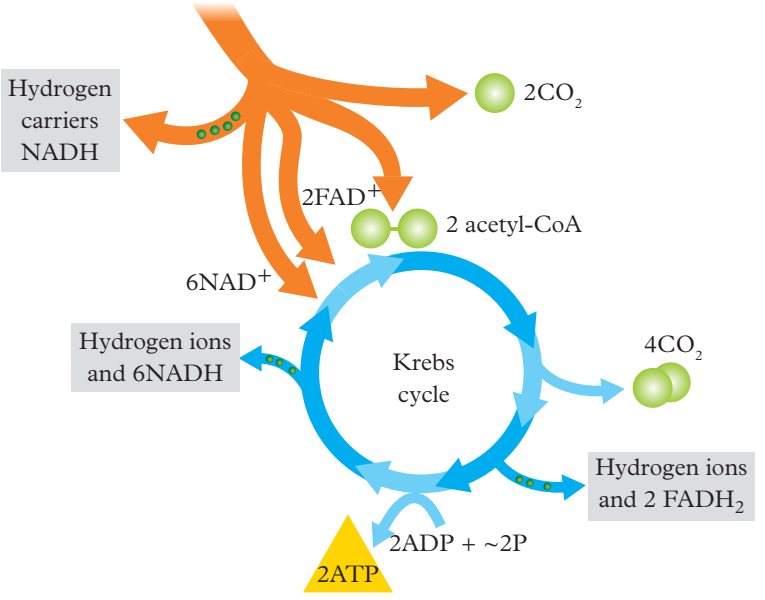


FIGURE 3 The Krebs cycle is a complex series of reactions and occurs twice. **Note:** You only need to know the inputs, outputs and location of the overall reaction.

Stage 3: Electron transport chain

Study tip

You do not need to remember the mechanisms of the biochemical pathways, just the location, inputs and outputs of each of the stages.

The process now moves to the inner membrane of the mitochondrion. This structure is highly folded to increase surface area for chemical reactions. A series of specialised enzymes associated with these reactions are embedded in the inner mitochondrial membrane.

Hydrogen ions formed in the previous steps are released from their NADH carriers along with high-energy electrons to the electron transport chain. At each step in the chain, energy is released from the electrons and is used in the formation of ATP. At this step, oxygen is used as the final electron acceptor, producing water molecules.

Depending on the type of cell, either 32 or 34 ATP molecules can be produced during electron transport chain reactions.

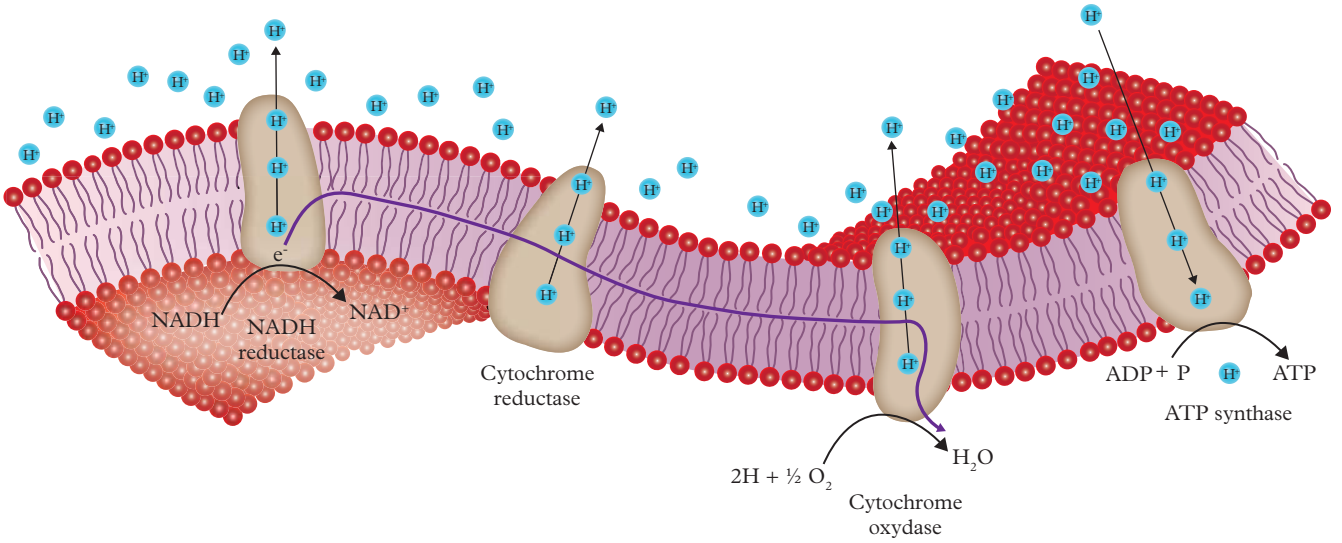


FIGURE 4 The enzymes embedded in the inner membrane of the mitochondrion carry electrons to the final electron acceptor that produces ATP molecules.

TABLE 3 Locations, inputs and outputs of the electron transport chain

Process	Location	Inputs	Outputs
Electron transport chain	Inner membrane of mitochondria (cristae)	$6O_2$ 10NADH $2FADH_2$ 32 or 34ADP	$6H_2O$ $10NAD^+$ $2FAD^+$ 32 or 34ATP

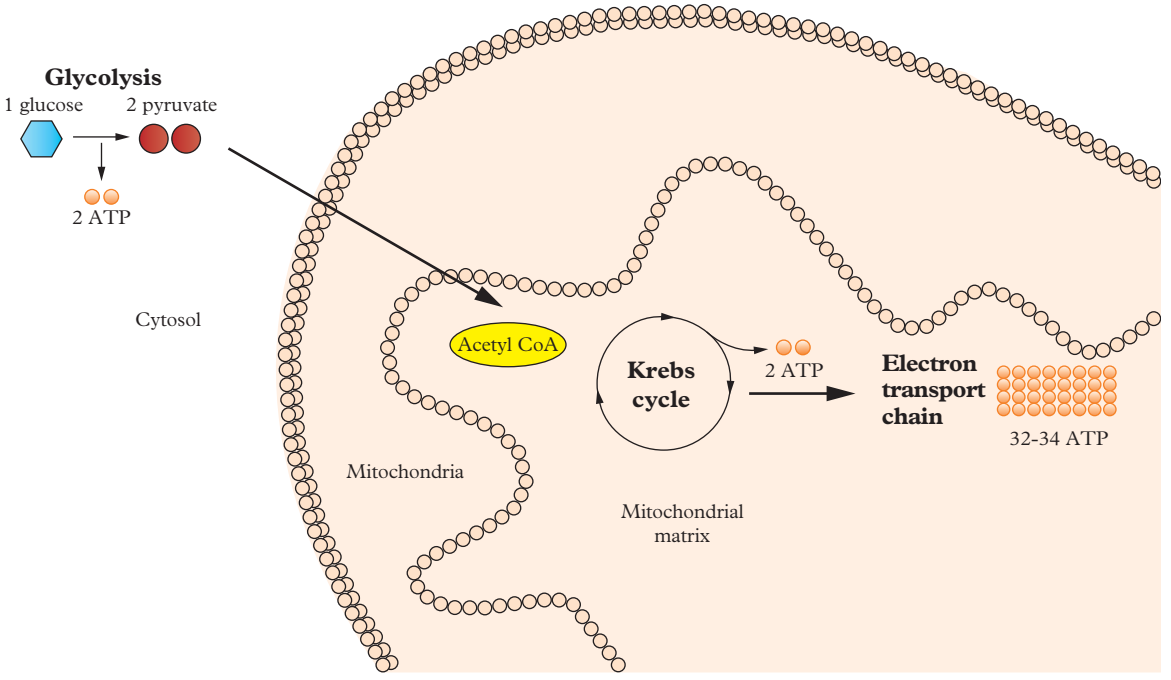


FIGURE 5 The stages of aerobic cellular respiration take place in the cytosol (glycolysis), mitochondrial matrix (Krebs cycle) and inner membrane of the mitochondrion (electron transport chain).

Cellular respiration of polysaccharides, lipids and proteins

Fats and amino acids can also be used as energy sources, after first being converted to coenzyme A, which can enter the Krebs cycle. The breakdown of fats for cellular respiration occurs when there is no more free glucose or glycogen in animals, or starch available in plants to convert to glucose. When all fat reserves have been depleted, the organism will then convert proteins for cellular respiration.

FIGURE 6 Starch is a major part of the human diet and gets converted into glycogen – an alternative energy source for cellular respiration.

CHALLENGE 6.1

Photosynthesis versus cellular respiration

In the light-dependent reaction of photosynthesis, 18 ATP molecules are produced. The process then continues to the Calvin cycle, with the products of photosynthesis then used in cellular respiration in photosynthetic organisms.

1 If ATP is being produced during the light-dependent reaction, would it still be necessary for the light-independent reactions of photosynthesis and aerobic cellular respiration to occur? Discuss.

CHECK YOUR LEARNING 6.1

Describe and explain

1 Define the term 'glycolysis'.

2 What is the chemical equation for aerobic cellular respiration?

3 **a** What are the locations of each of the three stages of aerobic cellular respiration?

b What happens in each of the stages?

4 Why is glycolysis considered an anaerobic reaction in aerobic cellular respiration?

Apply, analyse and compare

5 Produce a table to summarise the location, inputs and outputs of all stages of aerobic cellular respiration.

6 Carbon dioxide and water are by-products of cellular respiration.

a In which stages are they produced?

b How would these molecules leave the cell?

Design and discuss

7 Hypophosphataemia is a condition where phosphate levels in the blood are low. It can be caused by alcoholism or starvation. People suffering from hypophosphataemia are often very tired. Why would this be?

6.2 Anaerobic fermentation

KEY IDEAS

In this topic, you will learn that:

- anaerobic fermentation occurs in the absence of oxygen
- anaerobes are organisms capable of anaerobic respiration
- there are different types of anaerobic fermentation in animals, yeast and bacteria.

Video
Anaerobic fermentation

Oxygen is essential for the electron transport chain to produce ATP; however, oxygen is not always freely available. Some organisms, known as anaerobes, get enough energy from anaerobic respiration alone. They are able to undergo glycolysis and produce a net of two ATP molecules.

Types of anaerobes

complete anaerobe
an organism that gains its ATP exclusively through fermentation (also called an obligate anaerobe)

partial anaerobe
an organism that can gain its ATP through aerobic or anaerobic cellular respiration, depending on the conditions (also called a facultative anaerobe)

There are two types of anaerobes. **Complete anaerobes** live permanently in oxygen-deficient conditions. Some (including certain bacteria and some parasites) may be poisoned by being exposed to even a small concentration of oxygen. **Partial anaerobes** thrive in the presence of oxygen, but resort to anaerobic respiration if oxygen is absent or in short supply. Examples of partial anaerobes include worms living in the mud at the bottom of stagnant pools or oceans, and diving mammals such as whales that can stay under water for long periods of time. The length of time an organism can survive without oxygen varies – a matter of minutes for humans, while some organisms and plants can respire anaerobically for much longer periods.

FIGURE 1 Whales can stay underwater for extended periods of time since they are partial anaerobes.

Study tip

On the exam, never write just 'cellular respiration'. Be specific and either write aerobic cellular respiration or anaerobic cellular respiration.

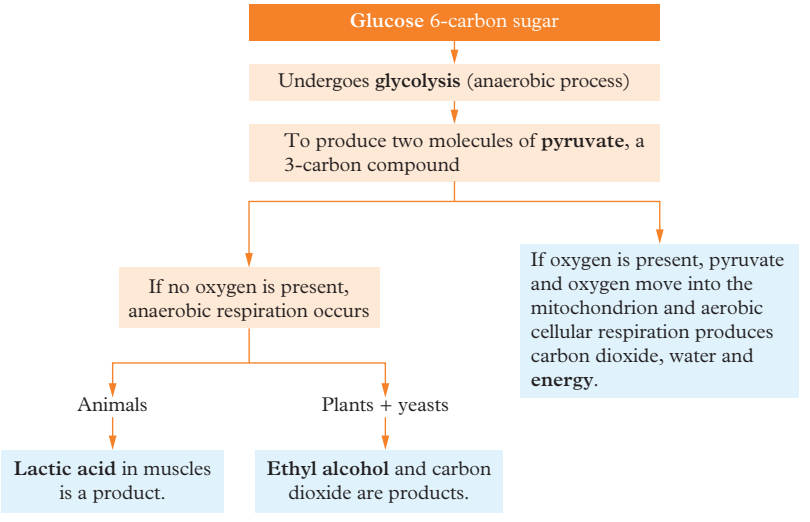


FIGURE 2 The aerobic respiration and anaerobic fermentation pathways.

Fermentation

fermentation
an anaerobic process of breaking down glucose in the absence of oxygen

lactic acid
$C_3H_6O_3$ produced from the anaerobic breakdown of glucose in animals

ethanol
C_2H_5OH produced from the anaerobic breakdown of glucose in plants and yeast

Fermentation is an anaerobic process that does not require oxygen to break down glucose. The pyruvate that is formed during glycolysis enters one of the fermentation pathways, depending on the organism. Animals undergo **lactic acid** fermentation, plants and yeasts undergo **ethanol** fermentation, and bacteria undergo different types of anaerobic fermentation.

Fermentation in animals

Lactic acid fermentation in animals can be represented by the following equation:

$$C_6H_{12}O_6 \rightarrow 2 \text{ lactic acid} + 2ATP$$

Glycolysis occurs, as usual, in the cytoplasm of the cell to produce two pyruvate molecules, two NADH and two ATP. Then in the absence of oxygen, lactic acid fermentation occurs in which two pyruvate are converted into two lactate molecules.

If a person undergoes vigorous exercise, more energy is required than can be supplied aerobically. This is because the breathing and circulatory systems cannot operate fast enough to sustain an adequate supply of oxygen. When oxygen levels in the blood start to run low, oxygen is released from myoglobin, an oxygen-storage molecule in the muscle. The levels of oxygen attached to the myoglobin are limited and eventually they become too low to sustain aerobic cellular respiration. Muscle fibres then provide energy anaerobically and produce lactate (which becomes lactic acid in solution). Like all acids, lactic acid is a metabolic poison and it can cause damage if it accumulates in large quantities.

When exercise is completed, the lactate can re-enter the aerobic cellular respiration pathway and be broken down into carbon dioxide and water. Alternatively, it is converted to a complex carbohydrate. Oxygen is needed for both processes. The amount of oxygen needed to remove the lactic acid is called the **oxygen debt**. Muscles can feel stiff when sufficient amounts of lactic acid accumulate.

oxygen debt
the amount of oxygen required to remove lactic acid from muscle tissue

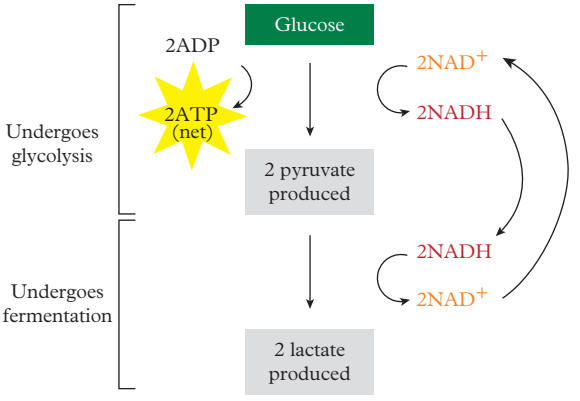


FIGURE 3 Glycolysis and lactic acid fermentation.

TABLE 1 Location, inputs and outputs of lactic acid fermentation in animals

Process	Location	Inputs	Outputs
Glycolysis	Cytoplasm	Glucose $2NAD^+$ 2ATP	2 pyruvate 2NADH 4ATP (net 2ATP)
Lactic acid fermentation	Cytoplasm of animal cells	2 pyruvate 2NADH	2 lactate (lactic acid) $2NAD^+$

FIGURE 4 Lactic acid is a by-product of fermentation in animals and too much is poison to the body.

CHALLENGE 6.2

Frogs in love

During spring, local wetlands and waterways ring out with the vigorous mating calls of frogs. These frogs have twice as much lactic acid in their systems as frogs not involved in the 'breeding chorus'.

1 Discuss why breeding frogs have more lactic acid.

FIGURE 5 The southern brown tree frog is common throughout Victoria.

Fermentation in yeasts, plants and bacteria

The natural fermentation of yeasts (single-celled fungi) and some bacteria and plants has been used for thousands of years in making products for human consumption, such as bread and beer. Fermentation in plants and yeasts is often referred to as ethanol or alcoholic fermentation.

Fermentation in plants and yeasts can be summarised by the following equation:

$$1 \text{ glucose} \rightarrow 2 \text{ ethanol} + 2CO_2 + 2ATP$$

This is represented by the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP$$

acetaldehyde
an organic compound that forms as an intermediate compound between pyruvate and ethanol during alcoholic fermentation

As with animals, glycolysis occurs in the cytoplasm of the cell to produce two pyruvate molecules, two NADH and two ATP. Then in the absence of oxygen, alcoholic fermentation occurs in which the two pyruvate molecules are converted into two **acetaldehyde** molecules (intermediate) and two carbon dioxide molecules. The acetaldehyde molecules are converted into ethanol (alcohol) via NADH.

Most bacteria are partial anaerobes that are able to switch between aerobic respiration and anaerobic fermentation, depending on oxygen availability. There are particular bacteria, such as Clostridia, that are killed when exposed to oxygen – these are known as complete anaerobes.

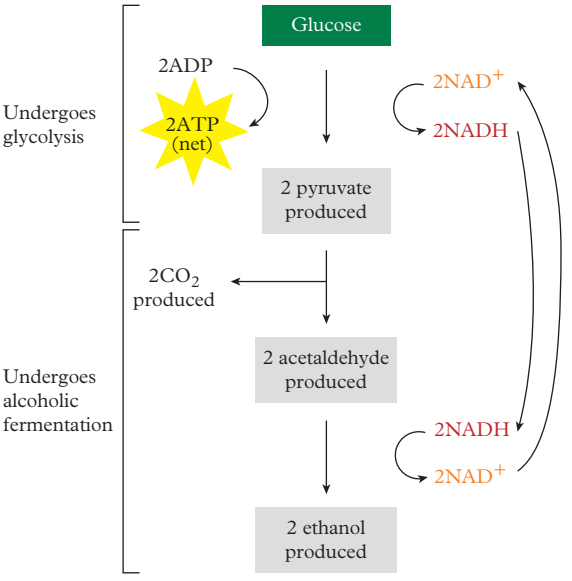


FIGURE 6 Glycolysis and alcoholic fermentation

TABLE 2 Location, inputs and outputs of alcoholic fermentation in yeast and fermentation in bacteria

Process	Location	Inputs	Outputs
Glycolysis	Cytoplasm	Glucose $2NAD^+$ 2ATP	2 pyruvate 2NADH 4ATP (net 2ATP)
Alcoholic fermentation	Cytoplasm of yeast cells	2 pyruvate 2NADH	2 ethanol $2NAD^+$ $2CO_2$

Anaerobic bacteria undergo glycolysis to produce two pyruvate, two ATP and two NADH in the cytoplasm. Various methods of fermentation are then used by these bacteria, and the different types of gas are produced from the fermentation reaction. The type of gas being produced can be used to identify the bacteria.

One type of fermentation in bacteria is anaerobic respiration using sulfate. These bacteria convert sulfate into oxygen and hydrogen sulfide, which is a green poisonous gas. This commonly occurs is sewers and swamps, but has also been identified in the ocean and deep sub-sea floor. Figure 7 shows a green hue in the ocean water along the coastline of Africa, where bacteria are undergoing anaerobic respiration using sulfate.

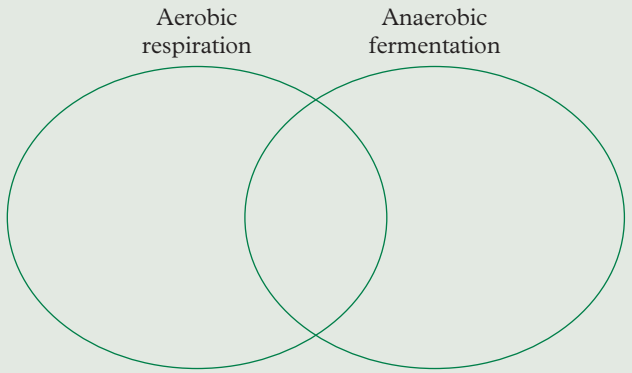
CHECK YOUR LEARNING 6.2

Describe and explain

1 Explain why all organisms are capable of glycolysis.
2 Where does anaerobic fermentation occur in animals, yeast and plants?
3 Describe the difference between complete and partial anaerobes.
4 When does lactic acid fermentation occur in animals? And why does it occur?

Apply, analyse and compare

5 Compare aerobic respiration and anaerobic fermentation using the Venn diagram shown on the right.



Design and discuss

6 Yeast are used in the fermentation of alcoholic beverages such as wine and beer. What safety concerns does this raise for workers in a brewery?

FIGURE 7 The green colour seen in the water off the coast of Africa is from hydrogen sulfide gas being produced by anaerobic bacteria.

6.3 Factors that affect cellular respiration

KEY IDEAS

In this topic, you will learn that:

+ factors that affect the rate of cellular respiration include temperature, glucose availability and oxygen concentration.

There are many factors that can affect the rate of cellular respiration. As discussed in Chapter 4, the rate of cellular respiration can be reduced by factors that influence enzyme action.

The key factors affecting the rate of cellular respiration include the following:

- temperature
- glucose availability
- oxygen concentration.

The rate of cellular respiration is usually measured by the amount of carbon dioxide gas produced.

Temperature

One of the ways the human body fights an infection is to develop a fever. While this is good for destroying the pathogen (bacteria or viruses), it can also damage cells by altering membrane fluidity and permeability. As mentioned in Chapter 4, enzymes have an optimum temperature range. At high temperatures, the shape of the enzyme's active site changes via denaturation. Above the optimum temperature range, the enzymes involved in cellular respiration will denature and cellular respiration (and therefore ATP production) will stop.

FIGURE 2 The Southern Ocean marine life can withstand extremely low temperatures and many organisms go into a state of hibernation, not growing for years.

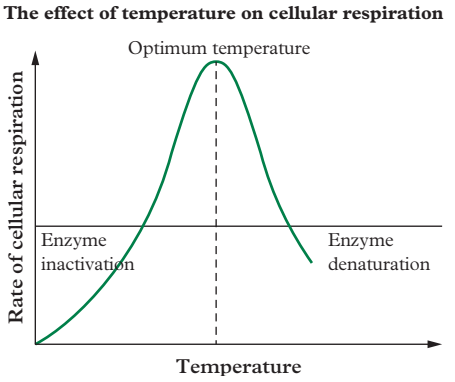


FIGURE 1 The effect of temperature on the rate of cellular respiration

At low temperatures, all molecules move more slowly. This makes it difficult for glucose and oxygen to bind with the enzymes responsible for cellular respiration. As a result, the overall rate of cellular respiration will slow, causing ATP production to slow. For example, in the Southern Ocean (at an average temperature of 2–4°C), many organisms display extremely slow growth rates.

Glucose availability

The availability of glucose has a strong effect on the rate of both aerobic respiration and anaerobic fermentation. Glucose is the main reactant of glycolysis, the first stage of cellular respiration. With low glucose availability, cells

will produce limited ATP. As glucose availability increases, the rate of cellular respiration increases, then plateaus, as shown in Figure 3. This follows the same curve as substrate concentration versus the rate of enzyme action. Eventually, a further increase in glucose availability will not increase the rate of cellular respiration. This occurs when the availability of enzymes becomes a limiting factor.

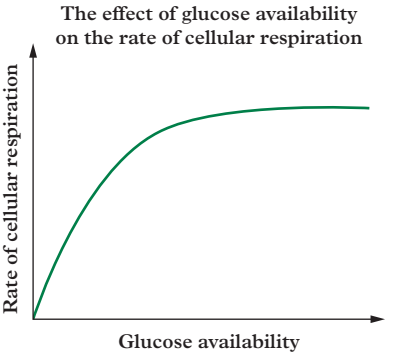


FIGURE 3 The effect of glucose availability on the rate of cellular respiration

Oxygen concentration

The oxygen concentration available to cells can greatly influence the rate of aerobic cellular respiration. Oxygen is a reactant in the electron transport chain, needed to combine with hydrogen ions to form water molecules. When no oxygen molecules are present, the carrier molecules (NADH and $FADH_2$) cannot unload their hydrogen ions and high-energy electrons. This then slows the Krebs cycle. As with glucose availability, an increase in oxygen concentration will increase the rate of aerobic cellular respiration until a point where the rate of aerobic cellular respiration does not increase any further, due to other limiting factors.

Oxygen concentration has no effect on the rate of anaerobic fermentation, since this reaction occurs in the absence of oxygen.

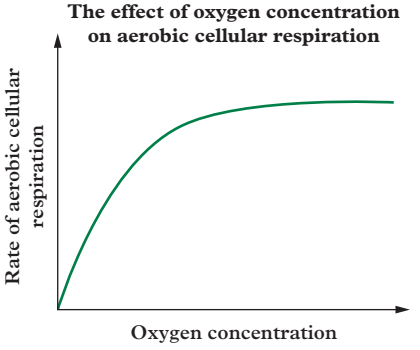


FIGURE 4 The effect of oxygen concentration on the rate of aerobic cellular respiration

CHECK YOUR LEARNING 6.3

Describe and explain

1 Why does temperature affect the rate of cellular respiration?

2 What occurs to the rate of cellular respiration when the temperature exceeds the optimum temperature range?

3 Explain why oxygen concentration doesn't affect anaerobic fermentation. Use the following terms in your response:
- glycolysis
- electron transport
- reactant.

4 Explain why glucose availability affects both aerobic respiration and anaerobic fermentation.

Apply, analyse and compare

5 **a** Draw a graph to represent the effect of oxygen concentration on the rate of aerobic respiration and the rate of anaerobic fermentation.
Hint: You should have two lines on your graph.

b Name and describe one limiting factor that would cause the plateau in the rate of aerobic respiration versus oxygen concentration.

Design and discuss

6 Consider the factors that affect enzyme action. What other factors (not including glucose availability, oxygen concentration or temperature) would affect the rate of cellular respiration? Explain.

CHAPTER 6

Review

Chapter summary

6.1
- There are three stages of aerobic respiration: glycolysis, the Krebs cycle and the electron transport chain.
- Mitochondria are the site of the Krebs cycle and the electron transport chain. Glycolysis takes place in the cytoplasm.
- If glucose is not available as an energy source for cellular respiration, proteins, fats, lipid and starch/glycogen can be used.

6.2
- Anaerobic fermentation occurs in the absence of oxygen.
- Complete anaerobic organisms are capable of anaerobic respiration only, while partial anaerobes can respire with or without oxygen.
- Animals anaerobically respire by converting glucose to lactate. Yeast and plants anaerobically respire by converting glucose to ethyl alcohol. Bacteria undergo different types of fermentation.

6.3
- Temperature, glucose availability and oxygen concentration all affect cellular respiration rates, similarly to how they affect enzymes.

Key formulas

Cellular respiration	$C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l) + 36 \text{ or } 38ATP$
Fermentation in animals	$C_6H_{12}O_6 \rightarrow 2 \text{ lactic acid} + 2ATP$
Fermentation in yeasts and plants	$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + CO_2 + 2ATP$

Revision questions

Multiple choice

1 Which of the following is not a product of the Krebs cycle?
- **A** Water
- **B** Coenzyme A
- **C** Carbon dioxide
- **D** ATP

2 Which product of pyruvate conversion enters the Krebs cycle?
- **A** Carbon dioxide
- **B** Glucose
- **C** Acetyl-CoA
- **D** Coenzyme A

3 The net number of ATP molecules produced during each stage of aerobic cellular respiration is:
- **A** 4 during glycolysis, 2 during the Krebs cycle, 32 or 34 during the electron transport chain.
- **B** 2 during glycolysis, 2 during the Krebs cycle, 32 or 34 during the electron transport chain.
- **C** 4 during glycolysis, 2 during the Krebs cycle, 2 during the electron transport chain.
- **D** 4 during glycolysis, 4 during the Krebs cycle, 32 or 34 during the electron transport chain.

4 $NADH$ and $FADH_2$ are electron carriers that are essential for the electron transport chain in aerobic cellular respiration. Which of the following is correct when describing $NADH$ and $FADH_2$?
- **A** $NADH$ and $FADH_2$ are hydrogen carriers, but afterwards donate their hydrogen so that it cannot be used again.
- **B** $NADH$ and $FADH_2$ are formed in previous stages of aerobic respiration and release a hydrogen ion and also an electron into the electron transport chain to drive the formation of ATP.
- **C** $NADH$ and $FADH_2$ are products of the electron transport chain to collect hydrogen ions from water molecules.
- **D** Both $NADH$ and $FADH_2$ release one hydrogen ion and one electron to the electron transport chain.

5 Where in a cell does the Krebs cycle occur?
- **A** Cytoplasm
- **B** Stroma
- **C** Mitochondrial cristae
- **D** Mitochondrial matrix

6 Abdi was trying to better his personal best result in the 100 m sprint. His first time was 11.3 seconds. He sprinted repeatedly with no rest between sprints. His time was getting longer and his muscles felt sore and fatigued. Which process was causing the soreness in his muscles?
- **A** Ethanol fermentation
- **B** Aerobic cellular respiration
- **C** Photosynthesis
- **D** Lactic acid fermentation

7 Which equation represents fermentation in animals?
- **A** 1 glucose $\rightarrow$ 2 lactate + 2ATP
- **B** 1 glucose $\rightarrow$ 2 ethanol + $2CO_2$ + 2ATP
- **C** 1 glucose $\rightarrow$ 3 lactate + 3ATP
- **D** 1 glucose $\rightarrow$ 1 ethanol + CO_2 + 1ATP

8 In the absence of oxygen, yeast anaerobically respires. What is the name of this process?
- **A** The Krebs cycle
- **B** Glycolysis
- **C** Lactic acid fermentation
- **D** Ethanol fermentation

9 Why does cellular respiration stop when the temperature exceeds an optimum temperature range?

A At high temperatures, enzymes move more slowly and their shape changes, affecting their ability to bind to substrates in the reaction.

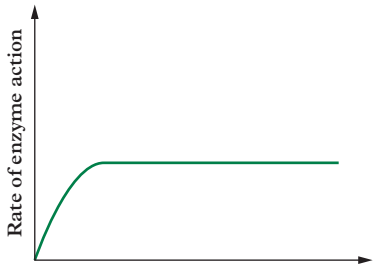
B At high temperatures, enzyme shape is permanently changed, in a process known as denaturation.

C At high temperatures, the substrates move too quickly to be bound to the enzyme's active site, ceasing the reaction.

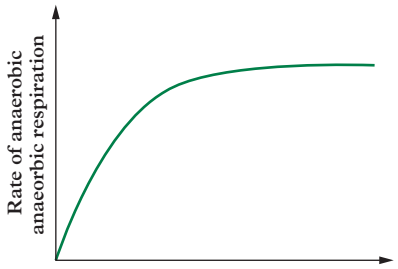
D At high temperatures, particles move faster, causing the molecules of the substrates to break apart so the enzyme has nothing to react with.

10 Which graph below represents the effect of oxygen concentration on the rate of aerobic cellular respiration?

A



B



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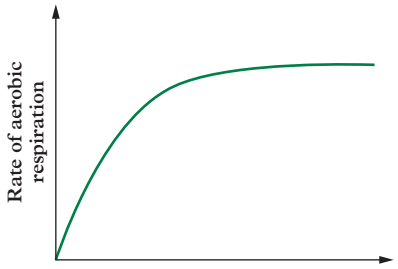
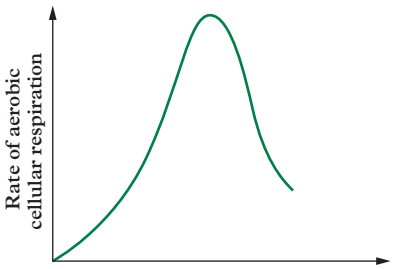


D



Short answer

Describe and explain

11 Summarise the main inputs, outputs and locations of the following processes:

- glycolysis
- Krebs cycle
- electron transport train.

12 In Figure 1, showing a mitochondrion, which steps of cellular respiration occur at sites A, B and C?

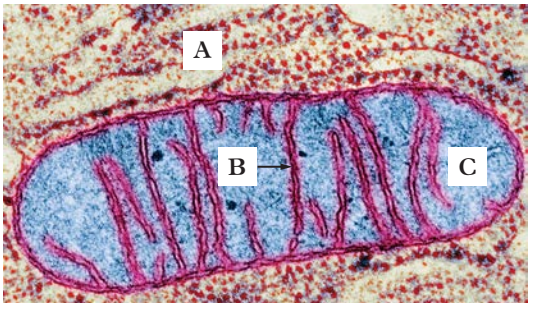


FIGURE 1 An electron micrograph of a mitochondrion

13 Explain what happens in the process of anaerobic fermentation in animals.

14 Explain what happens to the process of cellular respiration when the amount of glucose present in a cell is low.

15 Identify whether the following features are correct for aerobic respiration, anaerobic fermentation or both processes. **Hint:** Draw a table and tick the appropriate box for each feature.

- Affected by oxygen concentration
- Requires mitochondria
- Affected by glucose availability
- Produces ATP
- Requires coenzymes NAD^+ and FAD^+

16 **a** Draw a graph to represent the effect of temperature on the rate of cellular respiration.

b At three different points on your graph, explain the rate of cellular respiration.

Apply, analyse and compare

17 A runner's blood was tested for lactic acid concentration before, during and after a race.

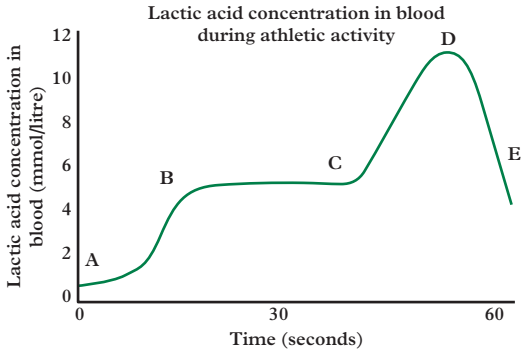


FIGURE 2 Graph of a runner's lactic acid concentration

a At which point on the graph (labelled A–E) does the runner complete the race? Justify your answer.

b Provide an explanation of anaerobic fermentation that accounts for the changing concentration of lactic acid in the runner's blood throughout the race.

c Predict what might happen to lactic acid concentration in the runner's blood if the race took place in a high-altitude area with low oxygen availability.

18 Compare aerobic cellular respiration and anaerobic fermentation in animals.

19 How many cycles of the Krebs cycle occur per glucose molecule? Justify your answer.

20 The electron transport chain produces 32 or 34 ATP, whereas glycolysis produces 2 ATP and the Krebs cycle produces 2 ATP. Why are most of the ATP from aerobic cellular respiration produced in the electron transport chain and not the other two stages?

21 When does cellular respiration of lipids and proteins occur? What is the importance of this process?

22 Explain how glycolysis can occur in both the aerobic and anaerobic cellular respiration pathways.

23 Several jars of strawberry jam were made and stored in a cupboard. When opened several months later, they were found to be frothy and smelled of alcohol.

a Analyse what has happened.

b Explain how this could have been prevented.

Design and discuss

24 The amount of cristae in a mitochondrion will affect the rate of aerobic cellular respiration. Explain why.

25 The white snakeroot plant (*Ageratina altissima*) produces the poison tremetol. This poison prevents the metabolism of lactate. Cows are known to eat this plant and consume the poison, which concentrates in their milk. If humans consume this infected milk, they begin to vomit, have abdominal pain and tremors, and their condition will worsen after exercise. Explain why these symptoms occur.

FIGURE 3 The white snakeroot plant contains a metabolic poison called tremetol.

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Chapter quiz
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Launch a quiz for your students on key concepts in this chapter.

Exam essentials

Responding to questions

In your exam, you may be expected to use data from the question in your response.

Use the data in your response

When a question provides data in a table, graph or image, then you should embed the appropriate data in your response. By doing so, you are demonstrating your understanding of the question and your ability to analyse the information provided.

The following question is taken from the 2013 VCE Biology Examination. Read the question carefully, then consider how the data is used in the responses.

QUESTION 1a (2013 Biology Written Examination)

Yeast is a single-celled, microscopic fungus that uses sucrose as a food source. An experiment was carried out to investigate cellular respiration by a particular species of yeast.

Yeast cells were placed in a container and a sucrose solution was added. An airtight lid was placed on the container. The percentages of oxygen and ethanol in the container were recorded over a one-hour period. The experiment was carried out at room temperature. The results are shown in the following table.

	Percentage of oxygen	Percentage of ethanol
at the start of the experiment	21	0
at the end of the experiment	18	4

a Explain any changes that have been observed in oxygen and ethanol levels within the airtight container.

2 marks

Source: 2013 Biology Written Examination Question 1a, Short answer, reproduced by permission © VCAA

Response 1

Ethanol levels rose from 0 to 4 since ethanol is a product of anaerobic fermentation. Oxygen levels decreased from 21 to 18 since oxygen is a reactant for aerobic respiration.

Data from question in understanding.

Good use of key terms.

This response would receive full marks because it clearly states and explains the change in ethanol and oxygen over the course of the experiment. Data from the question has been used to demonstrate understanding of the change in the molecules.

Response 2

Ethanol and oxygen levels changed over the course of the experiment because they are either required or produced in energy transformation reactions.

Does not specify how the levels have changed.

Should specify the reactions (using key terms).

This response is vague and does not state the change (increase or decrease) of ethanol or oxygen percentage. The explanation is not specific to each molecule and does not name the types of reactions.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 1c (2013 Biology Written Examination)

Scientists are looking at ways to increase the efficiency of photosynthesis in plants, including the way in which carbon dioxide is captured.

c i Name the stage of photosynthesis in which carbon dioxide is captured. 1 mark

The stage of photosynthesis during which carbon dioxide is captured is called the light-dependent stage. It is called the light-dependent stage because it requires the presence of sunlight.

ii The stage of photosynthesis in which carbon dioxide is captured requires other inputs.

Name two other inputs and describe the role played by each in this stage of photosynthesis. 2 marks

Water is required for the production of oxygen and NADH for electron transfer.

Source: 2013 Biology Written Examination Question 1c, Short answer, reproduced by permission © VCAA

Marking guide

Question 1 c i	- 1 mark for naming the light-independent stage.
Question 1 c ii	- 1 mark for identifying that ATP is required to provide energy for the production of glucose. - 1 mark for identifying that NADPH is required for the transfer of hydrogen ions.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

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CHAPTER 7

Biochemical pathways through biotechnology

Biochemical pathways are a series of chemical reactions that occur in all living organisms. Each chemical reaction is governed by enzymes and is dependent on the previous reaction occurring in the correct sequence. Any alteration in the sequence of reactions can result in a change that either benefits or damages the organism. Understanding the details in a biochemical pathway allows scientists to make changes that can improve the final outcome.

CRISPR-Cas9 is a complex molecule used by bacteria to defend themselves against viruses. This naturally occurring molecule has been used to modify the genetic material responsible for many of the biochemical pathways in bacteria, algae and yeast. Scientists are using CRISPR-Cas9 to improve on the biochemical reactions that occur in nature.

KEY KNOWLEDGE

→ potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields

→ uses and applications of anaerobic fermentation of biomass for biofuel production.

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

FIGURE 1 Molecular technologies are used to modify the biochemical pathways in algae to improve biomass production.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

7A What is CRISPR-Cas9?

7A Groundwork resource
CRISPR-Cas9

7B What is a genetically modified organism?

7B Groundwork resource
GMOs

7C Describe anaerobic fermentation in plants.

7C Groundwork resource
Anaerobic fermentation

PRACTICALS

HIGH-TECH PRACTICAL **7.1** Micropipette skills and knocking-in a gene

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

7.1 Improving photosynthesis with CRISPR-Cas9

KEY IDEAS

In this topic, you will learn that:

+ CRISPR-Cas9 technologies can be used to improve the efficiency of photosynthesis and crop yields.

For thousands of years, humans have tried to improve the organisms around them. Traditionally, this has occurred through the breeding of plants and animals with favourable desired characteristics. As a consequence, the number of organisms with desirable characteristics increased in each generation, increasing the number of genes. Historically, this breeding method is very inefficient, sometimes taking several generations for the desired traits to become prevalent throughout the population.

Recently, scientists have taken a more direct pathway to select and modify a desired trait in an organism. They do this by identifying the genes responsible for each trait and then use CRISPR-Cas9 to modify the genes of interest.

FIGURE 1 About 1100 years ago, carrots were purple, and it took over 500 years to selectively breed orange carrots with higher beta carotene.

CRISPR-Cas9 technologies

You were introduced to CRISPR-Cas9 in Chapter 3. CRISPR-Cas9 is made up of two parts.

- **CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)** – a series of short single-stranded DNA sequences gathered from viruses that have previously attacked the cell.
- **Cas9** – an endonuclease that acts like a pair of genetic scissors. Cas9 is guided to a specific CRISPR DNA sequence by a sequence of guide RNA (gRNA). Once the complementary RNA binds to the specific DNA segment, the Cas9 enzyme can cut the DNA. In this way, a bacterial cell can remember and defend against the virus if it is exposed to it again.

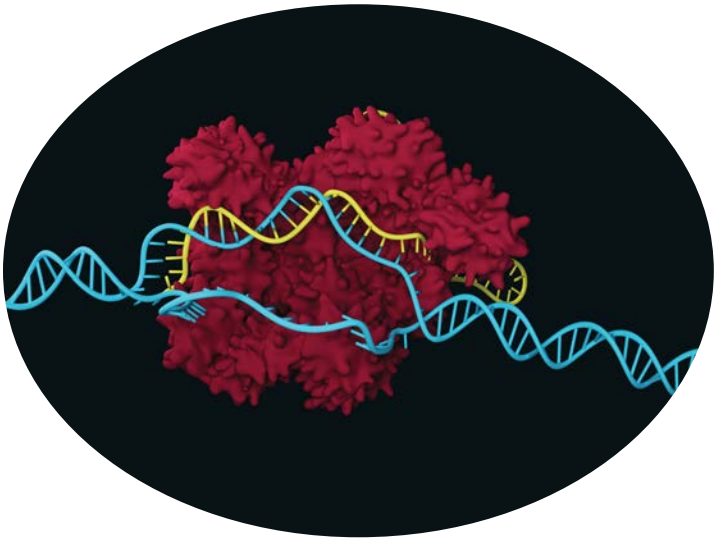

FIGURE 2 The CRISPR-Cas9 protein (red) uses a guide RNA sequence (yellow) to cut DNA (blue) at a complementary site.

Modifying organisms

To modify genes in a cell, scientists make a copy of the DNA (CRISPR) sequence they want to cut. This short sequence of RNA becomes the guide for the Cas9 enzymes, telling them where to cut the DNA. The guide RNA-Cas9 complex can be injected directly into a cell, or the genes for the CRISPR-Cas9 can be placed into a virus that can easily invade the cell.

When the virus infects the cell, the CRISPR-Cas9 genes are injected into the cell, where both genes are transcribed into RNA. The Cas9 gene is translated into protein enzymes and these form a complex with the CRISPR RNA. This complex of the CRISPR sequence and Cas9 enzymes can selectively identify, cut and modify specific genes in the cell's chromosome.

In the processes described here, the CRISPR-Cas9 complex is used to cause the initial selected break in the DNA, while the cell's own repair mechanisms are used to repair the damage.

FIGURE 3 A bacteriophage (virus) inject viral DNA directly into a bacteria cell.

Targeted knock-out

CRISPR-Cas9 can be used to modify the nucleotide sequence of a gene so that it can no longer function. To do this, scientists identify the genes that need to be deactivated and produce an appropriate CRISPR sequence.

1 The DNA sequence is placed in a virus, alongside the genes for the Cas9 enzymes.
2 When the virus is exposed to the cell, it invades the cell and releases the CRISPR-Cas9 genes.
3 The processes of transcription and translation produces the CRISPR-Cas9 complex, which identifies the selected sequence in the cell's specific gene.
4 Once the sequence of DNA that makes up the gene has been identified, the Cas9 enzymes cut it. This activates the cell's normal DNA repair mechanism for single nucleotides, which fixes the cut in a process called **non-homology end joining (NHEJ)**.

The cell's highly efficient NHEJ mechanism tries to repair the cut by replacing single nucleotides. But this process tends to make mistakes and its these mistakes that lead to gene knock-out, because the modified gene is no longer able to function.

non-homology end joining (NHEJ)
a mechanism that repairs double strand breaks in DNA when template DNA is absent

Targeted knock-in

The introduction of a new gene into the DNA of a cell is considered more difficult than interrupting normal gene function or knocking-out genes. Here, CRISPR-Cas9 is used to cut the double-stranded DNA and the cell's normal **homology directed repair (HDR)** mechanism is used. The target gene is also added to the cell as a DNA sequence with ends that are complementary to the cut double-stranded DNA.

homology directed repair (HDR)
a mechanism that repairs double strand breaks in DNA when template DNA is absent

1 CRISPR-Cas9 is used to cut the cell's double stranded DNA.
2 The target gene is added to a DNA sequence where the ends are the same as to the cell's cut DNA. This DNA sequence is inserted into the cell.
3 The cell employs its own repair mechanism called homology directed repair (HDR) and tries to rejoin the cut DNA, however in this process the targeted DNA sequence may be inserted as well since the ends are the same as the cut strands of DNA.
4 If this occurs, the targeted gene is knocked into the cell's DNA.

HDR is much less efficient compared with NHEJ, therefore targeted gene knock-in is more difficult than interrupting normal gene function or targeted gene knock-out.

Targeted knock-down

CRISPR-Cas9 complexes have also been used to interfere with the expression of regulatory genes. This modifies the expression of one or more coding genes, reducing the amount of protein being produced. The effects of the change in transcription of these genes can then be used to examine the importance of all the genes involved

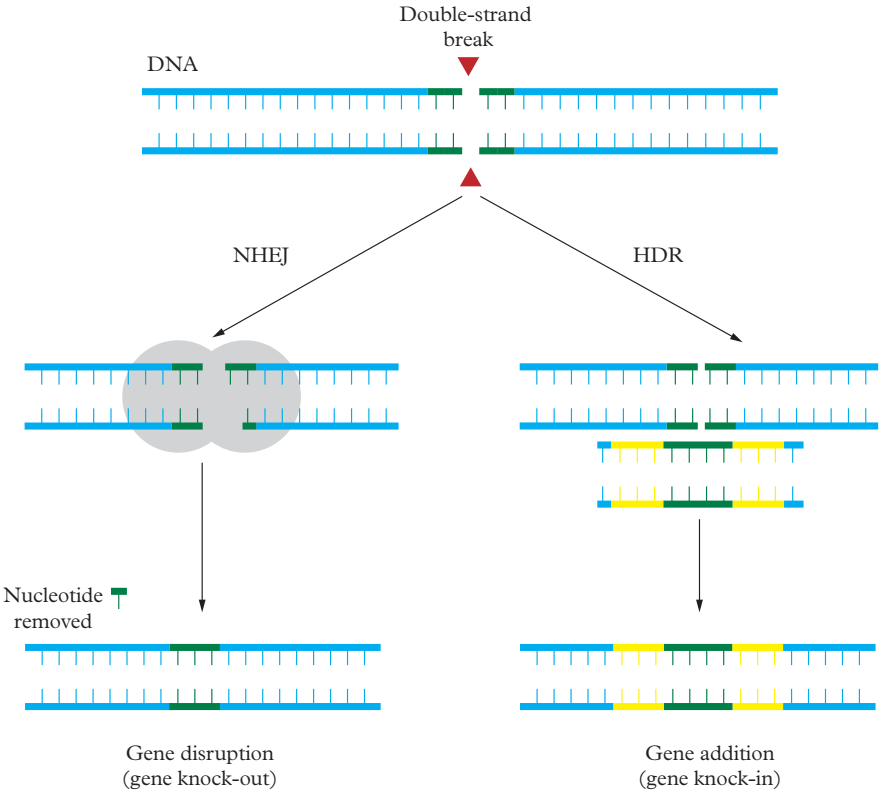

FIGURE 4 CRISPR-Cas9 complexes are used to produce the initial cut in the DNA sequence. The naturally occurring non-homology end joining (NHEJ) mechanism is used to knock-out genes, while the homology direct repair (HDR) mechanism is used to knock-in genes.

Study tip

A genetically modified organism (GMO) is an organism that has had its genetic material modified in some way. A transgenic organism is a genetically modified organism that has had a gene knocked-in from another organism.

Improving photosynthesis

The rate of plant growth and fruit production depends on the plant's ability to use light energy to convert carbon dioxide and water into glucose and oxygen. The amount of light supplied to a plant is rarely constant. Cloud cover and changing light levels can cause the amount of light to vary. When the plant is exposed to low light levels, it needs to be highly efficient, maximising the amount of light captured for photosynthesis.

In contrast, when the Sun is high in the sky, the plant can be exposed to high light intensity that is more than required for photosynthesis. If this happens for a long time, the plant will overheat, and the excess heat will block or inhibit photosynthesis and may even cause the leaves to become bleached. This ultimately causes a decrease in the rate of photosynthesis and a reduction in the growth rate of the plant.

When exposed to high intensity light, plants activate the non-photochemical quenching (NPQ) mechanism. When sunlight initially enters the chloroplast, it excites the electrons in the complex of proteins involving chlorophylls. If there is an excess of sunlight, the level of energy in this electron pathway can cause damage. The NPQ mechanism involves removing the electron's excitation energy and converting it into thermal (heat) energy. This heat can then be safely released, preventing damage to the plant.

When the amount of sunlight decreases due to cloud cover or shadows, the NPQ mechanism is switched off (or relaxed) so the plant does not waste any of the light available. The process of switching on the NPQ process can be efficient, occurring almost as fast as the pupil of an eye contracting when exposed to high light levels. Unfortunately, the relaxation of the NPQ process can be much slower, taking up to half an hour. This means for half an

hour after the shadow stops covering the plant, the plant will not be photosynthesising as efficiently as possible. Computer simulations suggest that this delay in switching off the NPQ could reduce crop productivity by 8–40%, depending on the plant type and sunlight conditions.

Using CRISPR to improve photosynthesis

CRISPR-Cas9 has been used to knock-in or increase the number of copies of the three main genes responsible for controlling the NPQ system. These genes (for the enzymes violaxanthin de-epoxidase and zeaxanthin epoxidase, and the PsbS protein, involved in a photosystem complex) enable the plant to rapidly switch off the NPQ system, limiting the amount of light energy lost through heat. This enables the plants to be more efficient in photosynthesising and producing glucose for energy or other carbon-based molecules.

FIGURE 5 Tobacco plants are used for many science experiments because of their ability to grow rapidly.

Trials using tobacco

Scientists used tobacco plants for the first trial because this plant was fast growing and had been used for many plant-based experiments in the past. This trial provided extensive information about other factors that affected plant growth, allowing the experiments to be effectively controlled. When extra copies of the three genes were knocked-in to the tobacco plants, it was observed that the NPQ process switched off faster than previously measured by carbon dioxide uptake. As a result, the plants were able to be more efficient in producing glucose, causing an average 14–20% increase in the dry weight (or **biomass**) of the plants. This was reflected in the plants growing taller with larger leaves and deeper roots.

biomass
the mass of living organisms in a measured area; used as a measure of a solid renewable energy source (e.g. wood logs) in the production of electricity

This process could be replicated in other food crops such as rice, soy and cassava, increasing growth rate and eventual yield.

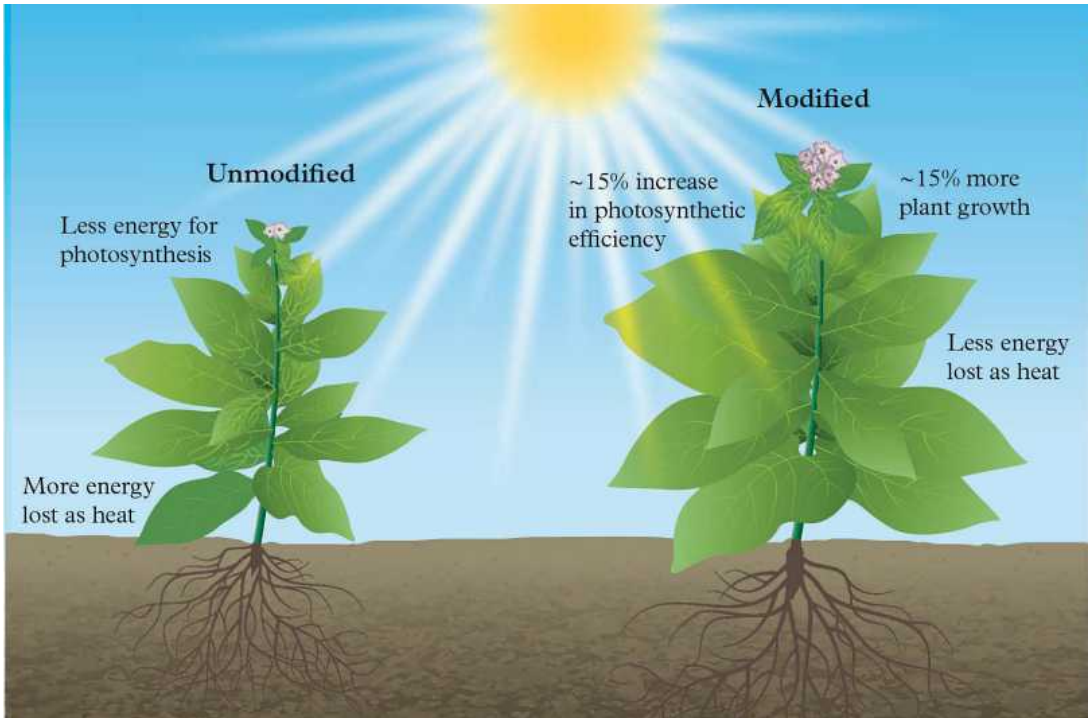

FIGURE 6 Adding extra copies of the genes controlling the NPQ process resulted in taller tobacco plants with bigger leaves and heavier roots.

Improving crop yield

There are other ways that the biomass produced by a plant can be increased. CRISPR-Cas9 has been used to modify genes that control the number and length of branches on tomato plants, preventing the branches breaking under the weight of the fruit. This allows more fruit to be grown, increasing the plant's yield.

Other plants can be modified to increase their resistance to pests and diseases. Rather than fighting the disease, the plants have more energy available to produce seeds. This ultimately increases crop yield.

FIGURE 7 The branches of tomato plants often break from the weight of the fruit.

CHECK YOUR LEARNING 7.1

Describe and explain

1 Explain how photosynthesis is related to biomass.

2 Why was the process of improving photosynthesis first trialled in a tobacco plant?

3 Explain the following terms:
- **a** Knock-in gene
- **b** Knock-out gene
- **c** Knock-down gene

4 Explain the difference between improving organisms through breeding and through CRISPR.

Apply, analyse and compare

5 What is the function of the different parts of the CRISPR and Cas9 complex?

6 Compare the following two processes: non-homology end joining (NHEJ) and homology direct repair (HDR).

Design and discuss

7 Use your knowledge of CRISPR-Cas9 technologies to support or refute the argument that 'the environment has selected the best adaptations'.

8 The deletion of the *Wxl* gene from corn has led to elevated levels of amylopectin and reduced levels of amylose. This allows the cornstarch produced from this corn to survive longer. Design a pamplet that describes how this corn was produced.

7.2 Anaerobic fermentation in biofuel production

KEY IDEAS

In this topic, you will learn that:

+ CRISPR-Cas9 can be used to modify and improve the anaerobic fermentation of biomass for biofuel production.

For many years, fossil fuels have been used as a source of energy. These fossil fuels such as coal, crude oil or natural gas are formed from the anaerobic decomposition of buried dead organisms. This is a limited and non-renewable resource since it can take many millions of years to produce, but can be consumed in a single day.

biofuel
the liquid or gas fuel formed from biomass

In contrast, there is renewed interest in the use of renewable energy sources such as biofuel. **Biofuel** is a source of chemical energy stored in the biomass of plant and animal material. If the biomass can be quickly produced, then it is considered renewable. As seen in Topic 7.1, CRISPR-Cas9 has been used to increase the amount of photosynthesis in some plants. The extra energy (glucose) produced as a result of this process is often used to increase the growth rate of the plant, ultimately increasing the biomass available for biofuel production.

Biofuel production

There are three main forms of biofuel: biodiesel, bioethanol and biogas. These three products are a result of anaerobic fermentation by yeast or bacteria.

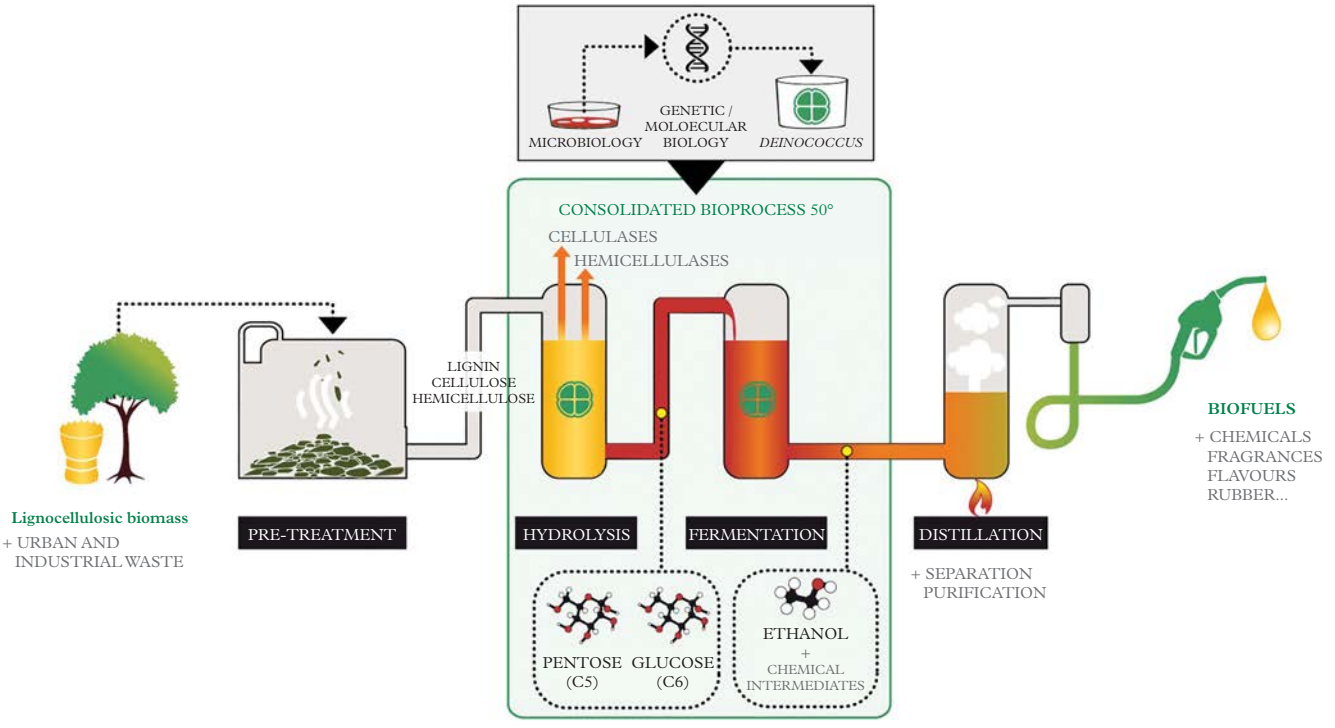

FIGURE 1 The process of biofuel formation can be improved by CRISPR-Cas9 technology, where the fermentation rate of bacteria is increased.

Using CRISPR-Cas9 to identify important genes

Yarrowia lipolytica (family Dipodascaceae) is a harmless aerobic yeast able to grow in cheeses, yoghurts and meat. This is due to its ability to use the enzyme lipase to digest and store fatty acids and triglycerides as a source of carbon. This store of carbon can be used in the production of biomass for biofuel production.

To identify which genes were important in the production and storage of carbon, scientists used CRISPR-Cas9 to systematically knock-out one gene at a time from the yeast. They identified 359 genes that were essential for the growth of the organism, those that break down ethanol, and four genes that are involved in the production of the carbon-rich lipids that could be used as a biofuel. CRISPR-Cas9 is currently being used to modify these genes to increase the biomass production of the yeast, or to increase the amount of ethanol produced as a result of anaerobic fermentation.

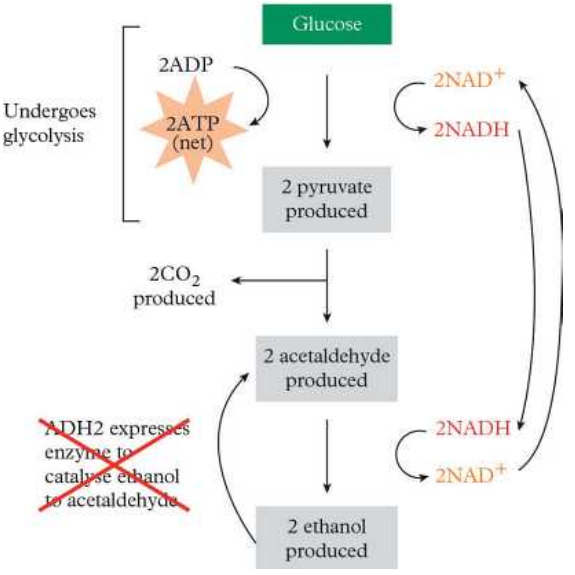

FIGURE 2 CRISPR-Cas9 technology allows the *ADH2* gene to be knocked-out, increasing the amount of ethanol produced for biofuel.

Preventing ethanol breakdown

One of the genes identified was the *ADH2* gene. Ethanol is toxic to yeast cells. The *ADH2* gene produces alcohol dehydrogenase II, an enzyme that allows the yeast to break down the ethanol to acetaldehyde, and prevent it from building up to a toxic level. Knocking-out this gene prevents the yeast from breaking down the ethanol (Figure 2), resulting in improved yields of **bioethanol** by up to 75%.

bioethanol ethanol produced by the anaerobic fermentation of organic matter in plants and animals

Biodiesel-producing algae

Algae are a group of photosynthesising aquatic organisms that includes seaweeds such as kelp or phytoplankton, and cyanobacteria. All algae lack true roots, stems or leaves and can be single-celled or multicellular. Although algae produce lipids for essential purposes such as membranes or lipoproteins, the level of fats is usually too low for the efficient production of **biodiesel**.

biodiesel the liquid fuel diesel that has been produced by the breakdown of plant and animal lipids

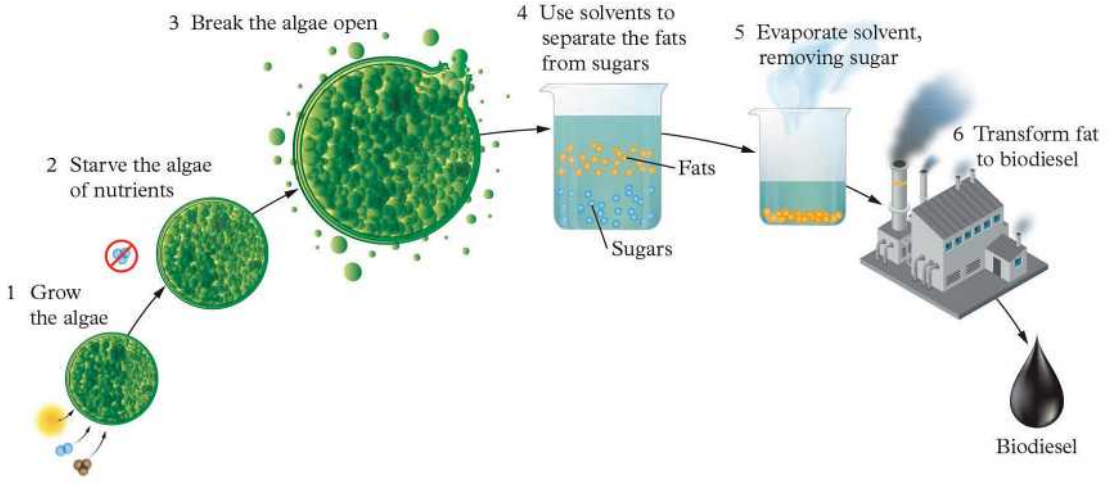

FIGURE 3 The fatty acids and triglycerides from algae can be used to generate biodiesel.

To remedy this, scientists have used CRISPR to knock-out the genes that limit the production of lipids. This causes the genetically modified algae to produce up to 5 g of lipid per metre per day – twice as much as the same algae in the wild. This lipid can be used as biomass in the production of biofuel.

Biogas production

Biogas is a term used to describe a mixture of gases (including methane) that can be used as a fuel to generate electricity. Biogas is generated through the fermentation of organic waste such as manure, sewage, food waste and agricultural waste. Often the organic waste requires pre-treatment with hydrolysis enzymes before the anaerobic bacteria digest it to produce the biogas. This pre-treatment could be bypassed if CRISPR-Cas9 was used to knock-in the hydrolysis enzymes into the bacteria. This would make the production of biogas more efficient.

FIGURE 4 A biogas generator

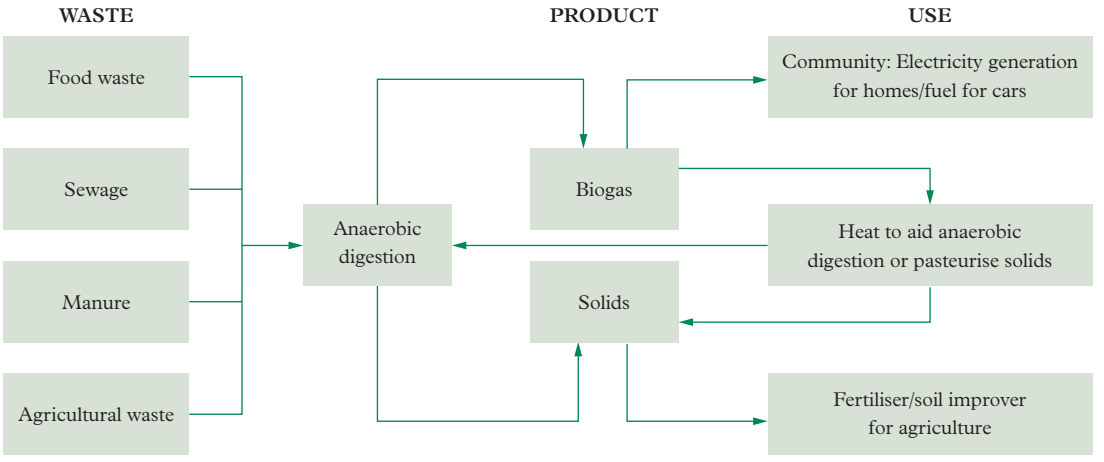

FIGURE 5 The process of biogas production

biogas
the combination of gases, including methane, produced by the anaerobic fermentation of organic matter from plants and animals

CHECK YOUR LEARNING 7.2

Describe and explain

1 Define the term 'biofuel'.
2 Describe the three types of biofuel.
3 Describe how CRISPR-Cas9 was used to identify the genes important in the production of biomass.

Apply, analyse and compare

4 How does an increase in biomass aid in the production of biofuel?
5 Compare the production of biodiesel by algae and the production of bioethanol by yeast.

Design and discuss

6 It is claimed that producing biofuel crops is harmful for the environment. Discuss why you agree or disagree with this claim. Justify your response with evidence.

CHAPTER 7

Review

Chapter summary

7.1

- CRISPR-Cas9 can be used to increase photosynthesis in plants by knocking-in more NPQ genes, ultimately increasing crop yields in plants.
- In targeted knock-out, CRISPR-Cas9 disrupts genes so that they are no longer able to function
- In targeted knock-in, CRISPR-Cas9 introduces a new gene

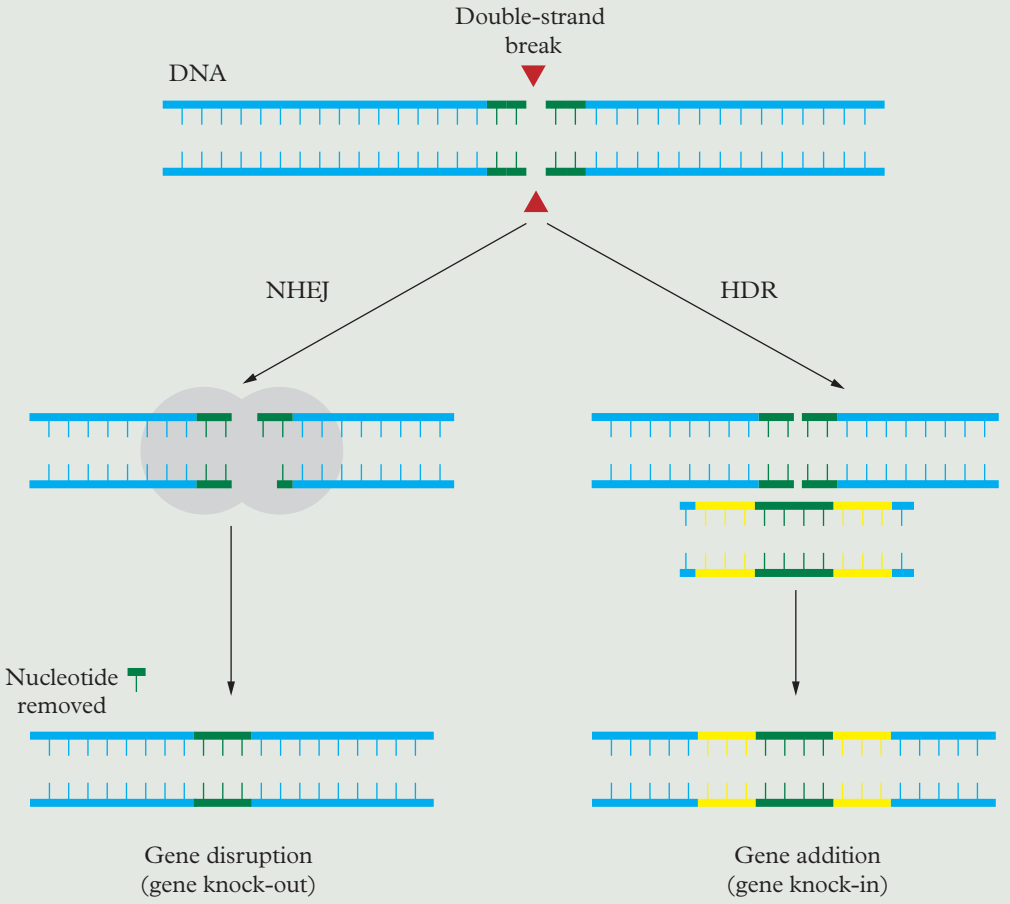

FIGURE 1 The CRISPR process

7.2

- CRISPR-Cas9 can be used to change and advance the anaerobic fermentation of biomass for biofuel production (such as biodiesel).

Revision questions

Multiple choice

1 The process of using CRISPR to add a gene to a chromosome is called:
- **A** knock-down.
- **B** knock-in.
- **C** knock-out.
- **D** knock-up.

2 The process of using CRISPR to decrease the amount of protein produced by a gene is called
- **A** knock-down.
- **B** knock-in.
- **C** knock-out.
- **D** knock-up.

3 The process of using CRISPR to prevent a gene from being expressed is called:
- **A** knock-down.
- **B** knock-in.
- **C** knock-out.
- **D** knock-up.

4 CRISPR is:
- **A** an enzyme that cuts DNA.
- **B** short repeated sections of DNA.
- **C** short repeated sections of RNA.
- **D** a protein with short repeating sections.

5 Cas9 is:
- **A** an enzyme complex.
- **B** a short section of DNA.
- **C** a short section of RNA.
- **D** a protein.

6 When exposed to light of high intensity, an unmodified tobacco plant will:
- **A** slow its rate of photosynthesis.
- **B** increase its rate of photosynthesis.
- **C** increase its rate of glucose production.
- **D** slow its rate of heat production.

7 Anaerobic fermentation in yeast produces:
- **A** oxygen.
- **B** glucose.
- **C** ethanol.
- **D** biodiesel.

8 Biodiesel is produced from:
- **A** the digestion of lipids.
- **B** ethanol.
- **C** methane.
- **D** the digestion of proteins.

9 Which of the following is transcribed and translated when introduced into a cell?
- **A** CRISPR only
- **B** CRISPR and Cas9
- **C** Cas9 only
- **D** Neither CRISPR nor Cas9

10 Which of the following are biofuels?
- **A** Biodiesel and biogas
- **B** Biodiesel and ethanol
- **C** Biogas and ethanol
- **D** Biogas, biodiesel and ethanol

Short answer

Describe and explain

11 Explain how CRISPR-Cas9 can be used to knock-out a gene.

12 Explain how CRISPR-Cas9 can be used to improve the biomass produced through photosynthesis.

13 Explain how CRISPR-Cas9 can identify a specific DNA sequence in a cell.

14 What is biogas and how does it form?

15 Describe how anaerobic digestion plays a role in biogas production.

16 Identify two sources of biomass that can be anaerobically fermented.

17 Describe two uses for biofuels that have been produced from biomass.

18 Explain how algae can be used to produce biofuel.

Apply, analyse and compare

19 Explain why an organism that has a knock-out gene is not classified as a transgenic organism.

20 Compare a genetically modified organism and one that has had a gene knocked-in.

21 Explain why it might be more difficult to knock-in a gene than to knock-out a gene.

22 Explain three ways CRISPR-Cas9 can be used to increase the yield of a crop.

23 Examine Figure 2 showing Australia's use of renewable energy in May, 2018.

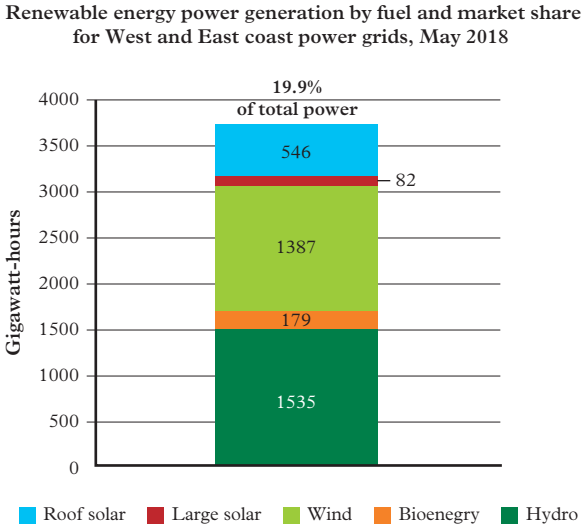

FIGURE 2 Australia's use of renewable energy in May, 2018

a Describe Australia's use of renewable energy sources in May, 2018.

b Compare Australia's use of bioenergy to other renewable energy sources.

c Describe one ethical reason that could account for Australia's level of bioenergy use.

d Discuss how the use of CRISPR to improve biofuel production could impact Australia's use of biofuels.

Design and discuss

24 Design an infographic to explain how CRISPR-Cas9 can modify the genetic information in a cell.

25 Recent reports in scientific literature suggest that the CRISPR sequence might not be as accurate as first thought. Discuss the possible implications if this is found to be correct.

26 Gene manipulation has been used to knock-in the *Bt* gene from the bacterium *Bacillus thuringiensis* to a cotton plant. This gene produces a protein that is toxic to insects. Discuss how this could increase the crop yield of cotton.

FIGURE 3 How could cotton benefit from genetic manipulation?

27 CRISPR-Cas9 has been used to identify a gene that increases the ability of mangroves to survive in salt-ridden environments.

a Suggest the approach scientists could have used to identify this gene.

b This gene was then inserted into other plants so that they could grow in high-salt soils. Suggest how CRISPR-Cas9 aided this process.

28 In 2020, Japanese scientists used CRISPR technology to improve the rate of photosynthesis in rice by editing the Rubisco enzyme.

Rubisco is an enzyme that acts as the initial catalyst for photosynthesis, which turns CO_2 into organic carbon. But Rubisco can have low catalytic activity and be inhibited by oxygen, which limits photosynthesis.

Rice is a C3 plant that uses regular biochemical pathways for photosynthesis.

C4 plants such as corn and sorghum, on the other hand, have acquired a mechanism to concentrate CO_2 (the C4 photosynthetic pathway).

The scientists wanted to test whether C3 plants could improve their rate of photosynthesis if they used a C4 photosynthetic pathway.

To investigate this question, they used CRISPR technology to genetically modify rice (a C3 plant). They transferred RbcS from a C4 plant (sorghum) into rice to increase the catalytic rate of rice Rubisco 1.5 times. Next, the rice RbcS gene was knocked out of the hybrid rice, leaving behind the sorghum RbcS.

The scientists tested the rate of photosynthesis in the unmodified rice and sorghum plants as well as the genetically modified rice plants.

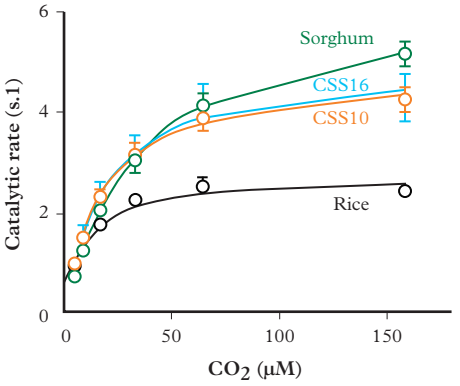

FIGURE 4 Catalytic activity of Rubisco: Graph showing the relationship between CO_2 concentration and the catalytic activity of Rubisco. CSS10 and CSS16 lines represent two trials of the sorghum RbcS incorporated/rice RbcS knock-out.

a Use the example of genetically modifying Rubisco in C3 plants to describe the process of CRISPR Cas9 gene editing.

FIGURE 5 Rice is a C3 plant.

b Suggest a hypothesis for the scientists' investigation.

c Identify the independent and dependent variables of the investigation.

d Examine the scientists' results in Figure 4. Compare the rate of photosynthesis in the three trials.

e Draw a conclusion for the study using the data presented. Do C3 plants improve their rate of photosynthesis if they are able to use a C4 photosynthetic pathway?

f The CSS (sorghum RbcS incorporated/rice RbcS knockout) is a genetically modified crop. Discuss one ethical implication of this study.

g Use the data provided to justify whether or not farmers should grow the hybrid CSS crop.

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Exam essentials

Responding to questions

In your exam, you may be expected to use the mark allocation and space provided as a guide to how much to write in your response.

Use the mark allocation as a guide

When answering questions, do not just write down everything you know about the topic; the examiner can only give you marks for information that directly answers the question. Writing more than you need to also wastes your time in the exam.

Sometimes a single word is enough to answer a question. The mark allocation can help you to identify how much information to provide. For example, if a question is worth 1 mark, you are probably not expected to write a large paragraph response.

The following question is taken from the 2018 VCE Biology Examination. Read the question carefully, then consider how to best used the mark allocation and the space provided to guide your response.

QUESTION 9c&d (2018 Northern Hemisphere Biology Written Examination)

The CRISPR technique is a gene editing method. It involves a protein called Cas9 and a short piece of guide RNA (gRNA). The gRNA leads Cas9 to a gene in the DNA that scientists wish to edit. Cas9 is an endonuclease that acts in a similar way to a restriction enzyme.

c What action is Cas9 expected to perform? 1 mark

DNA ligase is required for the next stage of the editing process.

d What role would DNA ligase play? 1 mark

Source: 2018 Biology Written Examination (Northern Hemisphere) Question 9c&d, Short answer, reproduced by permission © VCAA

Response 1

c Cut the DNA strands at a particular sequence.
d Join the DNA strands.

Succinct response that provides all information required by the question.

Each of these succinct responses would have received 1 mark.

Response 2

c Cas9 is an RNA guided enzyme that acts to cut the DNA at a particular sequence of the DNA strand, acting like molecular scissors in the process. It can do this in both natural and artificial CRISPR/Cas9 systems.

d DNA ligase is an important enzyme responsible for joining breaks in DNA. It is important for the CRISPR technique since it joins together the sequences that were cut by the Cas9 enzyme.

The question does not ask for this information.

No marks will be given for this additional information.

The question does not ask for judgements about the value of DNA ligase.

This is not required to answer the question. While correct, it is wasting the student's time to write this.

Although both of these responses would also receive full marks, they would not have received any extra marks for the additional detail, and they would have wasted time that could be spent answering other questions.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 9e (2018 Northern Hemisphere Biology Written Examination)

The CRISPR technique can be used to insert new genes or to delete sections of a gene.

While some scientists have recognised the potential benefits of using the CRISPR technique to edit genes in the future, other scientists have expressed concern.

e State one potential benefit some scientists may have recognised and one concern other scientists may have had. 2 marks

Benefit – making changes to the DNA of human embryos

Concern – incorrectly edit the DNA

Source: 2018 Biology Written Examination (Northern Hemisphere) Question 9e, Short answer, reproduced by permission © VCAA

Marking guide

Question 9 e	- 1 mark for identifying a suitable benefit, one of: • eliminate genetic disorders from humans prior to birth • increase success rate of IVF. - 1 mark for identifying a suitable concern, one of: • using embryos of research that are viable • producing genetically modified humans • introducing changes in the DNA that can then be inherited. (And other suitable responses in each case.)

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

Check your Student obook pro for these digital resources and more: pro

Video tutorial
Use the mark allocation as a guide

Weblink
Past examinations and examiners' reports

UNIT 3

Area of Study 1 – Practice SAC

Analysis and evaluation of a contemporary bioethical issue

This practice School-assessed Coursework (SAC) should take 50 minutes and covers Key Knowledge from Unit 3, Area of Study 1. If you have access to the *Biology for VCE Units 3 & 4 Student workbook*, you can practise your Key Science Skills by completing the Evaluating Ethics activities before attempting this practice assessment.

Mutant cows

Each year, 1.4 million Australian dairy cows produce 9.3 billion litres of milk. To prevent bruising and to keep both the cows and farmers safe, these cows must have their horns removed each year.

In 2015, an American genetics company announced that they had successfully genetically engineered two hornless Holstein bulls (male dairy cattle) by inserting a DNA sequence that stops Angus cattle from growing horns. This meant the Holstein bulls never fully developed their horns. The first hornless bull (Spotigy) was given to research, while the second (Buri) went on to produce 17 offspring. Their success was published in several scientific papers and Buri's sperm was shipped around the world to create herds of hornless dairy cows.

Early in 2019, the US Food and Drug Administration (FDA) identified that Buri (and his sperm) contained bacterial DNA. Instead of inserting the gene that led to hornlessness, the whole bacterial plasmid had been inserted by accident. This meant that Buri's genome contained the entire DNA sequence of the plasmid, including that of antibiotic resistance.

The company was embarrassed that they did not identify the error.

> 'It was not something expected, and we didn't look for it,'

said the CEO of the company that owns the animals.

The FDA scientists suggested that their find

> 'highlighted a potential blind-spot in standard genome-editing screening methods'.

FIGURE 1 A Holstein cow with its horns

1 Dehorning occurs when an animal's horn is removed by cutting through the bone and horn tissue. This is usually done without an anaesthetic. Suggest why the RSPCA (Royal Society for the Prevention of Cruelty to Animals) is in favour of breeding animals without horns.

2 Draw a labelled diagram that shows the process of introducing the DNA sequence from the Angus cows into the genome of the Holstein bulls.

3 Recreate and complete the table below to compare the similarities and differences between genetic engineering and traditional breeding methods.

Similarity between genetic engineering and traditional breeding methods	Difference between genetic engineering and traditional breeding methods

4 When the cows were first bred, the company claimed that Spotigy and Buri were not genetically modified organisms (GMOs). Instead, they claimed the cows were products of 'precision breeding' because the process replicated what nature could already do, but with greater precision.

a Do you agree or disagree?

b Explain your position, using evidence to justify your claims.

5 Would the bacterial DNA affect the milk produced by the cows? Use your understanding of genetics to justify your answer.

6 In 2018, the Brazilian government classified the hornless cattle as non-GMO and allowed the importation of some of Buri's sperm in an attempt to breed a herd of hornless cattle. A small herd of cattle were bred from the sperm before the bacterial DNA was identified in their genome. The Brazilian government now needs to decide what action to take with Buri's offspring.

a Identify two actions the Brazilian government could take.

b Recommend which option the Brazilian government should choose. Justify your decision.

7 In April 2019, the Australian government decided that site-directed nuclease (SDN1) techniques would not be classified as genetic modification. This process involves using gene editing to cut the DNA at a specific place and allowing the cell's natural DNA repair process to fix the damage, without intervention.

Would Buri's offspring be classified as genetically modified in Australia?

8 How is the SDN1 technique similar or different to mutation?

9 Suggest why the Australian government has allowed the use of SDN1 techniques to modify the genomes of organisms.

10 Would you drink the milk or eat the meat from Buri's offspring? Why or why not?

Area of Study 2 – Practice SAC

Comparison and evaluation of biological concepts, methodologies and methods, and findings from three student practical activities

This practice School-assessed Coursework (SAC) should take 50 minutes and covers Key Knowledge and Practicals 4.1, 5.1 and 6.3 from Unit 3, Area of Study 2. If you have access to the *Biology for VCE Units 3 & 4 Student workbook*, you can practise your Key Science Skills by completing the Experiment Explorer activities before attempting this practice assessment.

Photosynthesis, enzymes and cellular respiration

The photosynthesis of the single-celled organism *Chlorella* was determined by trapping the organism in an alginate ball. This biochemical pathway is controlled by a series of enzymes, one of which is Rubisco. Rubisco is the enzyme that catalyses the binding of carbon dioxide to RuBP as part of the light-independent reaction.

1 After completing Practical 5.1 on factors that affect photosynthesis, describe the purpose of the 'Dark negative control' and the 'Light negative control'.

2 Both the 'Dark negative control' (with no *Chorella*) and the 'Dark test' (with *Chlorella*) were covered in foil and placed under the light. Suggest why they were not placed inside a cupboard in the dark.

3 All experiments should be carefully controlled to ensure their validity. Describe what measures you used to control your experiment when you tested the effect of pH on the enzyme amylase during Practical 4.1.

4 Use your knowledge of molecular biology to explain how the amylase enzyme was affected by a pH outside its optimum range.

5 At high temperatures, the enzyme Rubisco is less effective, slowing the light-independent reaction. Explain how high temperatures could affect the rate of photosynthesis.

6 Describe why the rate of photosynthesis can affect cellular respiration in a plant.

7 Recently you completed an experiment on factors that affect aerobic cellular respiration. Use your results to describe the ideal conditions and nutrients for yeast to survive and grow.

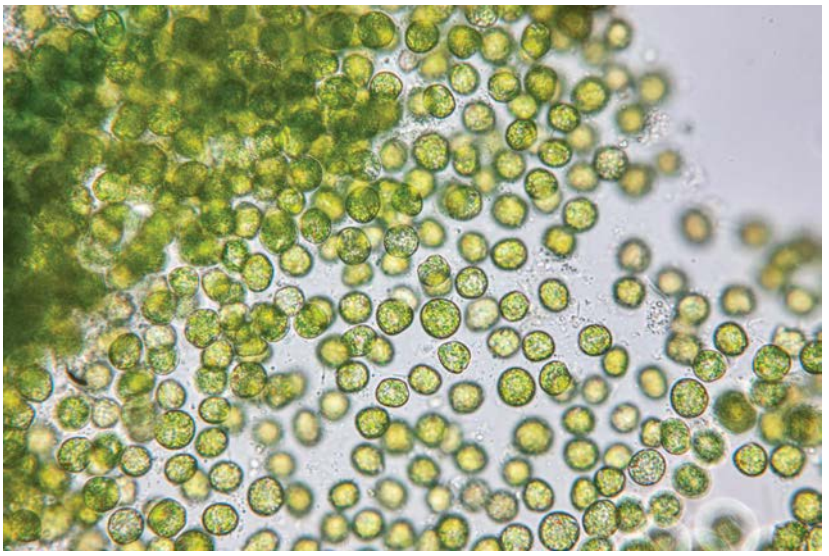


FIGURE 1 *Chlorella* is a single-celled algae able to photosynthesise through the action of enzymes.

8 After completing Practical 6.3 on the effect of temperature, sucrose concentration, pH and nutrient type on yeast, predict why a plant kept in a cupboard would fail to grow.

9 Suggest one way you could improve the accuracy and precision of one of your experiments on photosynthesis, cellular respiration or enzymes.

At high temperatures, RuBP binds to oxygen instead of carbon dioxide, producing the more toxic phosphoglycolate compound. The phosphoglycolate is cycled through the mitochondria in a process called photorespiration. This process uses ATP and reduces the effectiveness of photosynthesis. Genetic engineering has been used to knock-in a new Rubisco gene from red algae into tobacco plants. This new gene is more efficient at binding carbon dioxide to the RuBP.

10 Describe why improving the action of Rubisco could improve the growth rate of a plant.

FIGURE 2 The tobacco plant can bind carbon dioxide more efficiently with a gene from red algae.

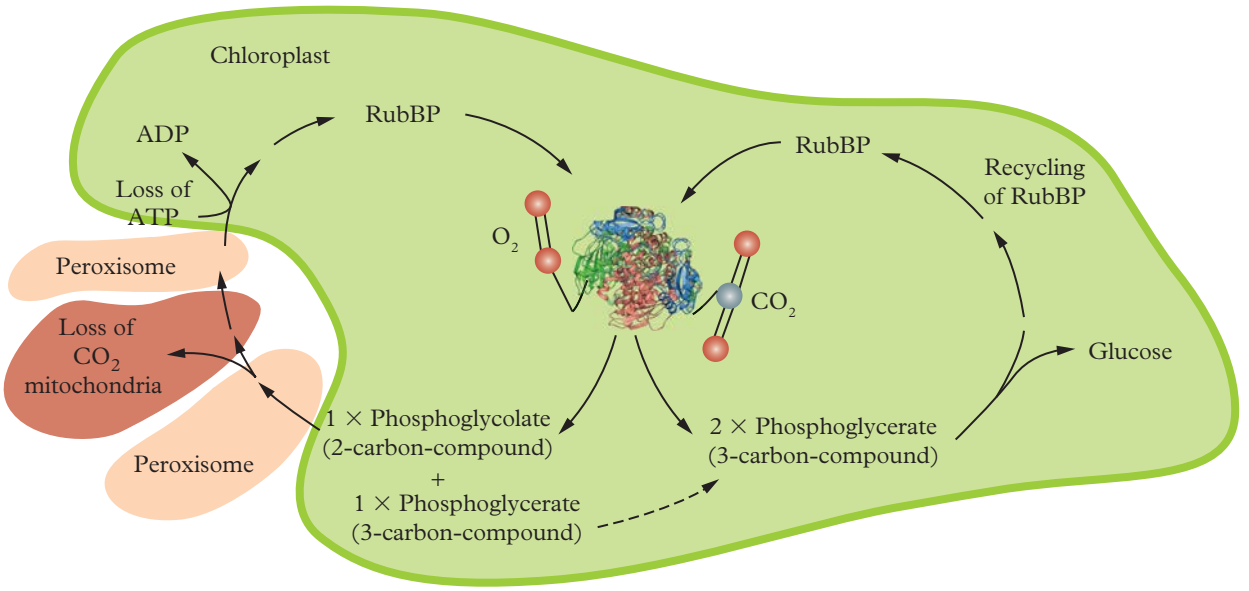


FIGURE 3 RuBP is a molecule that can bind carbon dioxide in the light-dependent reaction or oxygen in the photorespiration reaction.

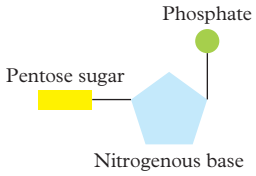
UNIT 3

Practice exam questions

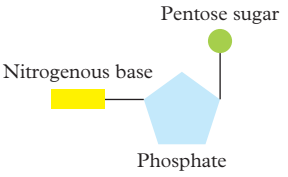
Multiple choice (Total = 10 marks)

1 Which of the following is the correct structure of a DNA nucleotide?

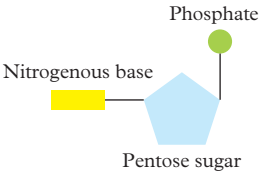
A



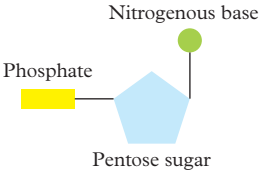
B



C



D



2 Myoglobin is a protein found in muscle cells that accepts oxygen from haemoglobin proteins in red blood cells. Which of the following does myoglobin not contain?

A α-helices
B Random coils
C mRNA codons
D β-pleated sheets

3 Which of the following enzymes is responsible for repairing DNA damage due to UV exposure?

A DNA ligase
B Endonuclease
C Reverse transcriptase
D DNA polymerase

4 In gel electrophoresis, the strands of DNA move through the gel because:

A DNA is negatively charged, and the end of the gel is positively charged.
B DNA is attracted to a magnet at the end of the gel.
C DNA is positively charged, and the end of the gel is negatively charged.
D DNA is repelled by a magnet at the start of the gel

5 Biotin (vitamin B7) is a water-soluble non-protein molecule used in a wide range of metabolic processes. Biotin binds to several carboxylases and can then metabolise fats and carbohydrates. Biotin is an example of:

A a holoenzyme.
B an apoenzyme.
C an enzyme.
D a coenzyme.

6 Which type of plants are more likely to lose fixed carbon through the action of Rubisco in hot, dry environments?

A CAM plants **B** C_4 plants
C Cacti **D** C_3 plants

7 A graph of the rate of cellular respiration versus glucose availability or oxygen concentration would plateau. However, a graph of cellular respiration versus temperature would not plateau, because:

A cellular respiration will increase as long as the temperature increases.
B the enzymes in cellular respiration will denature at a high temperature.
C the rate of cellular respiration continues to drop as the temperature increases.
D temperature doesn't affect the rate of cellular respiration.

8 In a low-oxygen environment, the rate of photosynthesis will:

A drop. **B** rise.
C stay the same. **D** not occur.

9 Glycolysis occurs in the cytoplasm.

Which of the following inputs and outputs are correct for glycolysis?

	Inputs	Outputs
A	2ATP Glucose ($C_6H_{12}O_6$) 2ADP	$6H_2O$ 32 or 34ATP $10NAD^+$ $2FAD^+$
B	$6O_2$ 10NADH $2FADH_2$ 32 or 34ADP	$6H_2O$ 32 or 34ATP $10NAD^+$ $2FAD^+$
C	2ATP Glucose ($C_6H_{12}O_6$) 2ADP $2NAD^+$	2 pyruvate 4ATP (net gain of 2ATP) 2NADH
D	2 pyruvate 2 coenzyme A $2\ NAD^+$	2 pyruvate 4ATP (net gain of 2ATP)

10 Which of the following organisms is not a potential target for the use of CRISPR-Cas9 to produce biofuels?

A Yeast **B** Algae
C Anaerobic bacteria **D** Fish

Short answer (Total = 20 marks)

11 Refer to the RNA codon table on page 51. (4 marks)

a Determine the code of the complementary mRNA strand and the amino acid sequence for the DNA sequence below. The first part of the answer has been completed. 2 marks

DNA strand	TACGTTTATTCCTCCCGCTTCAAAAA CCGTCGATCGCTAGCAACT
mRNA strand	AUG
Amino acid sequence	Met-

b Why would it be impossible to reconstruct the DNA sequence if given the amino acid sequence first? (2 marks)

12 Describe the relationship between each of the following. (2 marks)

a PCR and *Taq* polymerase 1 mark

b Gel electrophoresis and agarose gel 1 mark

13 Rice is the most widely consumed grain in the world. Golden rice is a genetically modified organism that contains two genes inserted into the rice genome: one from daffodils and one from soil bacteria. Golden rice produces high levels of vitamin A, an essential nutrient lacking in the diets of many people living in low-income countries. A lack of vitamin A can lead to blindness and frequent infections in children. (2 marks)

a Besides being genetically modified, can golden rice also be considered transgenic? Explain your answer. 1 mark

b Why was the development of golden rice seen as important to low-income countries? 1 mark

14 Consider the process of photosynthesis. (8 marks)

a What is the function of photosynthesis? 1 mark

b The overall simplified reaction for photosynthesis is:

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll + enzymes}]{\text{Sunlight}} C_6H_{12}O_6 + 6O_2$$

Use the chemical equation for photosynthesis to complete the following table. Include the correct number of molecules in the inputs and outputs column. 6 marks

Process	Location	Inputs	Outputs
Light-dependent reaction		$\_H_2O$ 18ADP $\_NADP^+$	$\_O_2$ _ATP 24NADPH
Light-independent reaction		$\_CO_2$ 18ATP _NADPH	$C_6H_{12}O_6$ $6H_2O$ _ADP $24NADP^+$

c The Calvin cycle (light-independent reaction) was previously known as the dark reaction. Why was it more accurately renamed the light-independent reaction? 1 mark

15 Explain one way that CRISPR-Cas9 is being used in biofuel research. 4 marks

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

FIGURE 1 Scientists recovering the remains of a young Mexican female skeleton aged 12 000–13 000 years old.

The human body is constantly under attack from other organisms, such as multicellular pathogens, harmful bacteria and non-living viruses and prions. Like a castle under siege, the body must have a system in place to prevent invasion by the attackers. The immune system adapts by remembering invaders so that they will not be able to cause damage a second time.

Occasionally, the immune system can make mistakes and overreact to harmless particles such as peanuts or pollen. In extreme cases, the body even starts to attack its own cells. Understanding how the immune system works allows us to 'hack the system', prepare for attacks with vaccinations, or even manipulate immune cells to recognise and destroy cancer cells.

Pathogens such as bacteria and viruses constantly mutate and change over time to hide from our immune systems. Both mutation and the ability of bacteria to transfer genetic material to other bacteria have led to their increasing ability to resist antibiotics.

Modern palaeontologists investigate evidence of evolution by scrutinising both living and fossilised organisms. These scientists also use similarities and differences in protein structures and gene mutations to explain how organisms change with time. The evolution of our own species has been examined from evidence of our early ancestors in Africa, to the movement of multiple species across Europe and Asia, and the migration of *Homo sapiens* into Australia.

A greater understanding of the mechanism of evolution has allowed us to manipulate how organisms change, though this provides an ethical challenge. What kinds of change are natural processes and how much change is caused by modern humans?

Outcomes

On completion of this unit, students should be able to:

→ analyse the immune response to specific antigens, compare the different ways that immunity may be acquired and evaluate challenges and strategies in the treatment of disease

→ analyse the evidence for genetic changes in populations and changes in species over time, analyse the evidence for relatedness between species, and evaluate the evidence for human change over time

→ design and conduct a scientific investigation related to cellular processes and/or how life changes and responds to challenges, and present an aim, methodology and method, results, discussion and a conclusion in a scientific poster.

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Area of Study 1	How do organisms respond to pathogens?	Chapters 8–10, pages 188–269; Practice SAC, pages 376–377
Area of Study 2	How are species related over time?	Chapters 11–14, pages 270–375; Practice SAC, pages 378–380
Area of Study 3	How is scientific inquiry used to investigate cellular processes and/or biological change?	Practice SAC, pages 381–385

CHAPTER 8

Antigens

All plants, fungi and animals are exposed to potential disease-causing agents every day. Some of these agents, such as bacteria, fungi and protozoans, are cellular, while others, such as viruses and prions, are classified as non-living organisms. To survive against pathogens, every organism needs to have defence mechanisms. This includes barriers to keep pathogens out, ways to identify foreign substances, and an appropriate response that is rapid and present from birth.

KEY KNOWLEDGE

- → physical, chemical and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants
- → 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
- → initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens

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FIGURE 1 Viruses come in all shapes and sizes, such as the COVID-19 SARS-CoV-2 virus, which created a global pandemic in 2020. Viruses get marked by antigens in the human body to create an immunity.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

8A Identify three ways diseases can spread between humans.

8A Groundwork resource
Spreading diseases

8B How does skin protect an organism from infection?

8B Groundwork resource
The first line of defence

8C What is a pathogen?

8C Groundwork resource
Pathogens

PRACTICALS

PRACTICAL **8.1** Plant defence mechanisms

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

8.1 Barriers as preventative mechanisms

KEY IDEAS

In this topic, you will learn that:

+ plants and animals have innate, non-specific barriers to prevent entry by pathogens
+ barriers that protect an organism can be physical, chemical or biological.

infectious disease a disease caused by a pathogen that can be transferred between hosts

innate inheritable feature, e.g. the innate immune system is present from birth

Infection diseases are caused by pathogens that are transferred from one organism to another. All organisms are exposed to potential pathogens. To survive against these pathogens, each organism must have several layers of defence. If all members of a species have inherited an identical mechanism or response when exposed to a stimulus, it is called **innate**. The innate immune system:

- is non-specific, meaning it responds to all foreign substances in the same way
- produces no memory cells (for long-lasting immunity).

There are many ways a pathogen can enter a multicellular organism. In animals, pathogens can enter through the eye, nose and mouth, the bladder and the reproductive tracts. In plants, pathogens can enter through stomata – the small openings that enable the exchange of oxygen and carbon dioxide into and out of the plant. Both plants and animals have evolved barriers to prevent potential pathogens from entering.

Barriers

There are three types of barriers that an organism can use to defend itself. These are physical, chemical and biological barriers. Physical and chemical barriers are often considered the **first line of defence**.

first line of defence the physical and chemical barriers that protect an organism from infection (such as intact skin and mucus)

Physical barriers

Physical barriers in plants and animals are non-specific and have evolved to keep out all pathogens.

Plant physical barriers

Plant physical barriers include:

- **Waxy cuticle** – the leaves of plants have an outer layer of wax called a cuticle that prevent pathogens from bonding to the surface of the plant.
- **Rigid cell wall** – this provides a structural barrier that prevents fungal and bacterial pathogens entering cells.

Study tip

Skin can be easily broken, allowing pathogens to enter. For this reason, skin only remains a barrier while it is intact.

Animal physical barriers

Animal physical barriers include:

- **Exoskeletons in insects** – this prevents pathogen entry.
- **The skin barrier** – skin cells are tightly packed, preventing foreign cells entering. A layer of dead cells on the skin's surface can also be shed, along with any pathogens clinging to them.

FIGURE 1 Ants have a hard exoskeleton that protects them from pathogens.

- **Mucous membranes** – mucus lining the nose, mouth, eyes, lungs or digestive tract traps pathogens that can then be ejected by mechanisms such as coughing or sneezing.

Chemical barriers

If a pathogen is able to get past the physical barrier, many organisms have chemical molecules they produce in order to protect themselves.

Plant chemical barriers

Plant chemical barriers include:

- **Toxins** – some plants produce molecules that are toxic to predators, including deadly odours, bitter tastes or poisons. Secondary metabolites are compounds that are poisonous to animals that ingest them. For example, tomato plants (*Solanum lycopersicum*) produce molecules that interrupt the breakdown of leaves in the digestive system of insects.
- **Chemical messengers** – some infected or damaged plant cells can send a chemical messenger to warn neighbouring cells. This may cause the surrounding cells to produce more toxic molecules or apoptose. If all the cells surrounding the infection die, the infected cell will be sealed off and the pathogen will be unable to spread further.

Animal chemical barriers

Animal chemical barriers include:

- **Enzymes** – for example, lysozymes in tears, saliva and breastmilk inhibit the survival and growth of bacteria.
- **Acids** – bacteria that land on skin are inhibited by the fatty acids secreted by skin cells. This is why washing your skin too often can sometimes encourage the growth of bacteria, such as those that cause pimples. The acid in the stomach has a pH of around 2, strong enough to digest most bacterial cells. Vaginal secretions and urine are also slightly acidic, while the fluid surrounding sperm contains defensins (chemicals that disrupt the structure of bacterial cell membranes) – and these are slightly alkaline.

Microbiota barriers

The **microbiota** are the range of microorganisms (including bacteria) that are found in and on all animals. On our skin and in our digestive and reproductive systems, natural flora (bacteria) provide a biological barrier against pathogens. Any pathogenic bacteria must compete with the microbiota for nutrients or space. Some microbiota can also control the local environment, making it difficult for pathogens to survive long enough to reproduce.

Natural bacterial flora act like grass on a school oval. If the grass is thick enough, then other seeds cannot make it through to touch the soil to get the nutrients or water they need to grow.

FIGURE 2 Foxglove (*Digitalis purpurea*) produces compounds that can affect the heart and can cause nausea, vomiting, convulsions or death.

microbiota
the microorganisms found in a particular environment, e.g. the skin or gut

CASE STUDY 8.1

The importance of gut microbiota

There has been increasing interest in the relationship between gut microbiota and the health of an organism. It has been known for some time that bacteria in the intestine are involved in the digestion of some nutrients to provide essential vitamins and minerals. Recent research suggests that the number and types of bacteria in the gut can influence levels of diet-induced obesity and heart disease.

FIGURE 3 Dave Hosking is one of many people using faecal matter transplants to rebalance their gut microbiota.

Faecal transplantation is the transfer of faeces from a healthy donor into the intestinal tract of a patient. While still in the testing stages for many diseases, it is used for patients who have reoccurring *Clostridium difficile* infections in their colon.

Colitis (*C. difficile*) is a complication of antibiotic therapy and can result in recurrent diarrhoea, abdominal cramping and sometimes fever. Although initial treatment of antibiotics can remove the pathogenic bacteria, in 30% of cases the infection will continue to return. A transplantation of faecal matter from a donor with a healthy gut microbiome has been shown to be more effective than antibiotics in these cases. It is thought that, through faecal transplantation, people suffering from colitis are able to restore a normal microbiota to the intestines.

Dave Hosking, lead singer of the Australian band Boy and Bear, is one of many who suffer from chronic dysbiosis (microbial imbalance). Dave manages this microbial imbalance through a daily faecal matter transplant.

CHECK YOUR LEARNING 8.1

Describe and explain

1 Explain what the first line of defence means when talking about the body's defence strategies.
2 Describe the features of an innate immune system.
3 Explain why skin must be intact for it to be considered a physical barrier.
4 Describe two ways that chemical barriers can prevent pathogenic infection in plants and animals.
5 Define 'microbiota'.

Apply, analyse and compare

6 Draw a Venn diagram that compares the physical barriers of plants and animals.
7 Use Case Study 8.1 to explain the role of microbiota in preventing infection in animals.

Design and discuss

8 Draw a diagram of a castle that has a series of defence barriers similar to an animal's innate immune system. Label and explain possible physical barriers, chemical barriers and biological barriers.

8.2 Inflammation

KEY IDEAS

In this topic, you will learn that:

- inflammation is a non-specific response by the immune system
- damaged cells release cytokines that attract other cells in the immune system
- activated mast cells release histamine
- macrophages, neutrophils and dendritic cells phagocytose foreign particles
- eosinophils amplify the cytokine response
- natural killer cells use cytotoxic granules to destroy target cells
- complement proteins act as chemoattractants and help immune cells recognise foreign matter
- interferons are signalling proteins made by cells infected by some viruses, and cause neighbouring cells to strengthen their defence against those viruses.

Despite the physical barriers that are present in all multicellular organisms, some pathogens are able to penetrate tissue or a cell's surface. The immune system of the organism needs a mechanism that allows it to fight these invading pathogens. The response must be fast, present from birth and respond to any pathogen that is not recognised as 'self'.

Inflammation response

cytokines
a large group of chemicals secreted by cells of the immune system – such as interferon, interleukin, and growth factors – that act as chemical messengers to stimulate other cells to respond to pathogens

inflammation
a physical condition in which part of the body becomes red, swollen, hot and sometimes painful as a reaction to injury or infection

mast cell
a cell commonly found in connective tissue containing many granules (including histamine); forms part of the immune system

If a small wooden splinter breaks the intact skin of a finger, it causes damage to the cells underneath. The damaged cells immediately release a series of chemical messengers (**cytokines**) to their neighbouring cells and into the blood. Within seconds, small platelets (small cell-like discs with no nucleus) gather at the damaged blood vessel and adhere to the sides. They become activated and release fibrin protein to connect with each other. This acts to plug any hole in the blood vessel.

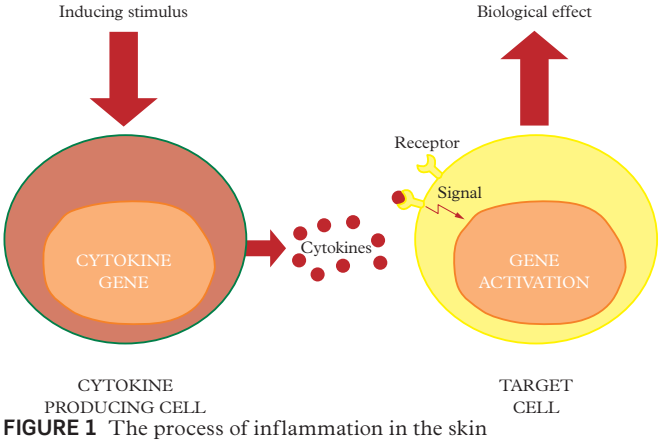


FIGURE 1 The process of inflammation in the skin

Within a few hours, the area can become itchy, hot, red and swollen. A number of white blood cells invade the area, primed to fight any foreign pathogens that may have entered through the broken skin. The key cells that contribute to this **inflammation** reaction are described below.

Mast cells

Mast cells are part of the inflammatory response that is triggered when an organism experiences cellular damage or an infection. They are easily identified by their large round nuclei and their granule-filled cytoplasm. Mast cells are released by the bone marrow and only mature when they reach their permanent site. Once there, they stand guard against

tissue
a group of cells that combine to achieve a particular function

damage or infection in the **tissue** (a group of similar cells that carry out a similar function). If they are stimulated or damaged, they release histamine and cytokines. Histamine is a chemical messenger (not classified as a cytokine) that binds to the receptors on blood vessel cells.

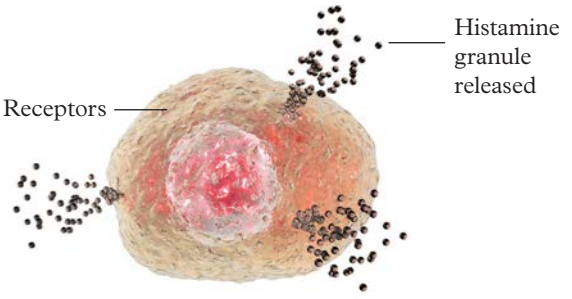


FIGURE 2 Mast cell releasing histamine granules

Histamine

When histamine binds to the epithelial cells that make up the blood vessel, the epithelial cells contract, leaving gaps in the blood vessel. This causes the fluid of the blood to start leaving and move into the tissue. Histamine also causes the smooth muscle cells surrounding the blood vessel to relax, making the blood vessel open wider (vasodilate). Both of these functions of histamine can result in swelling and redness from the increased blood supply and increased permeability of the blood vessels. If histamine release is excessive, it can result in a drop in blood pressure that can cause anaphylaxis.

Cytokines, also released by mast cells, are responsible for attracting other white blood cells to the area.

Macrophages

macrophage
a large phagocytic cell commonly found at sites of infection

Macrophages are white blood cells that are often called the 'big eaters' of the immune system – they engulf large cells. This immune cell is able to move through tissue, identifying any unwanted particles or foreign cells that do not have MHCI self-markers (see Topic 8.3).

FIGURE 3 Macrophages seek out bacteria and engulf them in a process called phagocytosis.

Macrophage membranes are highly flexible, constantly shifting to help the cell move along and around other cells. When they locate a foreign cell, their membrane surrounds it and seals it into a vesicle in a process called **phagocytosis**. Cells that perform phagocytosis are also referred to as **phagocytes**.

phagocytosis
the ingestion of bacteria or other material; the cell extends its membrane and wraps it around the bacteria, drawing it into the cell's cytosol

phagocyte
a type of white blood cell that can ingest foreign particles or bacteria, performing phagocytosis

1 The phagocyte forms a groove, which engulfs the foreign cell.

2 The phagocyte ingests the foreign cell, forming a package or vesicle around it.

3 A lysosome and the vesicle containing the unwanted cell fuse together.

4 The unwanted cell is 'lysed' or broken down by lysozymes within the lysosome.

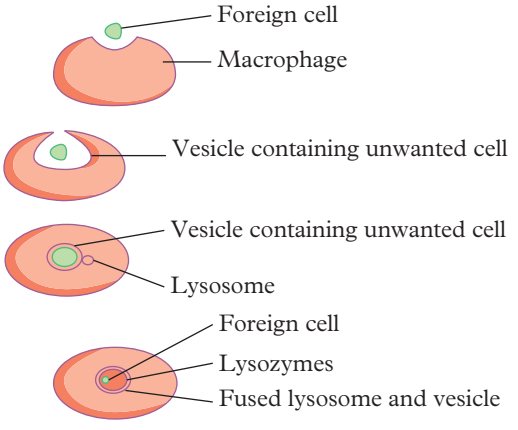


FIGURE 4 Phagocytosis involves the macrophage surrounding the foreign cell and forming a vesicle around it, before digesting the pathogen.

Once isolated inside the cell, the vesicle containing the foreign cell joins with another vesicle containing **lysozymes** (digestion enzymes) and the foreign cell is broken down and digested.

lysozyme
an enzyme in cells that is able to catalyse the destruction of the cell walls of certain bacteria

Macrophages are not just responsible for removing foreign cells. They are also responsible for removing apoptotic cells or blebs. They can digest more than 100 bacteria before they also apoptose.

Neutrophils

One of the most common white blood cells is the **neutrophil**. Like the macrophage, neutrophils are responsible for phagocytosing bacteria. Unlike the macrophage, neutrophils are normally found in the bloodstream, and are only attracted into the tissue by cytokines released by damaged cells or mast cells. They are the first responders to any infection – responding in minutes. When activated, they lose their circular shape and instead use a flexible membrane to engulf bacterial cells. Their life span is much shorter than a macrophage, only phagocytosing a few bacterial cells before undergoing apoptosis.

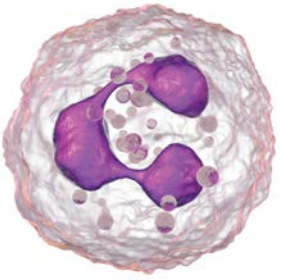


FIGURE 5 A neutrophil is often one of the first phagocytic cells that arrive at the site of an infection.

neutrophil
a type of phagocytic white blood cell that is one of the first cell types to arrive at the site of an infection

When macrophages and neutrophils apoptose after cleaning up an infection, they form a white substance called pus, which is comprised of the digestive remains of the infected material (dead white blood cells, tissue debris and bacteria).

Dendritic cells

Dendritic cells are more commonly found in tissue that has a higher chance of being exposed to pathogens. This includes the lining of the mouth, nose, lungs, stomach and intestines. Like many other white blood cells, they are produced in the bone marrow and move to the tissue before they mature. They are responsible for phagocytosing pathogens in their local area before taking them to the lymph node to be presented to B- and T-lymphocytes (see Chapter 9).

Dendritic cells are in constant communication with other immune cells through cytokines. These cytokines can activate other immune cells if needed.

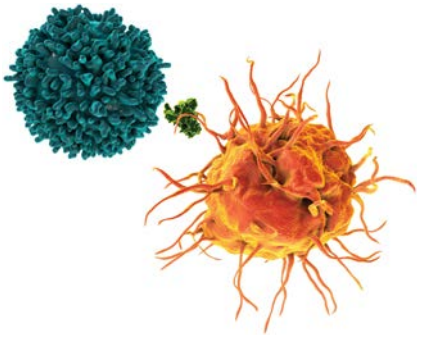


FIGURE 6 Dendritic cell presenting an antigen to a T-cell

dendritic cell
an antigen-presenting cell that processes antigen material and presents it on the cell surface to the T-cells of the immune system

Eosinophils

Eosinophils make up 1–3% of the white blood cells in the body. Like neutrophils, they mature in the bone marrow and travel through the blood to particular tissue such as the thymus, lower intestinal tract, ovaries, spleen and lymph nodes. The eosinophils will stay in the tissues of the body until they are needed. If cytokines are released by damaged mast cells or phagocytic cells, newly released eosinophils will move to the area in great numbers and respond by amplifying the cytokine chemical messengers in the area. This can sometimes cause tissue damage in an allergic reaction; however, this reaction is important in the event of multicellular parasites such as worms.

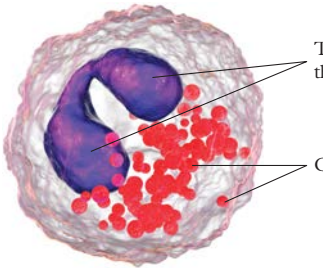
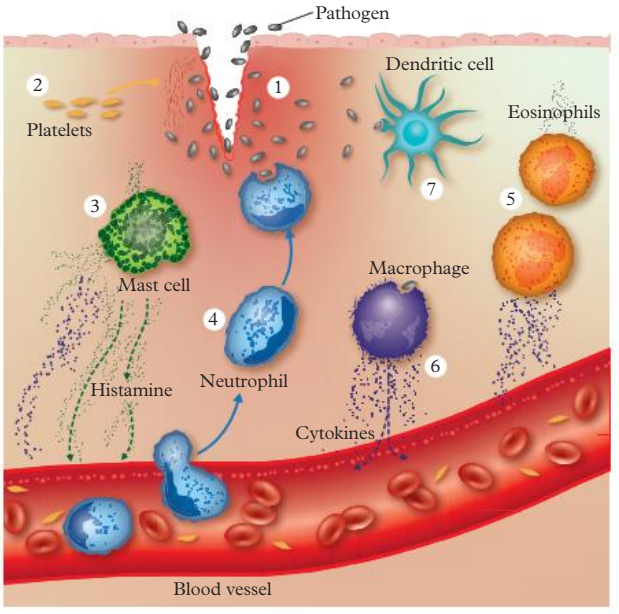


FIGURE 7 Eosinophils are often recognisable by the two lobes of their nucleus and the granules in their cytoplasm.

eosinophil
a type of white blood cell responsible for fighting multicellular parasites



1 Pathogens enter through the broken skin barrier. Damaged cells release cytokines to attract immune cells to the site of infection.

2 Platelets release blood-clotting factors to restore the skin barrier.

3 Mast cells release histamine to stimulate vasodilation and increase the permeability of capillaries. They also release cytokines.

4 Neutrophils are one of the first cells to arrive at the site of infection to phagocytose the pathogens.

5 Eosinophils move into the area and release cytokines to amplify the cytokine messengers.

6 Macrophages phagocytose pathogens and release cytokines.

7 Dendritic cells, macrophages and neutrophils phagocytose pathogens before presenting them on their surface to T- and B- lymphocytes.

FIGURE 8 The inflammatory response is a non-specific immune response to protect the body from pathogens at the site of tissue damage.

Natural killer cells

natural killer cell
a type of white blood cell and part of the innate immune system; these cells play a major role in the host-rejection of both tumours and cells infected by viruses

Natural killer cells are best known for killing cells infected with viruses or abnormally functioning cells such as cancer cells. After being produced in the bone marrow, they constantly patrol tissue, searching for self-markers (MHCI) that confirm whether a cell is foreign or self. If the inhibitory receptors on the natural killer cells locate the self-marker on a cell surface, the natural killer cell will move on. Cells infected with a virus or cancer cells can often lose their self-markers, causing the natural killer cell to recognise the cell as being foreign. This causes the natural killer cell to release cytotoxic granules containing perforin and granzymes, which causes the target cell to apoptose or lyse.

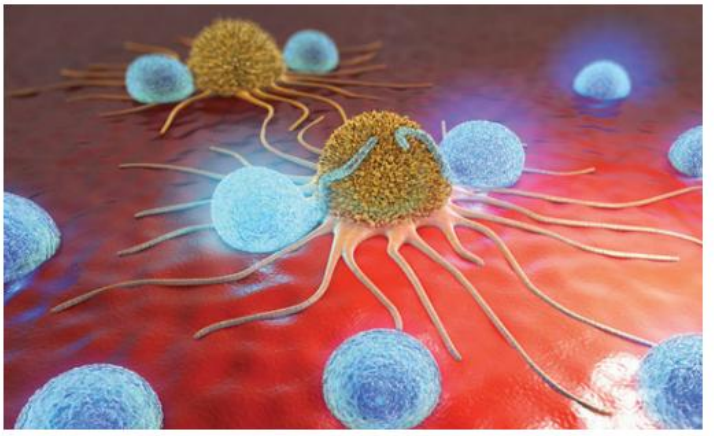


FIGURE 9 Natural killer cells identify cancer cells that have lost their self-markers (MHCI).

Complement proteins

complement protein
a complex system of more than 30 proteins that act together to help eliminate pathogens

There are many proteins in the plasma of the blood that contribute to the innate inflammation system. These **complement proteins** can easily move out of the blood vessels made permeable by histamine. The proteins can then become activated, starting a cascade reaction that results in them binding to pathogens and making it easier for macrophages and neutrophils to identify foreign cells. The complement proteins can also act as chemoattractants – that is, a chemical trail that other phagocytes can follow. In a few cases, the complement proteins can also cause damage to the plasma membrane of some bacterial cells.

Interferons

Interferons are a specific group of signalling proteins within the large cytokine group. More than 20 types have been identified in humans and other animals.

The name comes from their ability to interfere with the replication of several viruses. When a cell is infected by a virus, it manufactures and releases interferons. The interferons signal to neighbouring cells that a virus is present. The unaffected cells then increase their defences against the virus by producing an enzyme called protein kinase R and increasing the expression of MHCI markers on the surface of the cell.

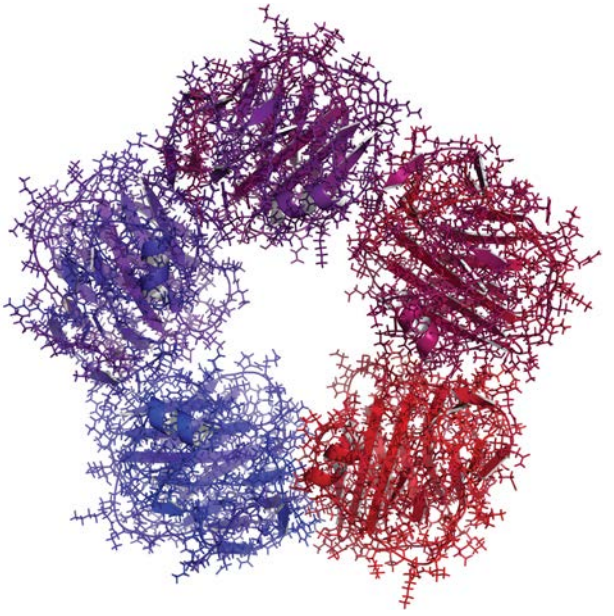


FIGURE 10 C-reactive protein (CRP)

interferons
a group of innate cytokines that are released by virus-infected cells or immune cells; they warn neighbouring cells of infection and activate other cells in the immune system

Interferons also activate immune cells, such as natural killer cells and macrophages, attracting them to the area of infection.

CHALLENGE 8.2

Should we treat a fever?

There are many different cytokines that are active in the inflammation process. Some of these cytokines (interleukin 1, interleukin 6 and tumour necrosis factor) act as pyrogens by binding to receptors in the hypothalamus of the brain and increasing the production of prostaglandin. This chemical messenger is responsible for temperature regulation of the body. An increase in prostaglandin can result in an increase in the body temperature of the patient (a fever). This has an important purpose because many pathogenic bacteria and fungi are unable to survive and reproduce at 39°C. At this temperature, many of the enzymes in the pathogenic organisms will denature and be unable to function. This increase of 2°C above the normal body temperature (37°C) is usually tolerated by human enzymes.

FIGURE 11 A fever is the increase of the body's internal temperature.

For many years, fevers were treated with drugs such as paracetamol that inhibit the production of prostaglandin and reduce fever by promoting heat loss through sweating and vasodilation. This decrease in body temperature limits the effectiveness of the immune system in destroying the cause of the infection.

1 Should low-level fevers be treated through the use of drugs such as paracetamol?

2 What steps could be taken if a fever reaches 40°C?

CASE STUDY 8.2

Cytokine storm

Cytokine release syndrome can occur as a result of a disease, infection, or some monoclonal antibody drugs. It occurs when a large number of immune system cells (including natural killer cells, macrophages and dendritic cells) are activated and release inflammatory cytokines. This attracts further immune system cells to the site of infection, which also start releasing cytokines. Severe cases of cytokine release syndrome are known as 'cytokine storms'. The initial symptoms of a cytokine storm are fever, muscle pain or fatigue. These symptoms do not usually need to be treated. If cytokines continue to be released, the blood pressure may drop, and the patient may require oxygen therapy and an injection of fluids. In severe cases, the cytokines can cause the immune system to attack the patient's own body, causing organ failure. This is thought to be the cause of previously healthy people dying as a result of influenza in 1918, SARS in 2003 and COVID-19 in 2020.

FIGURE 12 The Spanish flu epidemic from 1918–1919 was made substantially worse by cytokines attacking the patient's own body.

CHECK YOUR LEARNING 8.2

Describe and explain

1 Describe the characteristics and roles of the following immune system components.
   - **a** macrophage
   - **b** neutrophils
   - **c** dendritic cells
   - **d** eosinophils
   - **e** natural killer cells
   - **f** mast cells
   - **g** complement proteins
   - **h** interferons

2 Create a flowchart of the inflammation response, including which molecule or cell is involved and how they contribute to the response and protect the organism.

Apply, analyse and compare

3 Compare the functions of a neutrophil and a macrophage.

4 Compare the functions of cytokines and complement proteins.

5 Symptoms of inflammation include redness, swelling and pain. Use your knowledge of the immune system to explain each of these symptoms.

6 Use your understanding of the immune responses to explain how immune system cells are activated to release cytokines, and create the cytokine storms described in Case study 8.2.

Design and discuss

7 Many people suffer from hay fever each year. Their body reacts to an allergen and the inflammation response is initiated. Some take antihistamines to relieve the symptoms. Discuss the role of histamine and the inflammation response in developing hay fever.

8 In 2006, as part of a medical study in England, six healthy volunteers were injected with a new drug that stimulated their immune system. All six became critically ill with multiple organ failure, high fever and a systemic inflammatory response. Suggest a cause for this reaction.

8.3 Initiation of the immune system

KEY IDEAS

In this topic, you will learn that:

- all cells in a multicellular organism (except red blood cells) have a self-marker molecule that allows them to recognise self from non-self
- antigens are molecules that can be recognised by the immune system
- non-self antigens are molecules from outside the body that can initiate an immune system response
- self-antigens are cell surface molecules tolerated by the organism in which they are found, but will initiate an immune response if transplanted into another organism.

Each day our bodies are exposed to millions of pathogens that can harm us. They are in our food, the air we breathe and on the surfaces we touch. The role of our immune system is to identify any molecule or organism that is not self, and to remove or limit the damage to our body.

An effective immune system needs to:

- have barriers to stop substances from entering the body
- be present from birth
- work rapidly and not harm the host
- respond specifically to pathogens
- adapt if pathogens get past the initial responses.

For these things to occur, an immune system must first have a way of distinguishing which cells belong in the organism, and which cells are foreign. In this regard, the cells of a particular organism require self-marker molecules that enable them to recognise one another.

Antigens

antigen (Ag)
a molecule or part of a molecule that initiates a response by the immune system

A molecule or part of a molecule that is responsible for activating the immune response is called an **antigen (Ag)**. Antigen is short for antibody generator (and thus, can be shortened to the use of 'Ag'). Any type of molecule can act as an antigen. For example, it could be a molecule that is part of the cell wall of a bacterial cell, molecules injected by a bee sting or even the molecules on the surface of a pollen grain. These antigens are categorised differently, depending on their source and their potential for causing damage.

FIGURE 1 A bee transporting pollen. Both pollen and a bee sting hold antibody generators or antigens.

non-self antigen
a molecule on the surface of a foreign cell or body that initiates a response by the immune system

Non-self antigens

Non-self antigens are molecules found on the surface of a cell or other body that does not belong to the parent organism. As a result, the immune system will determine that the antigen is foreign and initiate an immune response to remove it from the body.

Self-antigens

Every cell has a range of molecules embedded in its membrane (such as proteins, sugars and lipids). These molecules are capable of acting as an antigen in the immune system. The molecules that are capable of initiating an immune response in another organism, but which are tolerated by the immune system of the parent organism, are called **self-antigens**.

self-antigen
a molecule that can initiate an immune reaction in another organism but not in the parent organism

In vertebrates, each organism has complex molecules (glycoproteins) that group together on the surface of each cell. These molecules are called the **major histocompatibility complex I (MHCI)**. MHCI is found on the surface of all cells except red blood cells and allows the immune system to identify each cell as belonging to the organism. This is especially important for the immune system, which relies on recognising and removing foreign cells rather than attacking the body's own cells.

major histocompatibility complex I (MHCI)
the group of glycoproteins on the membrane of cells that assist immune cells to recognise self from non-self

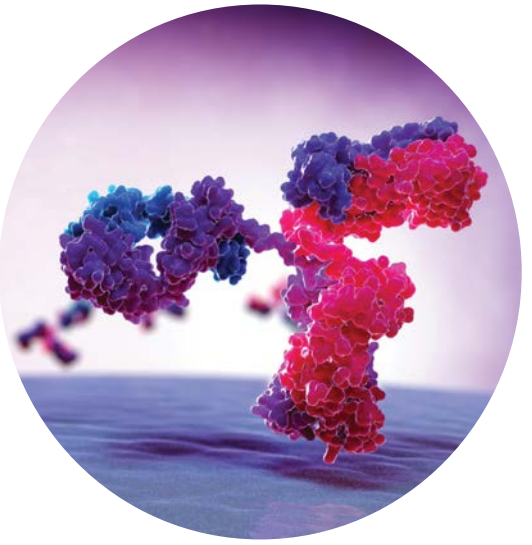


FIGURE 2 The MHCI on a cell surface allows all cells (including those in the immune system) to recognise self cells from non-self cells.

CASE STUDY 8.3

Avoiding the immune system

Many disease-causing pathogens, such as bacteria and viruses, have evolved to be able to invade host organisms and avoid detection by the host's immune system. Some bacteria secrete molecules that restrict the host's immune system.

Other bacteria will stay close to each other and secrete a slimy mixture of substances called a biofilm. This makes it difficult for the cells in the immune system to get close to the bacteria, interfering with the host's normal immune response. In this instance, a full immune response is not initiated.

All viruses, and some protozoans (such as *Plasmodium*, which causes malaria), are able to hide inside the host's own cells. Because the host's cells display the self-markers (MHCI) on their plasma membrane, the immune system is often not activated.

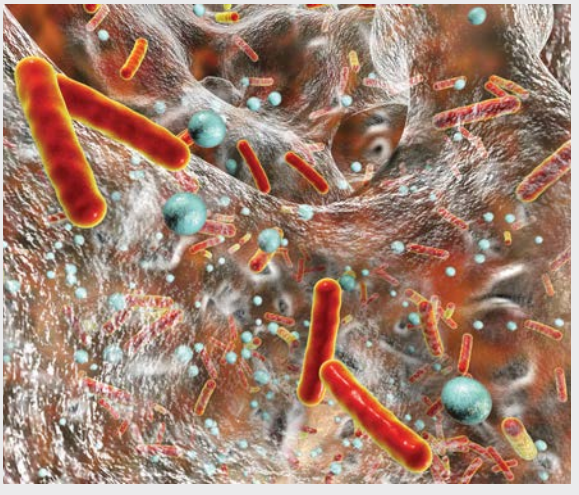


FIGURE 3 Bacteria can form a biofilm to avoid detection by the immune system.

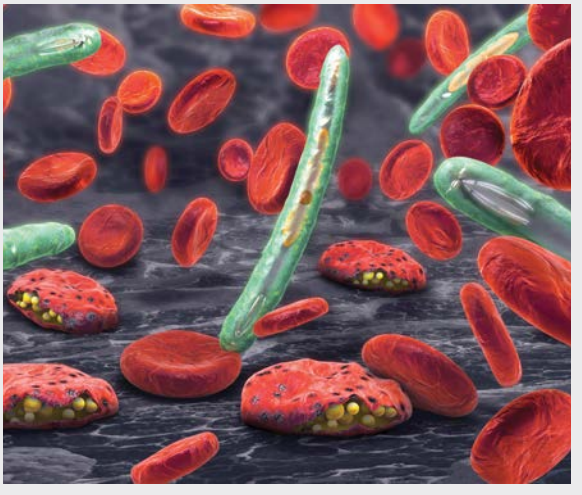


FIGURE 4 Single-celled *Plasmodium* are able to hide inside red blood cells to avoid detection by the immune system.

Antigen presentation

Macrophages, neutrophils and dendritic cells are all antigen-presenting cells. **Antigen presentation** occurs when:

1. an immune cell phagotyses an antigen and uses lysozomes to digest it into smaller particles
2. the antigen particles are combined with other molecules called **MHCII**
3. the complex that is formed is then presented on the surface of the immune cell's membrane.

The antigen-presenting cell displays the antigen complex, which then allows the antigen to be located by B- and T-lymphocytes in the lymph nodes. If the lymphocytes recognise the antigen, then the next level of the immune system is activated: the adaptive immune response. You will learn more about this in the next topic.

antigen-presenting cells
cells that present antigens to B- and T-lymphocytes to initiate the adaptive immune response (commonly macrophages, neutrophils and dendritic cells)

MHCII
major histocompatibility complex molecules normally found on antigen-presenting cells

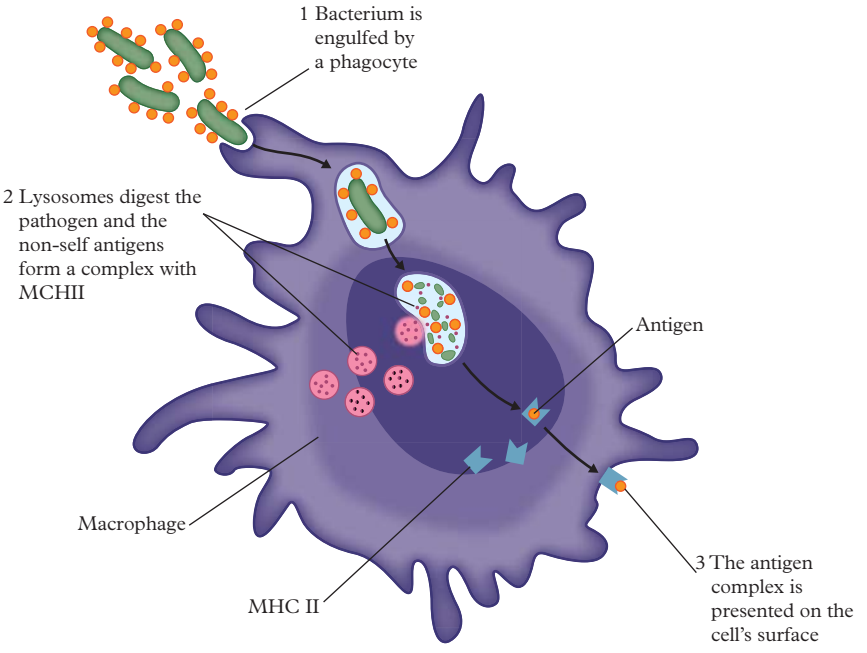


FIGURE 5 A dendritic cell phagotyses and presents an antigen on its surface for T-cells to recognise.

CHECK YOUR LEARNING 8.3

Describe and explain

1. Define the term 'antigen'.
2. What are the characteristics of an effective immune system?
3. Explain why an immune system needs to be able to detect self-markers.
4. Use Case study 8.3 to identify ways in which pathogens can avoid the immune system.
5. What is the major histocompatibility complex I and what is its role in the immune system?

Apply, analyse and compare

6. Compare a non-self antigen and a self-antigen.

Design and discuss

7. Discuss the similarities between a self-marker on the cells of an organism and the uniform worn by students in a school.
8. Many cancer cells are able to avoid detection by the immune system. Explain why this might occur.
9. Explain why the MHCI markers do not need to be checked before blood transfusions.

8.4 Pathogens and allergens

KEY IDEAS

In this topic, you will learn that:

- cellular pathogens include bacteria, fungi and protozoa
- non-cellular pathogens include viruses and prions
- allergens are initially harmless non-self antigens that cause an overreaction by the immune system.

disease
anything that interrupts the normal functioning of an organism

pathogen
an agent that causes disease

vector
an organism that carries a disease-causing pathogen from one organism to another

All organisms rely on their bodies to function effectively and to respond quickly to any changes in their environment. When an organism is no longer able to function normally, it is typically considered diseased. **Diseases** can be inherited (genetic), or they can be caused by a lack of nutrients (malnourishment) or an infectious **pathogen** (an agent that causes a disease). Many pathogens rely on **vectors**, organisms that carry the pathogen from one organism to another. Pathogens can be broken into two main groups: cellular pathogens and non-cellular pathogens. The COVID-19 SARS-CoV-2 virus is thought to have been transmitted from pangolins to humans. This means the pangolin was the vector.

FIGURE 1 Pangolins are thought to be the vector for the COVID-19 SARS-CoV-2 virus.

Cellular pathogens

cellular pathogen
a disease-causing organism made of cells (such as fungi, protozoa or bacteria)

Cellular pathogens are usually single-celled organisms that interrupt the normal functioning of their host organism. When cellular pathogens infect their host, they can rapidly divide and use nutrients that would otherwise be used by the host. They may cause a build-up of toxic waste products. Some cellular pathogens are also capable of releasing toxins to deliberately clear the area of host cell competition. There are three main types of cellular pathogens: bacteria, fungi and protozoa. Some multicellular organisms, such as mites, lice, ticks or worms, can cause diseases.

Bacteria

Many multicellular organisms such as humans rely on bacteria (e.g. *Escherichia coli*) to help them digest nutrients and produce essential vitamins and minerals. Some bacteria support the normal functioning of our immune systems. Despite this, there are many bacteria known to be pathogenic. These bacteria cause negative effects to other living organisms.

To cause a disease, a bacterial cell must first enter the host, reproduce and cause damage to the host cells. The most common pathway into a host organism is through the digestive or respiratory systems or through a break in the skin. Alternatively, mosquitos (family Culicidae) can act as a vector for bacterial infections. This is hypothesised to occur with the Buruli (or Bairnsdale) ulcer where mosquitos are thought to carry *Mycobacterium ulcerans*

between possums and humans. Once inside the host, **pathogenic** bacteria reproduce through **binary fission.** The single circular chromosome is initially copied and then the cell splits into two. Bacterial cells can double their number every 20 minutes, making the spread of pathogenic bacteria a relatively quick process. This requires a lot of nutrients and produces a lot of waste products – and these can also cause damage to surrounding host cells.

pathogenic
capable of causing disease

binary fission
when a prokaryote duplicates its chromosome asexually and then splits into two cells

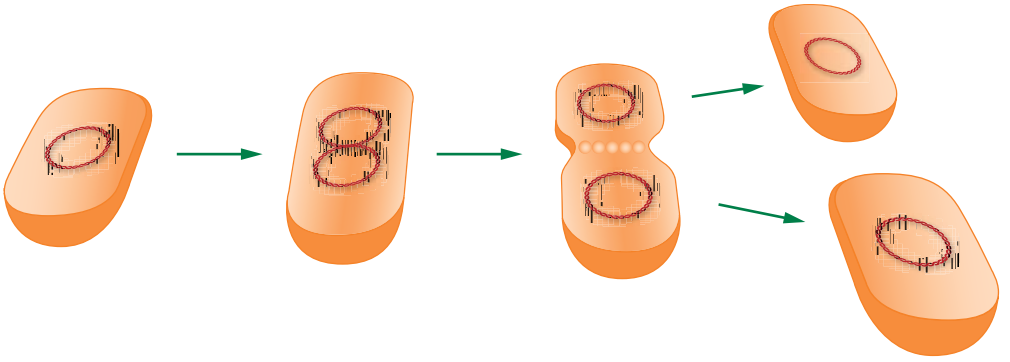


FIGURE 2 Bacterial cells reproduce through binary fission.

CASE STUDY 8.4A

Botox mechanism of action

The bacterium *Clostridium botulinum* produces a toxin that binds to a receptor protein on a neuron membrane. The botulinum toxin is then taken into the cytoplasm of the neuron where it binds to vesicles that are carrying the neurotransmitter acetylcholine. This means the vesicle cannot bind to the plasma membrane of the neuron. As a result, the neurotransmitter acetylcholine cannot be released, thereby preventing the signalling molecules from diffusing across the synaptic gap between a neuron and its nearby muscle cell. The toxin prevents the normal functioning of the muscle cells, causing them to become paralysed. While this is dangerous if the *Clostridium botulinum* bacteria are growing near neurons controlling the lungs or heart, the toxin can be used to treat migraines, in cosmetic treatments, or to prevent the overstimulated muscle movement that occurs in cerebral palsy.

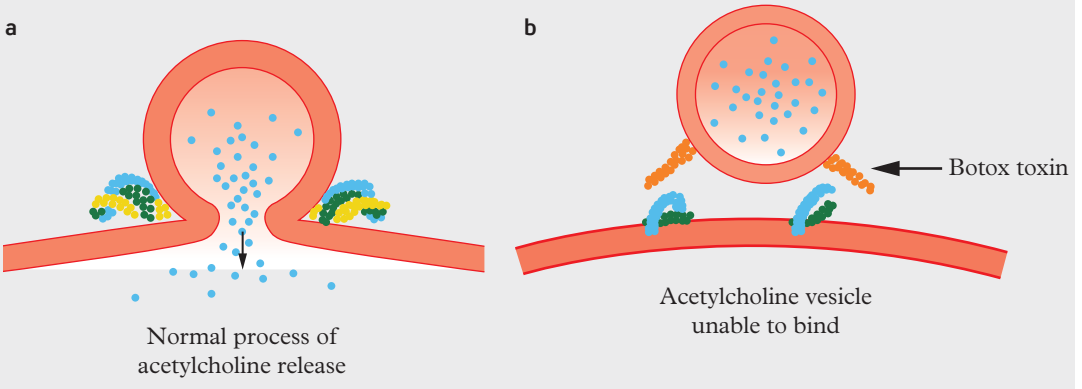
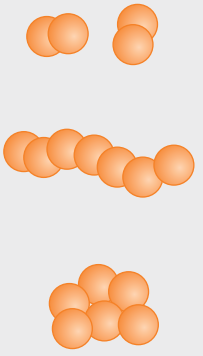
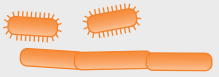


FIGURE 3 **a** Exocytosis of acetylcholine is part of the normal functioning of some neurons; **b** the presence of the *Clostridium botulinum* toxin prevents the vesicle carrying the acetylcholine from binding to the synaptic membrane.

Pathogenic bacteria can be identified by their shape, the conditions in which they live, or through the use of special stains. Table 1 identifies the five different bacterial shapes and includes some pathogenic examples.

TABLE 1 Common shapes of bacteria and examples

Bacterial shape	Examples
Cocci are spherical. 	• Diplococci join together in pairs. • *Diplococcus pneumoniae* causes pneumonia. • Streptococci join to form a chain. • Many *Streptococcus* species infect the upper respiratory tract. • *Streptococcus pyogenes* causes scarlet fever. • Different strains of *Staphylococcus aureus* (golden staph) cause boils, pneumonia, food poisoning and other infections.
Bacilli are rod-shaped. 	• *Bacillus typhosus* causes typhoid fever. • *Bacillus antracis* causes anthrax.
Spirilla are spiral-shaped. 	• The spirochete (a fine spirilla) *Treponema pallidum* causes syphilis.
Vibrio are comma-shaped. 	• *Vibrio cholerae* causes cholera.
Rickettsiae are rod-shaped. 	• Rickettsiae causes typhus. **Note:** They are similar in size to the smallest bacilli and cocci, but they are classified into a different group because they cannot survive outside living cells.

Fungi

fungus
a eukaryotic organism whose cells have organelles, a cell wall and hyphae, which needs to consume nutrients for cellular respiration (an example is yeast); plural fungi

Unlike bacteria, fungal cells are eukaryotic (they have a nucleus and organelles). **Fungi** often infect people who have a weakened immune system. Like bacteria, fungi can cause damage to their host's cells by depriving them of nutrients or producing waste or toxins.

All multicellular fungi have long filaments called hyphae, which can penetrate the skin surface of their host. They then secrete enzymes that digest surrounding molecules, and can then subsequently be absorbed by the hyphae. Longer strands called fruiting bodies release the spores that fungi use to reproduce.

One of the most common fungi is a member of the yeast family, *Candida albicans*. This yeast is an opportunistic pathogen, meaning that it grows quickly when the host's immune system is depressed.

CASE STUDY 8.4B

Koch's postulates

In 1890, German scientist, Robert Koch, developed a set of criteria to judge when a pathogen was the cause of a disease. The four statements he developed are as follows.

1 The pathogen must be present in every case of the disease.
2 The pathogen must be isolated from the host with the disease and grown in a pure culture.
3 The same disease must be reproduced when a pure culture of the pathogen is placed in a healthy host.
4 The pathogen must be able to be isolated from the newly infected host.

Although Koch originally developed these postulates when identifying bacteria, they have also been used by plant biologists when identifying if a fungus is the cause of an infection.

FIGURE 4 Sunflower rust (*Puccinia helianthi*) is a fungal infection that can be spread by contact, wind or rain.

Protozoans

Protozoans are single-celled eukaryotes that cannot be classified as fungi, plants or animals. Many of them are able to move by changing the shape of their bodies. Like fungi, they have a nucleus and organelles and need to live in a moist environment. Examples of disease-causing protozoans include dysentery-causing amoebas and malaria-causing *Plasmodium*.

FIGURE 5 A 3D illustration of the amoeba protozoan (*Entamoeba histolytica*). This protozoan is transmitted through contaminated drinking water and food. It can cause severe diarrhoea.

protozoan
a single-celled eukaryote that is able to move by changing the shape of its body (an example is the malaria-causing *Plasmodium*)

CASE STUDY 8.4C

Malaria

Malaria is caused by a protozoan that hides inside human cells to avoid the immune system. Mosquitos are the primary host (or vector) for the parasite because the *Plasmodium* undergoes sexual reproduction in the digestive tract of the female *Anopheles* mosquito. Humans are the secondary host of the parasite.

Malaria is endemic in many Mediterranean and tropical regions of the world. Between 200 and 400 million people have malaria, with 80% of cases occurring in Africa. Every year, 2.5 million people die of the disease, while those who survive end up with weakened resistance to other diseases.

There have been many attempts to control the spread of malaria. These include hormonal sprays to prevent the development of the aquatic mosquito larvae into adults, and sprays containing viruses that attack the larvae. Sterilised male mosquitoes have also been released so that they cannot fertilise female mosquitoes.

The race to find a robust vaccine for malaria is continuing. The protozoan that causes malaria is complex and so the vaccine to control malaria is also complex to make. You will learn more about vaccines in Chapter 10. Currently there is a low efficacy (low success rate) vaccine called Mosquirix that has been available since 2015.

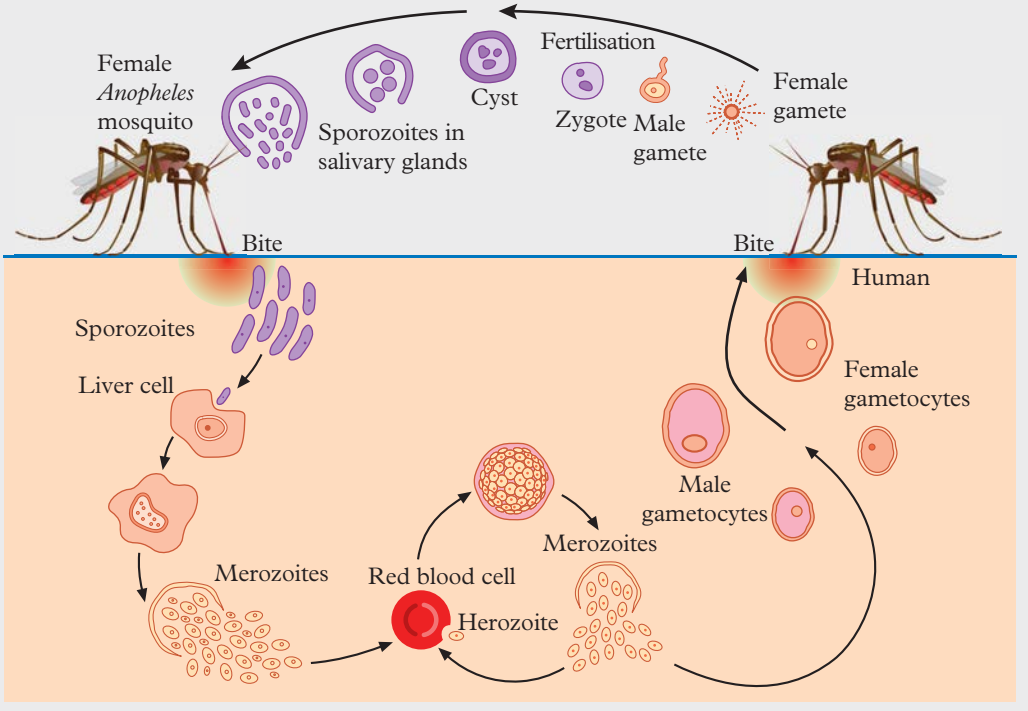


FIGURE 6 The life cycle of the protozoan *Plasmodium*

Non-cellular pathogens

Not all pathogens are living organisms. **Non-cellular pathogens** are classified as non-living, and therefore are not considered to be organisms.

non-cellular pathogens
non-living pathogens, e.g. viruses or prions

Viruses

Viruses consist of nucleic acid (either DNA or RNA) surrounded by a protein coat called a capsid. They are much smaller than bacteria and are unable to reproduce alone. Instead, they must insert their DNA or RNA into a living host cell and use the host's ribosomes and other organelles to replicate their nucleic acid and proteins.

virus
a non-living pathogen that consists of genetic material surrounded by a protein coat; it reproduces through the use of a host cell's organelles

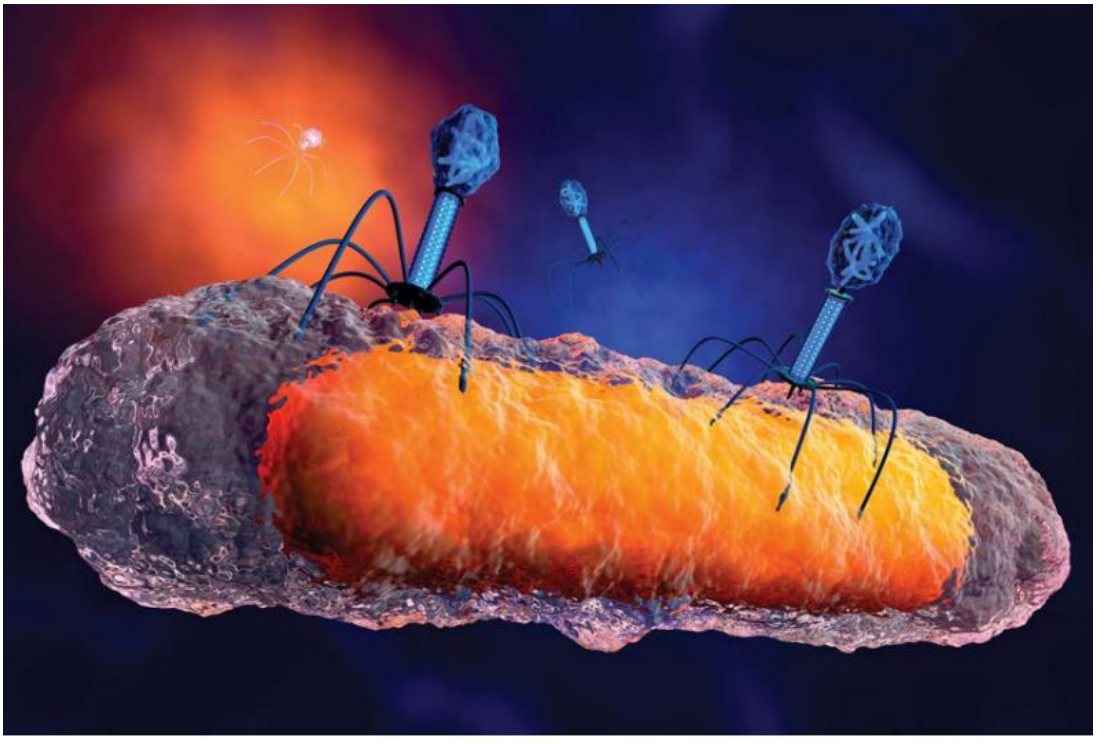


FIGURE 7 Bacteriophages are viruses that infect bacterial cells. Research has been conducted into using bacteriophages to fight bacterial infections in humans.

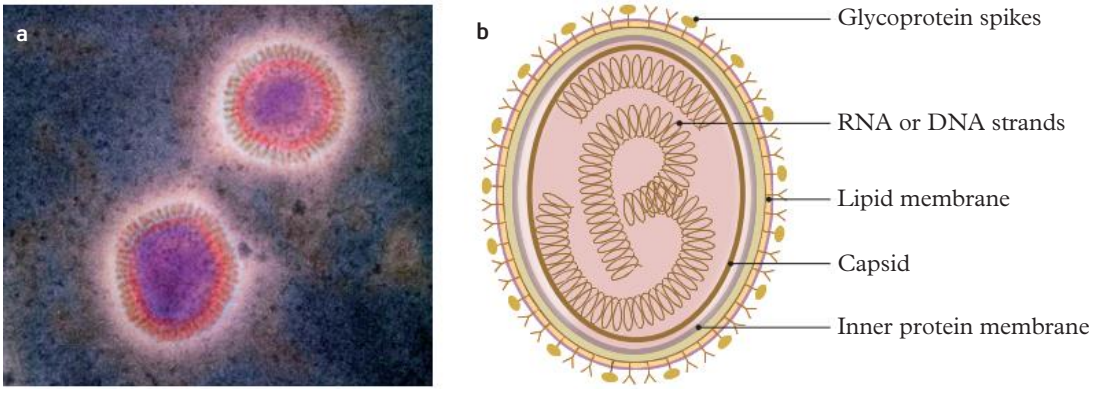


FIGURE 8 Influenza H1N1 virus: **a** an electron micrograph; **b** structure of the virus

Before a virus can enter a cell, it must bind to a molecule on the membrane's surface. If the virus cannot bond with the host membrane, the host's immune system may destroy it before it can replicate. Once it has bonded, there are many different ways the virus can enter the cell. The human immunodeficiency virus (HIV) is pulled directly into the cell, while the influenza virus is engulfed by the host cell and then breaks out of the vesicle into the cytoplasm. In contrast, the polio virus creates its own channel through the host cell's membrane.

Once inside, the virus particles disrupt the host's protein production so that new viral proteins and nucleic acids can be made. These molecules are then assembled into new virus particles, each of which is called a virion. The virions then leave the host cell, either by surrounding itself with the host's membrane, or through lysis (breaking the cell apart).

CHALLENGE 8.4

Phage therapy

The specificity of viruses for a particular species of bacteria has made them potential allies in the fight against bacterial infections. Bacteriophages are viruses that will only infect bacterial cells. Each bacteriophage (or phage) will recognise and bind to a specific molecule on the surface of a single species of bacteria. This means the phage can be used as therapy to treat infections. The phages specific to the bacteria can be taken orally or applied directly to the wound. Because the phage virus cannot enter human cells, it will enter the body and eventually lyse the bacterial cells that were causing the infection, without damaging the human cells.

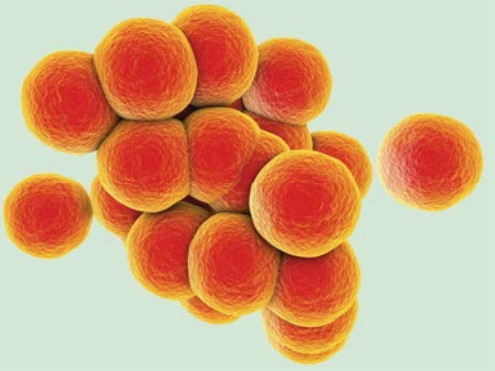


FIGURE 9 Golden staph (*Staphlococcus aureus*) bacteria

The specificity of the phages for a particular bacterial species is also a disadvantage. It means the species of bacteria needs to be identified before the correct phage can be used in the treatment. Many phage therapies used in Russia, Georgia and Poland currently contain a mixture of phages that are effective against the most common bacterial species. In 2019, the United States Food and Drug Administration approved the first clinical trials for the AB-SA01 phage, which is effective against *Staphlococcus aureus* (golden staph) infections.

1 Suggest why phage therapy is a good alternative to antibiotics.

Prions

Prions are misfolded infectious proteins that can cause normal proteins to change their arrangement and also become misfolded. Prions cause a number of neurodegenerative diseases that make cells in the brain apoptose, leaving large sponge-like holes. This gives the group of progressive and ultimately fatal conditions its name – spongiform encephalopathy.

prion
a misfolded infectious protein that can cause normal proteins to also become misfolded

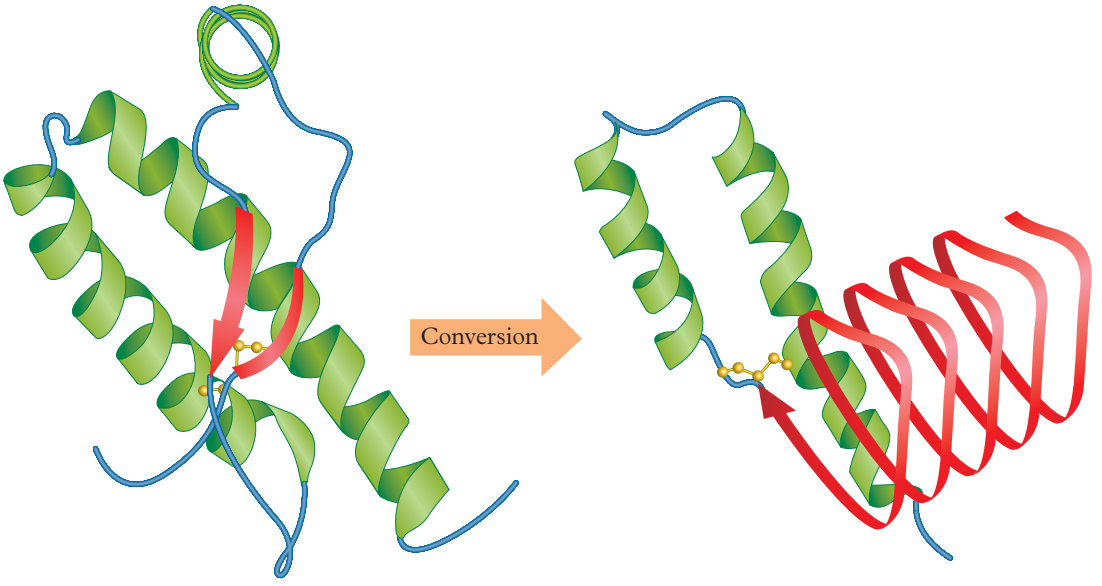


FIGURE 10 Prio Proteins ns (green) are infected by abnormal prion pieces (red), which convert the entire protein to the abnormal (prion) form.

The symptoms of various forms of spongiform encephalopathy include dementia, difficulty walking, hallucinations, confusion and fatigue. Diseases caused by prions have many names. In sheep it is called scabies, in cattle it is called mad cow disease, and in humans it is called kuru (in New Guinea) or Creutzfeldt-Jakob disease (CJD). The mechanisms for this group of diseases are yet unknown.

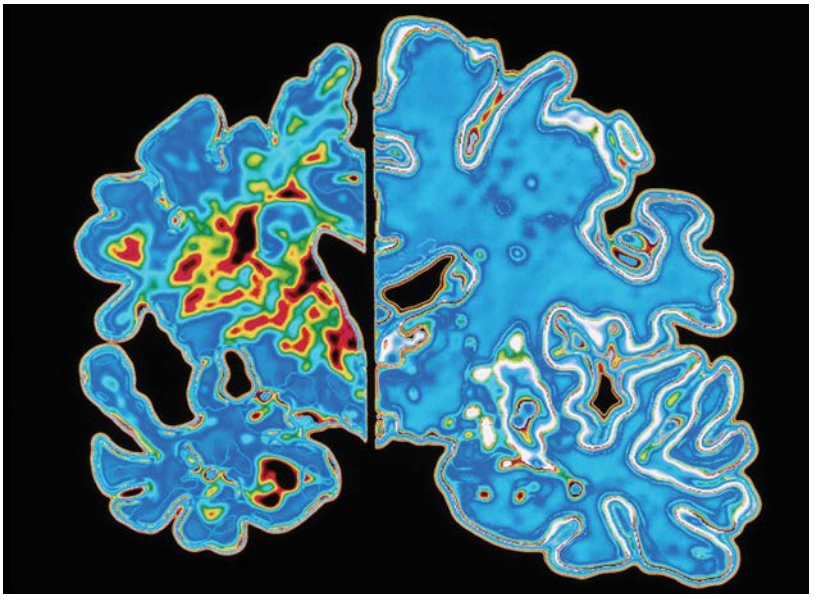


FIGURE 11 Comparison of a normal section of the brain (right) and an infected section with CJD (left)

CASE STUDY 8.4D

Mad cow disease

The scientific name for mad cow disease is bovine spongiform encephalopathy (BSE). The first case of mad cow disease in the UK was traced back to a farm in Sussex, England in December 1984. A cow on the farm was observed to have an arched back and severe weight loss. Within 6 weeks the cow had died. In the following months, other cows started showing symptoms. By 1987, four herds had been affected. At first it was thought that the infection was a small virus that was being transferred between the cows. In 1989, in an attempt to limit the spread of the disease, the British government paid the farmers to destroy the cows.

The origin and rapid spread of mad cow disease is now thought to be a result of the pressure on farmers to rapidly increase the muscle mass of their herd. This led them to feed the cows with protein supplements. The supplement used may have included protein processed from a sheep who suffered from scabies caused by the infectious prion. When the first cows were diagnosed with mad cow disease, they were sold to an abattoir to be made into protein supplements. This essentially made the cows cannibals.

It wasn't until 1990, when a farmer's cat was found to have the disease, that it was realised that mad cow disease could be passed between species. Although the ban on using bovine offal in baby food had been implemented, it was found that the disease could be passed on by eating meat contaminated by small pieces of spinal cord. In 1995, a 19-year-old was the first person to die of a variant of Creutzfeldt-Jakob disease (CJD), the human version of the disease. By 1996, another eight cases had been diagnosed. The British government introduced new measures, including destroying entire herds of cattle when a single cow was diagnosed with mad cow disease. Although the incidence of people diagnosed with the variant CJD has been reduced to zero, scientists are still cautious. In 2019, tests of archival human appendix tissue showed traces of the prions responsible for the disease. For this reason, people who lived in the UK during the mad cow disease outbreak cannot donate blood in Australia.

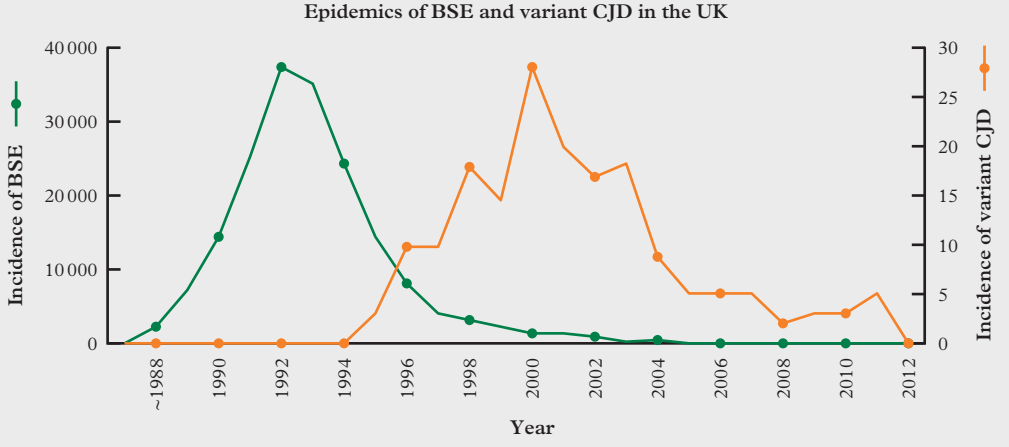


FIGURE 12 With the increase in incidence of mad cow disease, or bovine spongiform encephalopathy (BSE), there was a corresponding increase in the number of people diagnosed with the variant of Creutzfeldt-Jakob disease (CJD).

Allergens

Our bodies are exposed to many molecules, such as those found in dust. Our immune system should recognise these molecules as harmless and ignore them. Some immune systems overreact to some of these molecules, treating them as if they are pathogens. This hypersensitive reaction is often called an allergy, and the molecules that initiate the overreaction of the immune system

are called **allergens**. An allergic reaction can be dangerous to organisms because it can trigger a type of inflammation called hypersensitivity. Repeated exposure to an allergen can cause the immune system to produce special molecules called IgE antibodies (see Chapter 9). These antibodies bind to the surface of mast cells and act as extra receptors for the allergen. When the antibody receptors bind to the allergen, they activate the mast cell to release extra histamine, causing a heightened inflammation response (swelling and redness caused by leaky blood vessels). In extreme cases of repeated exposure, so much fluid is released that the blood pressure drops and an anaphylactic reaction can occur.

allergen
a normally harmless molecule recognised by the immune system that initiates an overreaction or hypersensitivity reaction

FIGURE 13 Allergic reactions, like hay fever, are the body's immune system overreacting to certain molecules such as pollen or dust.

CHECK YOUR LEARNING 8.4

Describe and explain

1 Define the following terms.
   - **a** Pathogen
   - **b** Disease

2 Not all diseases are caused by pathogens. Describe, using examples, two other causes of disease.

3 Define the term 'prion'.

Apply, analyse and compare

4 Use examples to compare viruses and fungi.

5 Use examples to compare cellular and non-cellular pathogens.

6 Read Case Study 8.4D.
   - **a** Explain how mad cow disease managed to evade each of the infected cows' first line of defence.
   - **b** Explain why people who lived in the UK during the mad cow disease outbreak cannot donate blood in Australia.
   - **c** Examine the graph in Figure 12.
     - **i** Compare the incidences of bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD).
     - **ii** Give a reason for the differences in incidences of BSE and CJD.
     - **iii** Consider the way the graph has been drawn and evaluate the presentation of the data. Could the two data sets be drawn differently?

Design and discuss

7 Some people have been found to be immune to HIV because they have a mutation in the protein on the cell membrane to which the virus binds. Explain how this mutation makes them resistant to infection.

8 *Plasmodium* causes malaria and avoids the immune system by hiding inside liver cells and red blood cells. This led a student to describe it as a virus. Use your knowledge of protozoans to explain why the student is incorrect.

9 Some scientists now suggest that a virus is a living organism. Do you agree or disagree? Use your knowledge of viruses to justify your answer.

10 Can an allergen be a pathogen? Use your knowledge of the immune system to justify your decision.

CHAPTER 8

Review

Chapter summary

8.1
- Plants and animals have different physical and chemical barriers that protect them from incoming pathogens.
- Barriers that protect an organism can be physical, chemical or microbiota (biological).

8.2
- Inflammation is an immune response that occurs when a pathogen has entered the body.
- Damaged cells release cytokines that attract other cells in the immune system.
- Activated mast cells release histamine, causing vasodilation and increased cell permeability.
- Macrophages, neutrophils and dendritic cells phagocytose (engulf) foreign particles.
- Eosinophils amplify the cytokine response.
- Natural killer cells use cytotoxic granules to destroy target cells.
- Complement proteins act as chemoattractants and help the immune cells recognise foreign matter.
- Interferons are signalling proteins made by cells infected by viruses, causing neighbouring cells to strengthen their defence against those viruses.

8.3
- All cells in a multicellular organism (except red blood cells) have a self-marker molecule that allows them to recognise self from non-self.
- Antigens are antibody molecules that can be recognised by the immune system.
- Non-self antigens are molecules from outside the body that can cause an immune system response.
- Self-antigens are cell surface molecules tolerated by the organism in which they are found, but which will cause an immune response if transplanted into another organism.

8.4
- There are different types of cellular pathogens, including bacteria, fungi and protozoa.
- There are a number of different bacterial shapes that can help identify pathogenic bacteria.
- There are different types of non-cellular pathogens, including viruses and prions.
- Our immune system treats allergens the same way it treats pathogens, even though they are initially harmless non-self antigens.

Revision questions

Multiple choice

1 A physical barrier in plants includes:
- **A** intact skin.
- **B** toxic molecules in sap.
- **C** chemical messengers that attract insects.
- **D** hairs on a leaf.

2 A chemical barrier in mammals includes:
- **A** microbiota on skin.
- **B** intact skin.
- **C** lysozymes in tears.
- **D** chemical messenger that attracts insects.

3 Which immune system cell produces histamine?
- **A** Macrophages
- **B** Neutrophils
- **C** Mast cells
- **D** Eosinophils

4 Prions are:
- **A** a type of virus.
- **B** a type of protein.
- **C** a type of bacterium.
- **D** a type of fungus.

5 Which immune system cells are the first to arrive at the site of infection from the blood?
- **A** Neutrophils
- **B** Red blood cells
- **C** Macrophages
- **D** Mast cells

6 An example of a microbiota barrier in mammals includes:
- **A** bacteria on skin.
- **B** acidic urine.
- **C** waxy cuticle on a leaf.
- **D** macrophages in tissue.

7 Viruses:
- **A** are living organisms.
- **B** can reproduce without a host cell.
- **C** can enter any host cell.
- **D** consist of genetic material with a protein coat.

8 What process does a neutrophil or macrophage use to ingest (eat) a bacterial cell?
- **A** Phagocytosis
- **B** Chemotaxis
- **C** Exocytosis
- **D** Cytokines

9 An allergen is:
- **A** an antigen.
- **B** a toxic molecule that initiates an immune response.
- **C** a pathogen.
- **D** a harmless molecule that initiates an immune response.

10 Malaria is a type of:
- **A** bacterium.
- **B** virus.
- **C** prion.
- **D** protozoan.

FIGURE 1 Malaria is typically transmitted through mosquito bites.

11 Binary fission is:
- **A** a way for bacteria to reproduce by splitting into two separate cells.
- **B** a way for viruses to enter the human body.
- **C** a type of antigen.
- **D** the release of histamine granules from mast cells.

12 Which of the following features make it easy to identify a mast cell?

A No nucleus and antigen presentation

B A large round nucleus and granule-filled cytoplasm

C A tail (flagellum) and granule-filled cytoplasm

D A small nucleus and antigen presentation

Short answer

Describe and explain

13 Use an example to explain each of the following terms.

a Infectious disease

b Cellular pathogen

c Non-cellular pathogen

14 Describe three ways a bacterial infection can cause damage to host tissue.

15 Explain why using pesticides to kill mosquitos can help to control the spread of malaria.

16 Identify one physical and one chemical defence barrier in humans.

17 Describe the roles of the following cells in the immune system.

a Eosinophils

b Mast cells

c Dendritic cells

d Natural killer cells

18 Define what interferons are.

19 Outline the steps in the inflammatory response.

20 What is an 'antigen-presenting cell? Explain using an example.

21 Define the terms 'self-antigen' and 'non-self antigen'.

Apply, analyse and compare

22 Explain why workers who serve food must wash their hands after blowing their nose in a tissue.

23 Figure 2 shows a close-up view of a dwarf birch (*Betula nana*) leaf. The dwarf birch has hard, leathery, waxy leaves.

FIGURE 2 Close-up view of a dwarf birch leaf

a Describe how the physical features of the leaf provide the first line of defence for the plant's immune system.

b Suggest how a pathogen could potentially evade the dwarf birch's first line of defence.

24 Explain why viral infections can be more difficult for the immune system to fight than bacterial infections.

25 Explain how viruses can spread from one plant to another.

26 Describe the biological cause of an anaphylaxis response.

27 Identify the difference between the following:

a Chemical and physical defensive strategies in animals

b Cytokines and mast cells

c Receptors and histamine granules

d Complement proteins and interferons

28 Apply your knowledge of pathogens to determine whether allergens are a pathogen or not.

FIGURE 3 Pollen is a common allergen.

29 Conjunctivitis can occur when a person has a bacterial infection in their eye. Symptoms include redness, swelling, tear production and the production of pus. Explain the mechanism for each of these symptoms.

FIGURE 4 Red eyes are a symptom of conjunctivitis.

30 Identify the causes of the reaction shown in Figure 5.

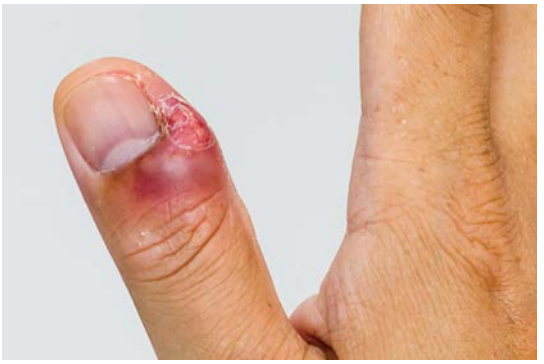


FIGURE 5 Fingernail infection

Design and discuss

31 Discuss the difference between an antigen and an allergen.

32 Design a labelled diagram that shows the process of inflammation. Include the following in your diagram:

- Macrophages
- Neutrophils
- Eosinophils
- Dendritic cells
- Natural killer cells
- Mast cells
- Complement proteins.

33 Natural killer cells will attack some cells infected with a virus. Discuss why it is important that natural killer cells do not destroy all viral-infected cells.

34 In 1982, Barry Marshall and Robin Warren (winners of the Nobel prize) were studying the causes of stomach ulcers. A bacteriaum, *Helicobacter pylori*, was found in many of the pathology samples from patients. After purifying the samples, Marshall drank a purified form of the bacteria. Within a few days he developed a severe stomach ulcer. They were able to isolate the specific bacteria from his stomach. Use your knowledge of Koch's postulates to identify why Marshall swallowed the bacterial sample.

35 A farmer needed to decide between three species of apple to grow in her orchard. She asked a team of scientists to help her determine which species of apple would be most resistant to a new type of fungus that had been found in the region.

Design a controlled experiment that could determine which species of apple was the most resistant to the fungus.

Exam essentials

Responding to questions

In your exam, you may be expected to provide more than one example or explanation.

Read the question carefully

In the stressful environment of an exam, it is very easy to rush your answer and not re-read the question and your final response. This can lead to missing key elements in the question (such as mentioning physical barriers instead of chemical barriers) or using the wrong definition or explanation for a cell type.

It is worth practising reading a question three times:

- The first time is to understand the general information that is covered.
- The second reading is to check the key information that is needed in the answer.
- The final reading of the question is after you have written the answer. This checks that you have covered all the important information that might be required by the examiner.

The following question is taken from the 2020 VCE Biology Examination. Read the question carefully using the guide described above, then consider whether the response is missing any key elements.

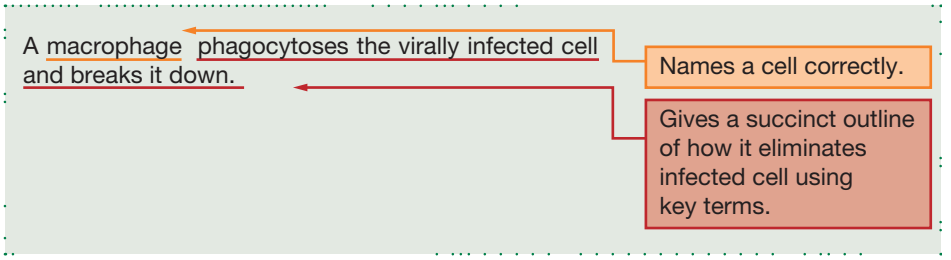
QUESTION 5a (2020 VCE Biology Written examination)

a The human immune system uses several different types of cells to eliminate virally infected cells.

Name **one** of these cells and outline how it eliminates virally infected cells. 2 marks

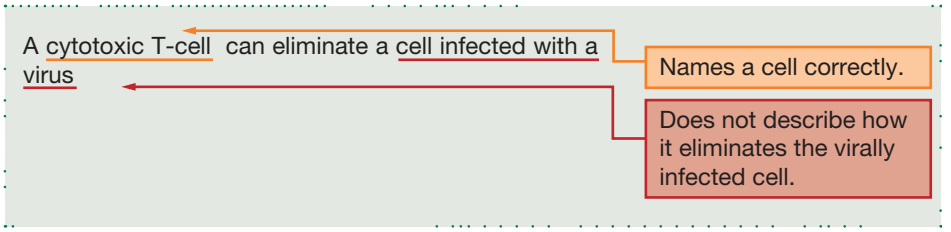
Source: 2020 Biology Written Examination Question 5a, Short answer, reproduced by permission © VCAA

Response 1



This answer would receive 2 marks since it correctly identifies the cell and outlines how it eliminates the virus-infected cell. An alternative answer could have described the cytotoxic T-cell releasing chemicals that break down or induce apoptosis in the infected cell.

Response 2



This response would receive one mark for identifying a cell, but it does not provide all the information required by the question. (Cytotoxic T-lymphocytes are covered in Chapter 9.)

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 3 (2018 Biology Written Examination)

Plants are a rich source of nutrients for many organisms, including bacteria, fungi and viruses. Although plants lack an immune system that is comparable to animals, plants have evolved chemical barriers to stop invading pathogens from causing significant damage.

a Describe **two** chemical barriers that could be present in a plant that is protecting itself from an invading pathogen. 2 marks

The waxy cuticle on the leaf

Enzymes

b Humans have a sophisticated immune response to invading pathogens.

State **two** ways that pathogens are prevented from entering the internal environment of the human body. 2 marks

Skin and mucus

Source: 2018 Biology Written Examination Question 3, Short answer, reproduced by permission © VCAA

Marking guide

Question 3 a	These responses must be chemical barriers. - 1 mark for the secretion of a toxin or odour that is harmful or unfavourable to the pathogen. - 1 mark for enzymes (chitinases, oxalic acid, phenols, saponins and glucanases) that affect normal pathogen functioning.
Question 3 b	- 1 mark for any two of the following: • intact skin • nose lined with thick sticky mucus • fine nasal hairs to trap pathogens • lysozymes in tears and saliva.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

CHAPTER 9

Immunity

When a pathogen is able to break through the body's physical, chemical and biological defence systems, the non-specific innate immune system tries to remove and destroy it. This system does not have any memory of particular pathogens and can sometimes fail to remove the pathogen. When this occurs, the immune system needs to be able to adapt and modify its response depending on the type of pathogen it needs to fight. This tertiary response requires a series of checkpoints where antigens are presented, and cells that are able to recognise and destroy infections are created. The lymphatic system offers this function, providing a place for phagocytic cells to present the antigen to specific immune cells.

KEY KNOWLEDGE

- → 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
- → 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
- → the difference between natural and artificial immunity and active and passive strategies for acquiring immunity

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GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

9A What is an antigen?

9A Groundwork resource
Antigens

9B What do macrophages, neutrophils and dendritic cells all have in common?

9B Groundwork resource
Antigen presentation

9C Draw a labelled diagram of a macrophage phagocytosing a bacterial cell.

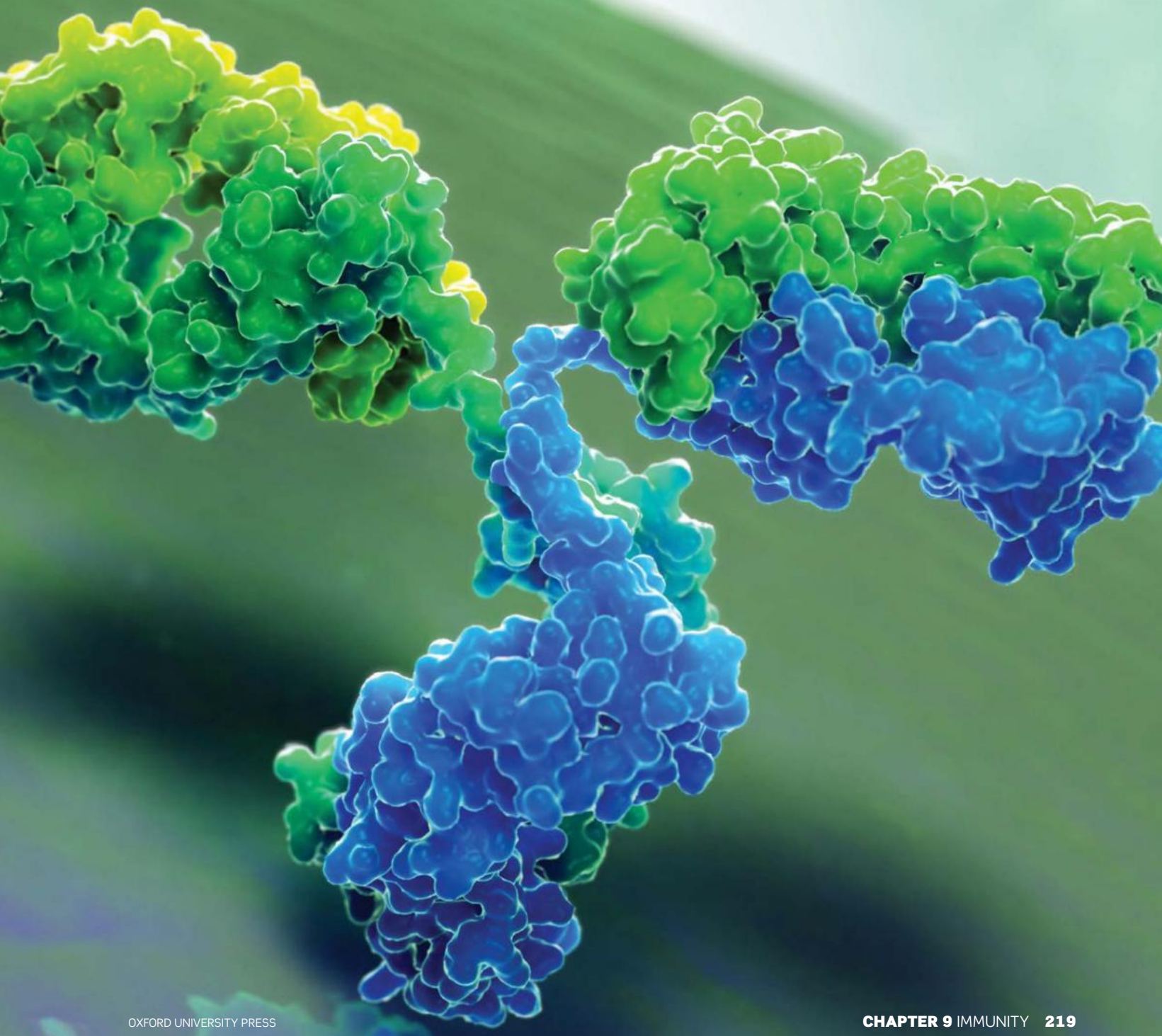
9C Groundwork resource
Phagocytosis

FIGURE 1 Some immune cells produce a protein called an antibody that will bind to one specific antigen.

PRACTICALS

PRACTICAL **9.1** Blood typing

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.



9.1 The lymphatic system

KEY IDEAS

In this topic, you will learn that:

- the lymphatic system provides transport for cells and molecules of the immune system
- antigens are presented to T- and B-lymphocytes in the lymph nodes.

Video
The role of the lymphatic system

When a pathogen makes it past the physical, chemical and microbiota barriers of the immune system, an inflammation response is initiated. This includes triggering mast cells to release histamine, resulting in vasodilation and increased permeability of blood vessels. The fluid that leaves the blood collects in the surrounding tissue, causing it to swell. Eventually, the blood vessels return to normal, leaving the fluid filling the cavities between cells. The lymphatic system is responsible for returning this fluid to the blood supply.

The lymphatic system consists of lymphatic vessels and the primary and secondary lymphatic tissues.

Lymph vessels

lymphatic system
a network of tissues and organs that transport lymph, amongst other things, throughout the body

lymph vessel
a thin-walled vessel, like a blood vessel, that carries lymph

The **lymphatic system** is considered an open circulatory system since it drains and collects fluids. The lymph capillaries drain any extra interstitial fluid, called lymph, from between cells in the tissue, similar to street drains in cities. Once the lymph is in the **lymph vessels**, it gets pushed forward each time the surrounding skeletal muscles move. Valves in the vessels prevent the fluid from flowing backwards. The fluid then moves into the lymph nodes before being returned to the blood supply.

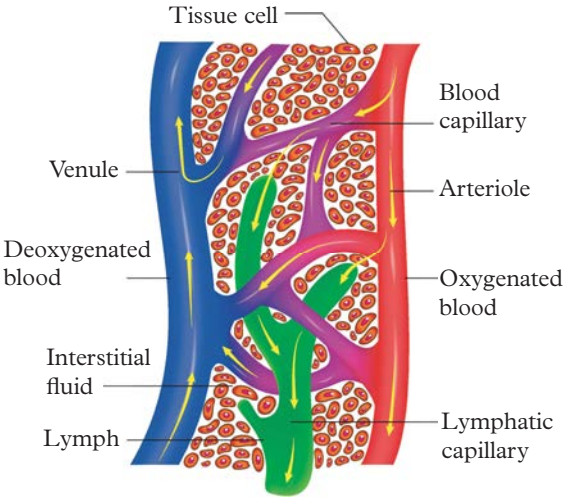


FIGURE 1 Interstitial fluid is returned to the blood through the lymphatic system (green).

If a person is sitting still for a long time and not moving their leg muscles, interstitial fluid cannot move through the lymphatic vessels and instead begins to build up in the tissues and lymph vessels. This can cause the ankles and lower parts of the leg to swell. Once the leg muscles are moving again, the lymph fluid will start to disperse and the swelling will go down. This is one of the reasons you are reminded to get up and move around on long haul flights.

Primary lymphatic tissues

bone marrow
spongy tissue in bones that contains stem cells for the development of red and white blood cells and blood platelets

Bone marrow is found in the centre of large bones. It contains stem cells, which produce white blood cells. The white blood cells include macrophages, neutrophils, dendritic cells and B- and T-lymphocytes. All of these cells, except for T-lymphocytes, mature in or shortly after leaving the bone marrow. Refer to Chapter 8 for more detail on these cells.

The **thymus** is a small, irregularly shaped gland located just under the breastbone. Its function is to allow the T-lymphocytes to mature and to remove the cells that do not recognise self-markers. This is important since immune cells that recognise and destroy cells with self-markers can result in the immune system attacking the body. The thymus will typically reach full size at the age of one and continue to increase activity levels until puberty.

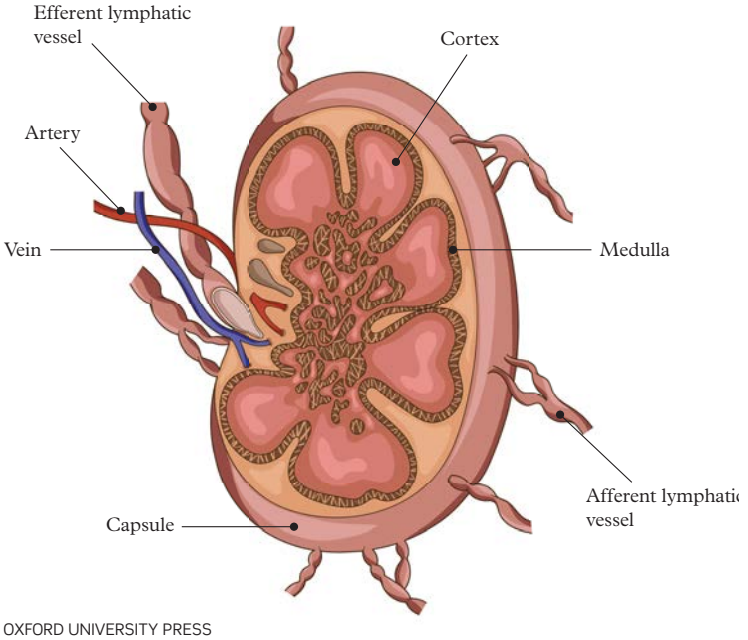
Secondary lymphatic tissues

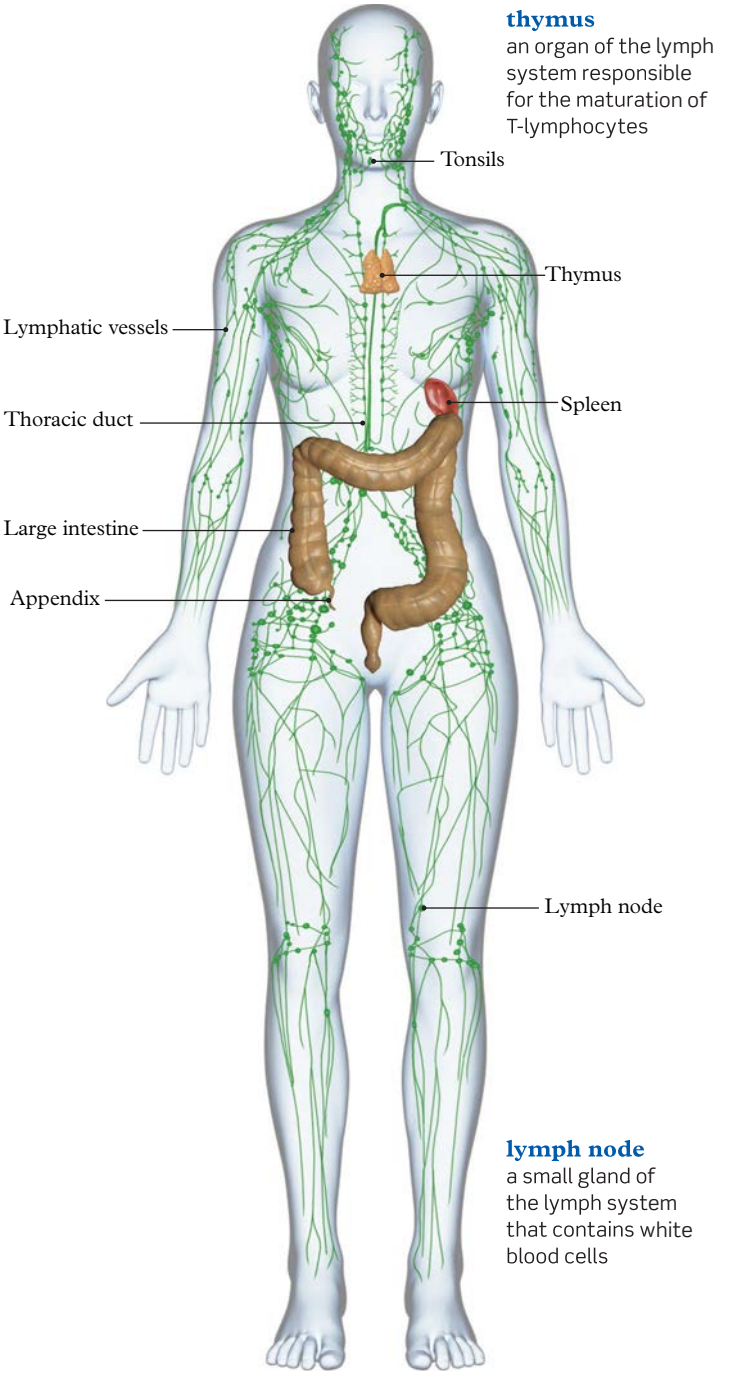
There are a number of secondary lymphatic tissues, including:

- appendix – promotes communication between the immune system and intestinal bacteria
- spleen – controls the number of red blood cells in the body
- tonsils – filter bacteria and viruses that may be in food or air
- **lymph nodes** – where the memory B- and T-cells of the immune system are exposed to antigens.

Lymph nodes

Lymph nodes are located at key points along the lymph vessels. They provide a place for the memory cells of the immune system to search for antigens that are presented on the surface of antigen-presenting cells.





thymus
an organ of the lymph system responsible for the maturation of T-lymphocytes

lymph node
a small gland of the lymph system that contains white blood cells

FIGURE 2 The lymphatic system consists of primary lymph tissues (thymus and bone marrow) and secondary lymph tissues (lymph nodes, spleen, tonsils).

FIGURE 3 Lymph enters the lymph node via the afferent lymphatic vessels, and drains via the efferent lymphatic vessels.

Antigen-presenting cells

As you learned in Chapter 8, macrophages, neutrophils and dendritic cells all act as antigen-presenting cells. When these cells engulf a bacterial cell, virus or single antigen molecule, they use lysozymes to digest it into small particles. These antigen particles are combined with other molecules (called MHCII) to form a complex that is presented on the surface of the membrane. The antigen-presenting cell displays the antigen complex to B- and T-lymphocytes in the lymph node. If one of the lymphocytes recognises the antigen, the adaptive immune response is initiated.

CASE STUDY 9.1

Lymphoedema

Lympheodema occurs when the lymph nodes are removed or damaged as part of cancer treatment. This can cause a blockage in the lymphatic system, preventing the lymph fluid from draining. As a result, the fluid in the tissues can build up, causing the local area or limb to swell. While this can restrict movement of the limb and cause some discomfort, the biggest risk of lymphoedema is the possibility of infection. The antigen-presenting cells cannot travel through the blocked lymph vessels to the lymph node. Therefore, the movement of all lymphocytes is affected, increasing the risk of an infection developing in the area.

FIGURE 4 Removal of the lymph nodes as a result of surgery for cancer can result in lymphoedema.

CHECK YOUR LEARNING 9.1

Describe and explain

1 Describe the purpose of the lymphatic system.
2 What is the function of a lymph node?
3 Name three cells that can act as antigen-presenting cells.

Apply, analyse and compare

4 Compare the function of the thymus and bone marrow in cells within the lymphatic system.
5 Using your knowledge from Chapter 8, compare MHCI and MHCII molecules.
6 Use Case study 9.1 to explain the role of lymph nodes.

Design and discuss

7 Many venoms that are injected from a snake bite will move through the lymphatic system as well as the blood. The current treatment for snake bite is to immobilise the limb. Discuss why this treatment is effective.
8 When surgeons remove cancer tissue from a patient, they often remove the surrounding lymph nodes. Suggest a reason for this procedure.

9.2 The adaptive immune response

KEY IDEAS

In this topic, you will learn that:

- the adaptive immune system can recognise previous pathogens and has specialised white blood cell groups, B- and T-lymphocytes, and antibodies
- threats to the immune system can be extracellular (bacteria) or intracellular (viruses and some bacteria)
- B-lymphocytes differentiate into plasma cells and memory B-lymphocytes
- plasma cells produce antibodies that are specific to the antigen causing the disease/infection.

If a pathogen evades the innate immune system, the adaptive immune system responds. The adaptive immune system:

- learns to recognise a specific pathogen
- produces cells and antibodies to specifically target that pathogen.

The first time that the adaptive system is exposed to the pathogen, the response is slower and the host organism will display symptoms of the disease. If the adaptive system has been exposed to the pathogen before, then the response can be much faster and the symptoms displayed by the host may be very mild or non-existent.

The adaptive immune system is comprised of specialised white blood cells. These can be divided into two main groups, called B- and T-lymphocytes. These cells are able to store the memory of antigens that they have met before. If the immune response detects the antigen, it can trigger the production of cells that can target and destroy the antigen.

Like all immune cells, the B- and T-lymphocytes are generated from stem cells in the bone marrow. But they differ in that:

- B-lymphocytes mature before they leave the bone marrow
- T-lymphocytes must travel to and mature in the thymus before they are released into the rest of the body.

Once activated, each of these cells is highly specific to one type of antigen. When they recognise their specific antigen, they will rapidly clone themselves (clonal proliferation) to produce thousands of identical cells that respond to that antigen. When the antigen is removed from the body, the cloned cells will apoptose, leaving only a few cells that retain the 'memory' of how to respond if the antigen returns.

FIGURE 1
B-lymphocytes were first discovered in the bursa of Fabricius, an organ found only in birds.

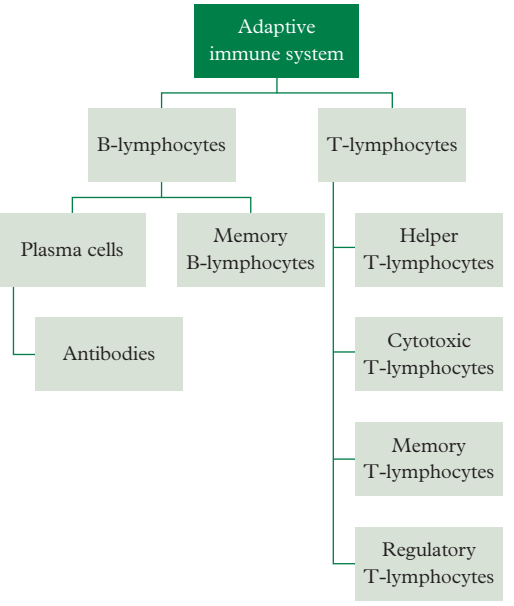


FIGURE 2 The structure of the adaptive immune response.

Extracellular and intracellular threats

extracellular threat
pathogens that enter the host organism and survive outside of the host's cells

intracellular threat
pathogens that invade host cells to survive and reproduce, e.g. viruses

The immune system needs to be able to respond to extracellular and intracellular threats. **Extracellular threats** are pathogens (such as bacteria) that are able to move about the host organism and grow and reproduce within the host. They must first find their way into the organism and avoid the innate immune system and the adaptive immune system to survive. These threats are usually easily detected by the cells of the immune system and the host organism will usually recover after a short illness.

Intracellular threats include viruses and some bacteria that are able to hide inside the host's cells. This allows them to evade the innate and adaptive immune system. An example of this is the human immunodeficiency virus (HIV) responsible for acquired immunodeficiency syndrome (AIDS) in humans. HIV targets helper T-lymphocytes (CD4+ cells) and enters these cells. The virus then replicates inside the cells and causes them to die. Helper T-lymphocytes are the cells able to detect cells infected with viruses and signal to other cells to respond (covered in Topic 9.3).

B-lymphocytes

naive B-cell
a B-cell that has not been exposed to an antigen; it will become either a memory B-cell or a plasma cell that secretes antibodies specific to the antigen that was originally bound to it

B-lymphocytes are a special group of white blood cells. Cells that have not yet been exposed to an antigen are known as **naive B-cells**. They require two signals before they can become activated.

- First signal – an antigen binding to the cell's complementary receptor. This receptor is a membrane-bound antibody produced by the B-lymphocyte. Once the antigen binds to the receptor, it takes the antigen into the cell before digesting it and presenting it on the membrane surface. Unlike other antigen-presenting cells, B-lymphocytes will only present the antigen that is complementary to its receptor.
- Second signal – contact with a group of cytokines called interleukins, which are released by an activated helper T-lymphocyte. If the second signal is not received from the helper T-lymphocyte, then the B-lymphocyte will apoptose. This is one way the immune system becomes tolerant to the self-antigens found on the organism's own cells.

Once activated by the two separate signals, the B-lymphocyte undergoes clonal proliferation or clonal expansion (rapidly producing copies of themselves). These cloned copies then differentiate into either plasma cells or memory B-lymphocytes.

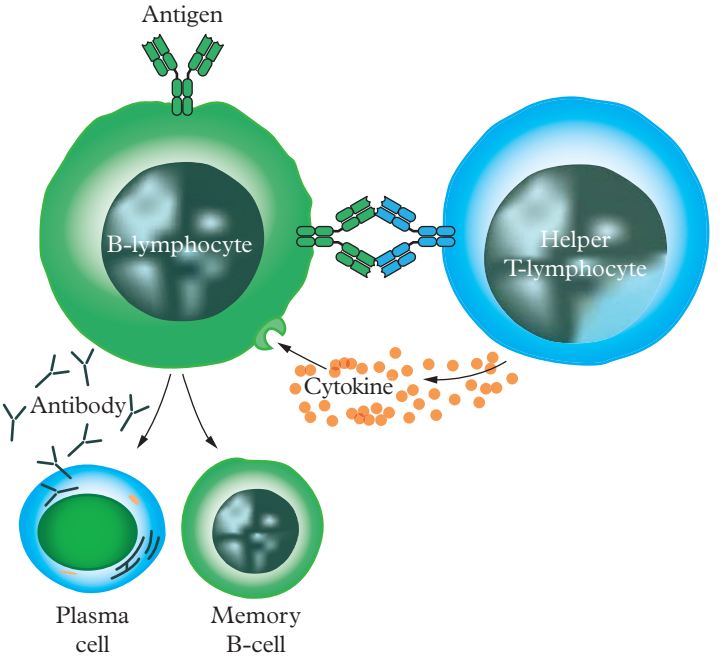


FIGURE 3 B-lymphocytes require the antigen to bind to its complementary receptor (signal 1) and contact with cytokines from a helper T-lymphocyte (signal 2) before it will differentiate.

Plasma cells

Once a B-lymphocyte has been activated it is ready to differentiate into a plasma cell. **Plasma cells** are mature B-lymphocytes that are able to produce large amounts of identical **antibodies**. When activated, the membrane receptors of the naive B-lymphocyte clump together, activating internal cell messengers. This causes the cell to respond by initiating the transcription and translation of the genes to produce the specific antibody. These antibodies are then released by the cell. The mechanism that results in the production of a specific antibody by cloned plasma cells is called the **humoral immune response**.

plasma cell
a mature B-lymphocyte that produces large amounts of identical antibodies against a specific antigen

antibody
a large, Y-shaped protein produced by plasma cells; used by the immune system to neutralise pathogens (also known as an immunoglobulin)

humoral immune response
immunity that is mediated by antibodies and complement proteins

Antibodies

Antibodies, or immunoglobulins, are used by the immune system to bind to specific antigens. The antibodies are quaternary structure proteins (refer back to Chapter 2 to refresh your knowledge on protein structures) with two heavy chains and two light chains arranged in a 'Y' shape.

- Each top tip of the 'Y' is identical and will only bind to a specific complementary antigen. Each plasma cell will produce antibodies with a single-shaped antigen-binding site. This makes each antibody specific to a particular antigen.
- The hinge region between the two antigen-binding sites is flexible, allowing the antibodies to bind to two antigens at the same time. This cross-linking of antigens can bind pathogens together into a clump (called agglutination), preventing them from escaping other white blood cells such as macrophages, or lymphocytes such as cytotoxic T-lymphocytes.
- The base of the 'Y' molecule is conserved for all antibodies. Its role is to signal and activate the rest of the immune system.

There are five main classes of antibodies that are released at different times in the immune response. Some, such as immunoglobulin G (IgG) and immunoglobulin A (IgA), are able to pass through breast milk to a baby. Others, such as immunoglobulin E (IgE), are responsible for the hypersensitivity response of allergies.

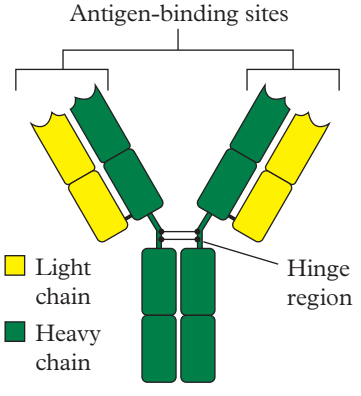


FIGURE 4 Antibodies are quaternary structure proteins with two heavy chains and two light chains. The hinged regions of antibodies provide the flexibility to link pathogens together.

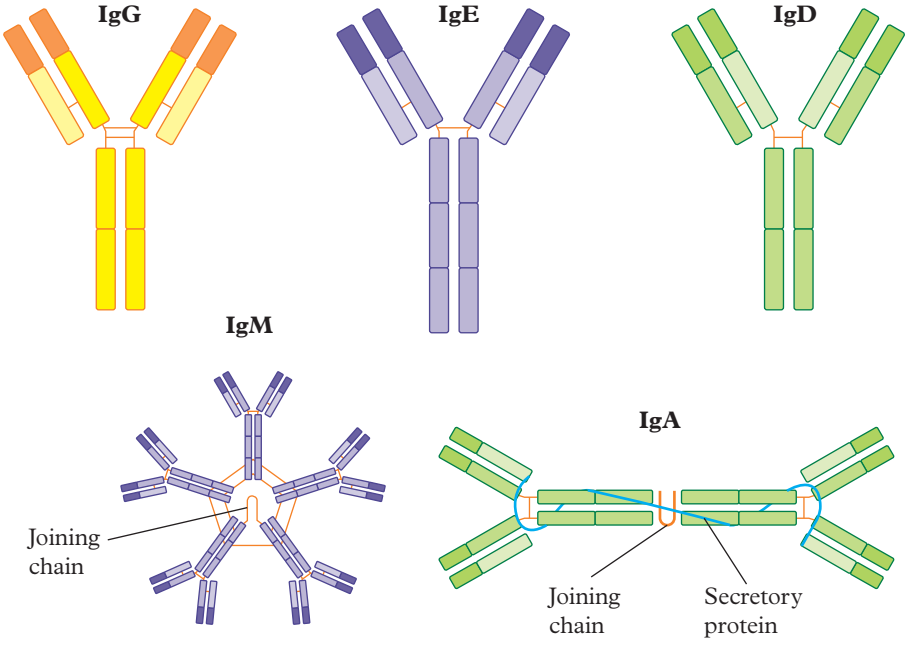


FIGURE 5 There are five main types of antibodies or immunoglobulins. Each plasma cell will produce a single kind of antibody.

Memory B-lymphocytes

memory B-lymphocyte a cell that 'remembers' the antigens presented by a particular pathogen for rapid antibody production in future infections

Memory B-lymphocytes are produced in response to a specific antigen and are retained as a memory of prior exposure to that specific antigen. The presence of these cells in localised lymph nodes is important because it allows the immune system to respond quickly if it is exposed to the same pathogen again.

When a memory B-lymphocyte is produced, antibodies bind to the plasma membrane and act as receptors. When a complementary antigen binds to the antibody receptors, the receptors pass the message into the cell. In many cases, the memory B-lymphocytes act as antigen-presenting cells and activate the helper T-lymphocytes. This can accelerate the immune response when exposed to a pathogen for the second time.

clonal proliferation the selection and reproduction of only one type of cell

The memory B-lymphocytes reproduce (undergo **clonal proliferation**) and differentiate into considerably more plasma cells capable of producing a larger number of antibodies more quickly than the first exposure. This rapid response prevents the infection from spreading throughout the body. It is this aspect of the immune system that can contribute to allergies becoming worse with each exposure to the allergen. Every time a person is exposed to the allergen, the memory B-lymphocytes are activated into plasma cells and produce larger and larger amounts of IgE. These IgE antibodies continue to bind to mast cells and result in higher levels of histamine produced with each exposure. In most cases, memory B-lymphocytes will age and be removed.

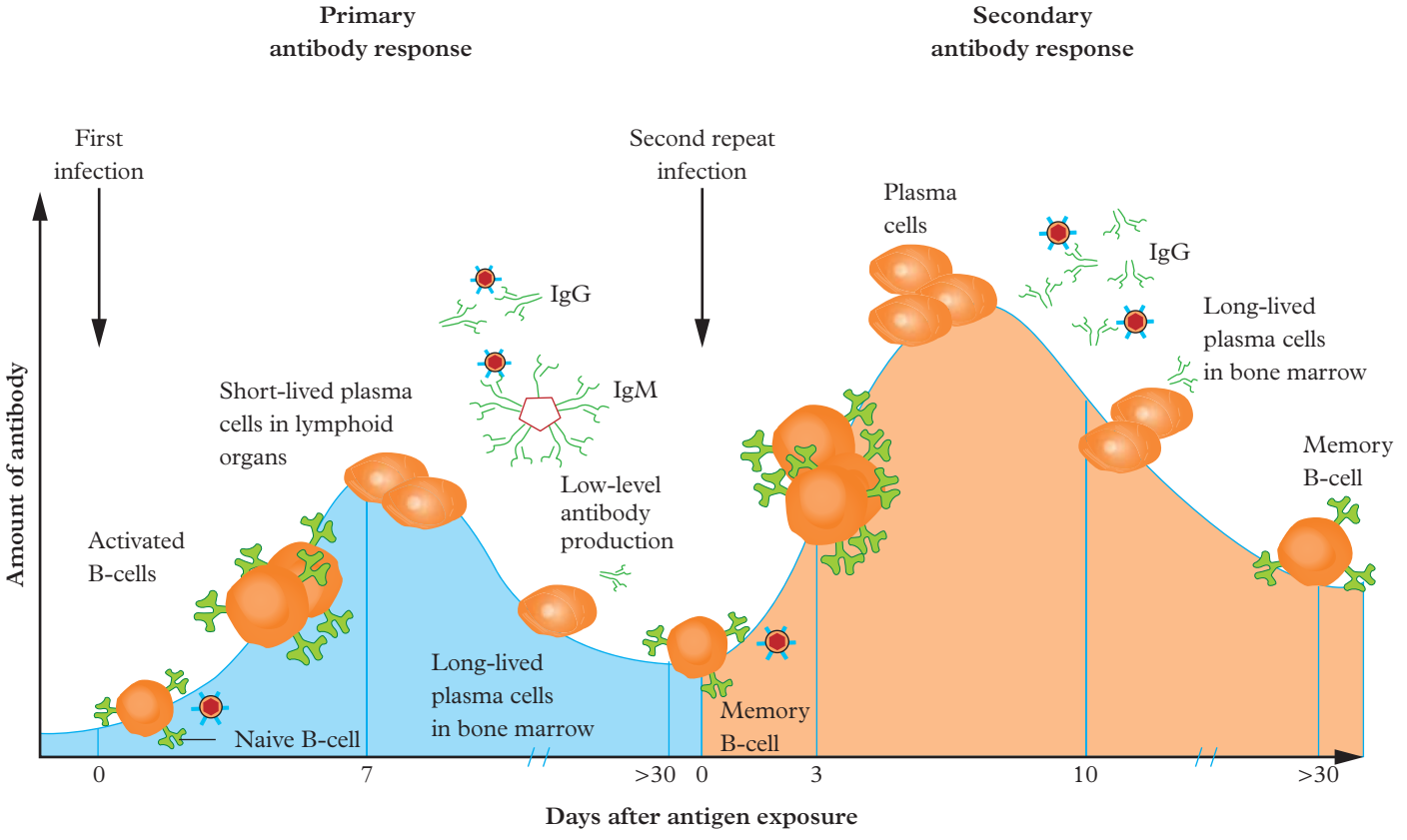


FIGURE 6 The second time the antigen is presented, the memory B-cells are able to produce a large number of antibodies in a short time period.

CASE STUDY 9.2

Measles infection weakens the immune system

The measles virus is one of the most contagious diseases that exists. It can linger in the air for up to two hours after an infected person has left. It can cause a high fever, a runny nose and a recognisable red rash. It can also be deadly if the infection leads to encephalitis (brain inflammation) or pneumonia.

The disease's activity was recently tracked by inserting a green fluorescent protein gene into the virus and tracing it through a monkey host. As the modified virus invaded cells to reproduce, the fluorescent protein was also produced, allowing scientists to track the disease. The fluorescent protein appeared in the memory cells located in the lymphoid tissue. As the infection spread, it settled on the surface lining of the lungs and nose, allowing it to move into the air with every cough and sneeze.

Once the monkey's immune system was able to clear the infection, the memory B- and T-lymphocytes were destroyed, removing the adaptive immune response. This left the monkey susceptible to other infections that it had previously been immune to. This is similar to reactions found in humans. People who become infected with measles will be more susceptible to other infections in the following years.

In contrast, as measles vaccination becomes more common, the number of childhood deaths from other diseases shows a corresponding decrease due to the retention of the memory cells.

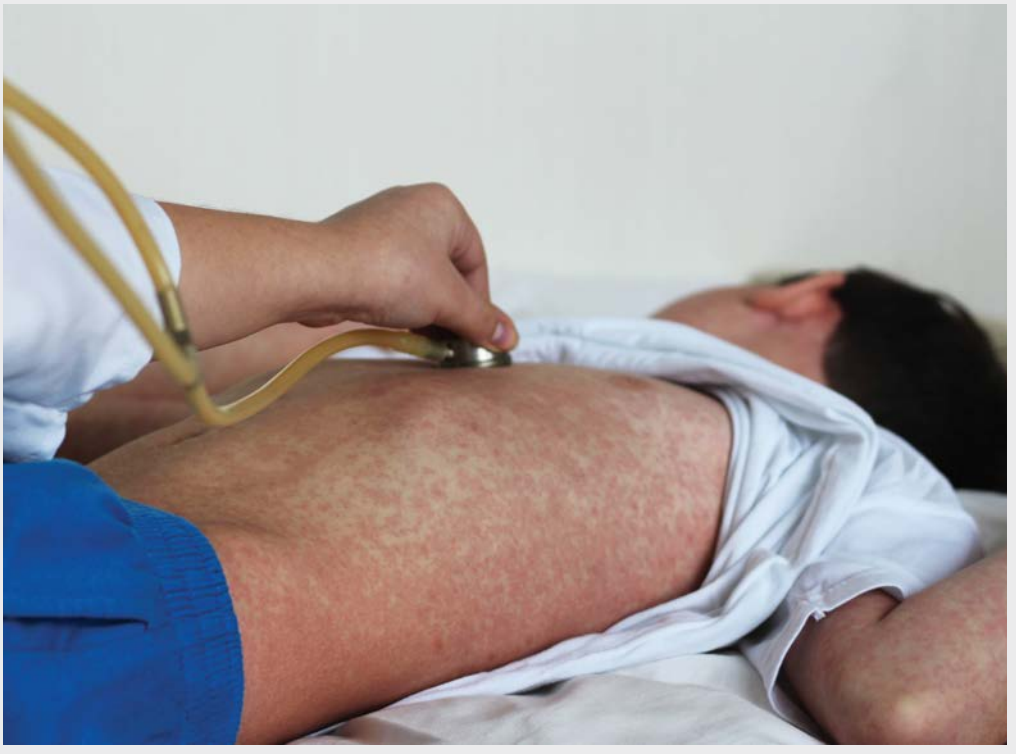


FIGURE 7 Childhood measles can deplete the adaptive immune response.

CHALLENGE 9.2

Testing B-lymphocytes

Scientists wanted to test the hypothesis that a given B-lymphocyte makes only one specific antibody against an antigen. They injected an animal simultaneously with two different antigens. After waiting for the immune system to respond, they took a sample of blood from the animal. From the blood, they isolated single plasma cells and tested whether each one produced antibodies to one or both of the antigens.

1 Predict the results you would expect for the B-lymphocytes based on your knowledge of the immune system. Justify your predictions.

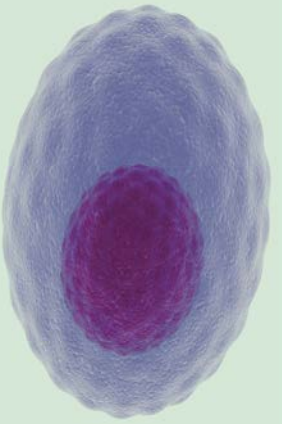


FIGURE 8 A B-lymphocyte plasma cell

CHECK YOUR LEARNING 9.2

Describe and explain

1 Describe the adaptive immune response.

2 Describe what is meant by the quaternary structure of an antibody.

3 Draw a labelled diagram of an antibody. Draw an antigen that is complementary to the antigen binding site.

4 Explain how antibodies can prevent the spread of an infection.

5 Describe the two signals required to activate a B-lymphocyte.

6 Which antibody can pass from mother to baby via breast milk?

FIGURE 9 Breast milk can pass certain antibodies from the mother to the child.

7 Describe how a naive B-lymphocyte can become a memory B-cell.

Apply, analyse and compare

8 Contrast plasma cells and memory B-lymphocytes.

9 Explain why you can become sick if you are exposed to a pathogen for the second time.

Design and discuss

10 Ready Case study 9.2 and discuss the importance of preventing the spread of a measles infection within a population.

11 Explain why repeated exposure to an allergen can result in increasingly severe allergic reactions.

12 Draw a graph that shows the level of antibodies produced by the body at the first exposure and second exposure to an antigen.

13 a Which antibody is able to pass from a mother to an unborn foetus?

b Discuss the importance of this to the foetus.

9.3 Helper T-cells and cytotoxic T-cells

KEY IDEAS

In this topic, you will learn that:

- helper T-lymphocytes control the adaptive immune response
- cytotoxic T-lymphocytes attack cells that are damaged, dysfunctional or infected with a virus.

Video
Helper T-cells and cytotoxic T-cells

T-lymphocytes

helper T-lymphocyte
a type of T-cell that plays an important role in the immune system; stimulates the activity of other immune cells by releasing cytokines

cytotoxic T-lymphocyte
a type of T-cell that kills cancer cells and virus-infected cells

T-lymphocytes are an important component of the adaptive immune system. Immature T-lymphocytes travel from the bone marrow to the outer cortex of the thymus. Once there, they are exposed to a range of different molecules that are naturally found in the body. Any T-lymphocyte that tries to start an immune response to these molecules will apoptose. This prevents the T-lymphocytes from starting an immune response against the cells in the rest of the body. Having matured, they move to the inner parts of the thymus and differentiate into four major classes: **helper T-lymphocytes**, **cytotoxic T-lymphocytes**, memory T-lymphocytes and regulatory T-lymphocytes. For VCE Biology, you only need to learn about helper T-lymphocytes and cytotoxic T-lymphocytes.

Helper T-lymphocytes

Helper T-lymphocytes are responsible for activating other lymphocytes in the adaptive immune system.

Helper T-lymphocytes need two signals before they can be activated. Both these signals are provided by the antigen-presenting cells (i.e. macrophages, neutrophils or dendritic cells).

- First signal – the antigen-presenting cells present the antigen to the helper T-lymphocytes in the lymph node. If one of the helper T-lymphocytes has a complementary (matching) receptor to the antigen, it becomes partially activated.
- Second signal – The antigen-presenting cell releases a cytokine before the helper T-lymphocyte becomes fully activated. If the second signal is not received then the helper T-lymphocyte will apoptose.

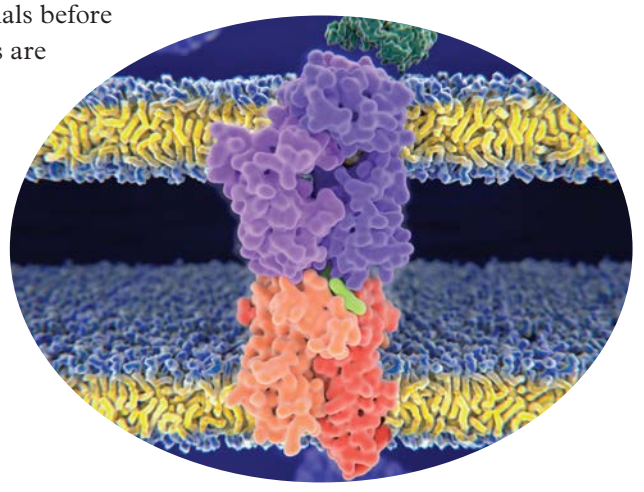


FIGURE 1 An antigen (green) is presented as part of the MHCII complex, where it is recognised by a complementary T-cell receptor.

Once activated, the helper T-lymphocytes undergo clonal proliferation or clonal expansion. The increased number of cloned T-lymphocytes means many more helper T-lymphocytes are responsible for producing a number of different cytokines. The cytokines activate specific B-lymphocytes and cytotoxic T-lymphocytes that destroy any cells displaying that antigen.

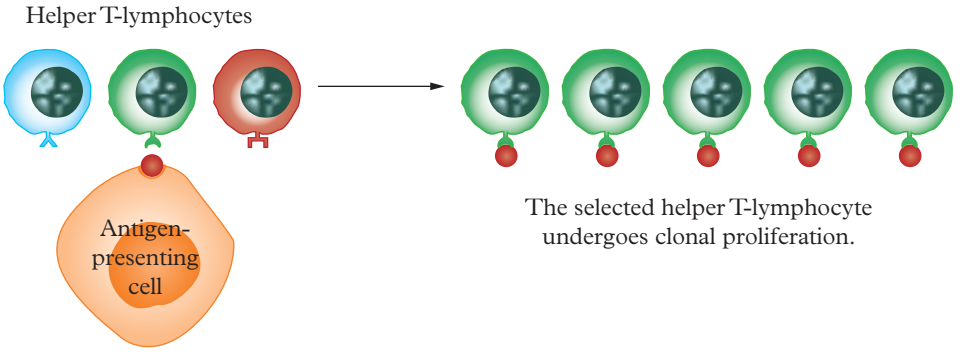


FIGURE 2 The helper T-lymphocyte that has a receptor complementary to the presented antigen will undergo clonal proliferation. This produces many helper T-lymphocytes.

Study tip

T-lymphocyte and T-cell are interchangeable terms. They mean the same thing.

Cytotoxic T-lymphocytes

Cytotoxic T-lymphocytes are most commonly located in the lymph nodes. They kill cancer cells, virus-infected or damaged cells. These cells also require two signals before they can be fully activated.

- First signal – is direct contact with a specific antigen offered by the antigen-presenting cells. Because the cytotoxic T-lymphocytes are selective, the antigen presented must be complementary to the receptor on the cytotoxic T-lymphocyte.
- Second signal – the antigen-presenting cell will then produce cytokines to activate the cytotoxic T-lymphocyte.

Cytotoxic T-lymphocytes also become partially activated when a virus–infected cell or damaged or dysfunctional cell is unable to produce a complete self-marker or MHCI molecule. Small parts of the viral protein coat on the surface of the infected cell can also activate the cytotoxic T-lymphocyte. In this case, the second cytokine signal will be produced by antigen-presenting cells that are phagocytosing viral particles or by the damaged cell itself.

Once activated, the cytotoxic T-lymphocytes undergo clonal proliferation and start identifying pathogens or damaged cells. If they bind to a self-cell, the cytotoxic T-lymphocyte will activate the death receptor pathway of the cell, initiating apoptosis. Cytotoxic T-lymphocytes will also produce cytotoxic granules that contain perforins and granzymes. These molecules can form protein channels in their target cell and trigger the apoptotic pathway. Both these mechanisms result in the controlled cell death of the cell in question.

When helper T-lymphocytes activate cytotoxic T-lymphocytes to attack damaged cells or pathogens, it is called **cell–mediated immunity**.

cell–mediated immunity
the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen

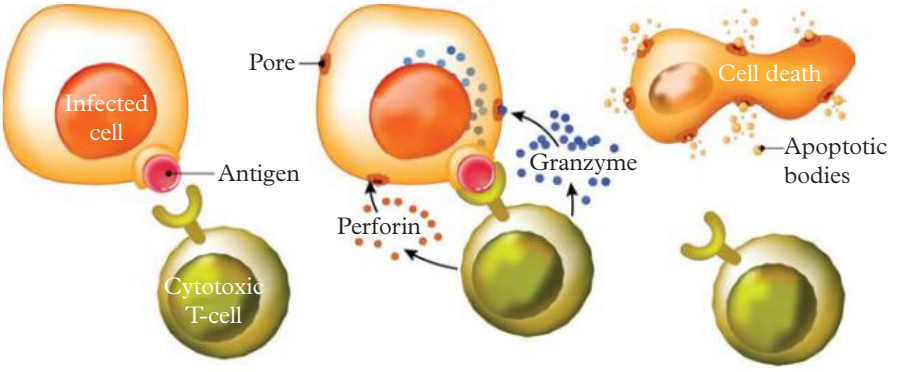


FIGURE 3 Cytotoxic T-lymphocytes produce cytotoxic molecules (perforin and granzyme) to initiate apoptosis in the infected cell.

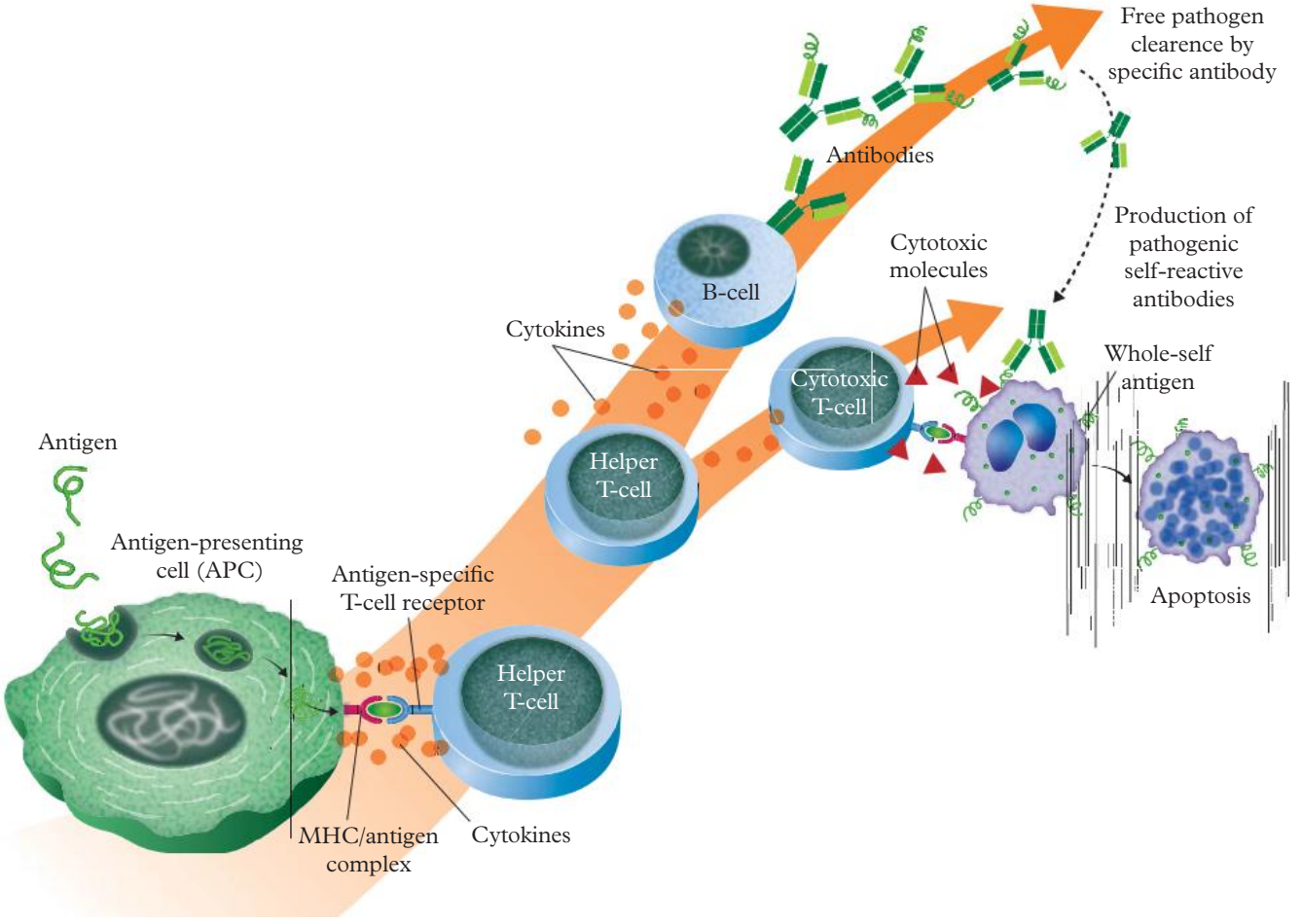


FIGURE 4 The cell-mediated immune pathway involves antigen-presenting cells activating helper T-cells, which then activate cytotoxic T-cells.

CASE STUDY 9.3

Transplantation immunity

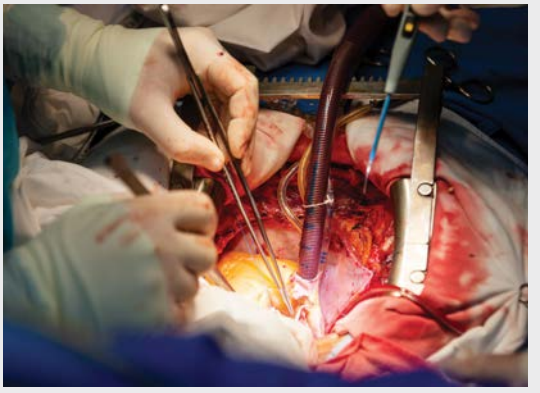


FIGURE 5 Supressing the recipient's immune system is important to reduce rejection of the donor tissue.

Transplantation is the process of removing cells, tissues or organs from one body (donor) and placing them in another (the recipient). This can be complicated since the immune system of the recipient will recognise the donor cells as foreign and attack them. Doctors will try to limit the number of antigens in the donor cells by matching the immune capability of the recipient. This can be difficult because the pool of donors who have consented to the use of their organs or tissue is small, and there will always be some degree of rejection (when the recipient's immune system mounts an immune response against the donor tissue). Often patients can wait over two years for a partially matching donor to become available.

Host-versus-graft disease occurs when the recipient's immune system recognises the transplanted tissue as foreign. To reduce the risk of this occurring, patients are provided with immunosuppressive drugs that will dampen their immune response. These drugs are usually targeted against the immune cells that are most active against foreign cells (cytotoxic T-cells).

Drugs such as cyclosporin and tacrolimus act to limit cytotoxic T-lymphocyte activation, preventing clonal proliferation and the release of cytotoxic molecules.

Graft-versus-host disease is rare and occurs when mature immune cells are already present in the recipient's tissue. This may occur during a bone marrow transplant. These cells are activated by the donot's tissue, undergoing clonal proliferation before attacking other parts of the recipient's body.

CHECK YOUR LEARNING 9.3

Describe and explain

1 Name the two types of T-lymphocytes and describe their role in the adaptive immune system.
2 What does the 'T' in T-lymphocyte stand for? Why is this significant?
3 Where do T-lymphocytes originate from in the body?

Apply, analyse and compare

4 Compare the function of the helper T-lymphocyte and the cytotoxic T-lymphocyte.

Design and discuss

5 Discuss the possible consequences of helper T-lymphocytes failing to perform their function correctly.
6 Use Case Study 9.3 to discuss why cytotoxic T-lymphocytes are targeted by drugs in transplantation recipients.

9.4 Natural versus artificial immunity

KEY IDEAS

In this topic, you will learn that:

- natural immunity occurs when the immune system has been exposed to the pathogen
- artificial immunity occurs when the immune system has been exposed to artificially produced samples of antigen
- active immunity occurs when the organism produces memory cells against an antigen
- passive immunity occurs when the organism is provided with antibodies from another source, and no memory cells are produced.

immunity
the resistance of an organism to an invading pathogen and its harmful effects

natural immunity
occurs when an organism is exposed to a live pathogen, develops the disease, and develops immunity to the pathogen

artificial immunity
occurs when an organism is intentionally exposed to a pathogen and develops immunity to the pathogen

active immunity
occurs when an organism produces antibodies in response to an antigen

natural active immunity
active immunity from exposure to a pathogen that causes disease in the organism, and the production of antibodies and memory cells to that pathogen

adjuvant
an ingredient used in some vaccines that initiates an inflammatory response, increasing the subsequent immune response

attenuated
a vaccine that contains a viable pathogen with reduced virulence

The production of memory cells can result in the ability to resist further infection from a pathogen. An organism that has produced these memory cells is said to have **immunity** against the pathogen. Organisms can acquire this immunity by direct exposure to the pathogen (**natural immunity**) or by indirect exposure to the pathogen (**artificial immunity**).

Active immunity

When a person has produced their own memory B- and T-lymphocytes, they are said to have **active immunity**. This type of adaptive immunity can take up to 3 weeks to develop and can last many years. There are two ways active immunity can develop: naturally and artificially.

Natural active immunity

Direct exposure to the pathogen through infection will result in macrophages presenting antigens from the pathogen to cells in the adaptive immune system. The helper T-lymphocytes will recognise the antigen that is presented and release cytokines that will activate both the cytotoxic T-lymphocytes and the B-lymphocytes. This activation from direct exposure to the pathogen will result in the active production of memory B- and T-lymphocytes to form **natural active immunity**.

Artificial active immunity

Organisms can be indirectly exposed to a pathogen through vaccination. Vaccinations contain two main ingredients: antigens and **adjuvants**.

The antigens are usually prepared from the digested parts of the pathogen or a weakened (**attenuated**) strain of the pathogen so that it does not cause the symptoms of the disease. Both forms of the vaccine contain the antigen molecules that will initiate a response by the immune system resulting in the production of memory B- and T-lymphocytes.

FIGURE 1 Vaccinations are a way of creating artificial immunity to a disease.

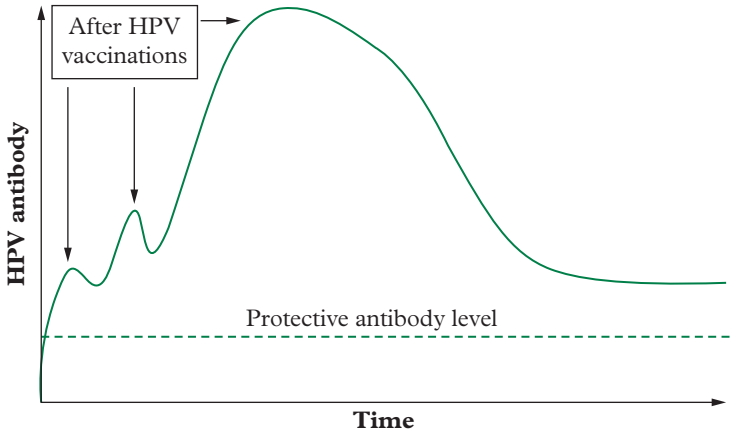


FIGURE 2 The level of human papillomavirus (HPV) antibodies increases after each successive booster vaccination.

Adjuvants are substances that support and stimulate the immune system to respond to the injected antigens. In human vaccines, the adjuvant could be aluminium salt, lipids or sugars. These molecules can cause a small inflammatory response that increases blood flow to the area of the vaccination and in turn the immune cells that help support the adaptive immune response. Adjuvants can also increase the number of antigen-presenting cells that can trigger the immune response.

Because the vaccination does not contain the actual pathogen, it is considered an indirect artificial form of active immunity. An organism that has been vaccinated has **artificial active immunity**.

artificial active immunity active immunity initiated by a vaccine containing antigens, which then causes the production of antibodies and memory cells to that antigen

passive immunity where an organism receives antibodies to a pathogen rather than producing them through their own immune system

natural passive immunity occurs when a mother passes her antibodies across the placenta or in breast milk to her developing child

Not all vaccinations provide lifelong immunity. Booster vaccines provide an opportunity for the memory cells to respond, forming new helper T-lymphocytes, plasma cells and cytotoxic T-lymphocytes. This secondary exposure also increases the number of memory cells in a patient. This will result in an even faster and larger response from the immune system if the organism is eventually exposed to the pathogen.

Passive immunity

Sometimes it is too dangerous for a person who has a depressed immune system (immunocompromised) – such as a those being treated for cancer or a newborn baby – to be directly or indirectly exposed to an antigen. In these situations, a person is provided with antibodies from another source. These antibodies can bind to the antigens and prevent them causing damage to the body. This type of immunity, where no T- or B-cells have been initiated and no memory cells are produced by the organism, is called **passive immunity**.

Natural passive immunity

All mammals are able to produce milk to feed their young. When the mother is exposed to a pathogen, she produces antibodies against the antigens found on the pathogen. Some antibodies are able to pass into the breast milk and are received by the baby. Many antibodies are also able to pass through the wall of the uterus to the developing child, conferring protection from pathogens that the mother has been exposed to. Because the antibodies are produced as a result of direct exposure to the pathogen, they are described as natural. When the antibodies are provided to the baby, it will be protected despite not producing any memory cells themselves. This means the baby has **natural passive immunity**.

FIGURE 3 A mother is able to pass on natural passive immunity to offspring through her milk.

Artificial passive immunity

When humans are bitten by a snake or spider, they may be given an antiserum. Antiserum is produced when an antigen similar to the venom is injected into an animal (most commonly a rabbit, horse or chicken). The animal is not hurt; however, their immune system is activated and their immune system starts producing antibodies specifically for the toxin. These antibodies can be found in the liquid serum part of their blood. When the serum is collected from the animal and the antibodies purified, this forms an antiserum.

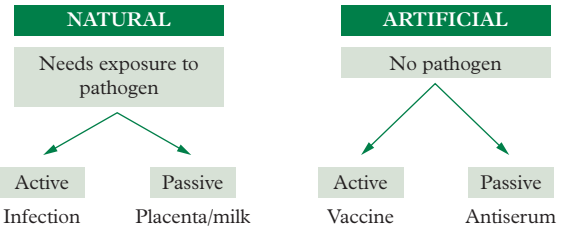


FIGURE 4 Immunity can be achieved by four different mechanisms.

Video
Active versus passive immunity

An organism that has been bitten can then be injected with the antiserum. The antibodies contained in the serum will then bind to the venom, inactivating it and preventing it from causing damage. The antibodies can survive for several weeks in the organism, providing temporary protection from the antigen. Because immunity was produced as a result of an artificial antigen, and no memory cells are produced by the protected person, this immunity is called **artificial passive immunity**.

artificial passive immunity occurs when antibodies are passed from one individual to another, usually by intravenous injection

CHALLENGE 9.4

Variolation

Variolation is a form of inoculation that was used as the first method to provide immunity against smallpox. This process involved rubbing powdered smallpox scabs or fluid from the pustules into scratches made in the skin of a healthy individual. The goal was to provide the individual with a mild form of the disease so that they would be protected (immune) when exposed at a later date. This method was first used in China and the Middle East, before being introduced to England in the 1720s.

1 What is the difference between vaccination and variolation?
2 Use the terms 'active', 'passive', 'natural' and 'artificial' to describe the types of immunity provided by vaccination and variolation.

CHECK YOUR LEARNING 9.4

Describe and explain

1 Define the following terms.
   a Active immunity
   b Passive immunity
   c Natural immunity
   d Artificial immunity
2 Provide examples for each type of immunity shown in the table below.

	Active	Passive
Natural		
Artificial		

Apply, analyse and compare

3 What are the advantages of being vaccinated?
4 Analyse the advantages of a mother breastfeeding her baby.

Design and discuss

5 Discuss the ethics of using animals to produce antiserum.
6 Some people claim that vaccinations are not natural. Discuss how you would respond to such a claim.

CHAPTER 9

Review

Chapter summary

9.1
- The lymphatic system's role in the immune system is to transport cells through the body.
- Antigens are presented to B- and T-lymphocytes in the lymph nodes.

9.2
- An adaptive immune system can recognise previous pathogens and has specialised white blood cell groups, B- and T-lymphocytes, and antibodies.
- There are both intracellular and extracellular threats to the immune system.
- B-lymphocytes differentiate into plasma cells and memory B-lymphocytes.
- Plasma cells produce antibodies that are specific to the antigen causing the disease/infection.

9.3
- Helper T-lymphocytes control the adaptive immune response and once they are initiated they go through clonal proliferation.
- Cytotoxic T-lymphocytes attack cells that are damaged, dysfunctional or infected with a virus and initiate apoptosis.

9.4
- Natural immunity occurs when an organism is exposed to a pathogen and naturally recovers and forms immunity.
- Artificial immunity is provided when an organism is vaccinated against a pathogen. The vaccination contains antigens and adjuvants.
- Active immunity occurs when an organism produces memory cells against an antigen.
- Passive immunity occurs when an organism is provided with antibodies from another source, and no memory cells are produced. This can be natural through a mother's milk, or artificial through antiserum to a snake or spider bite.

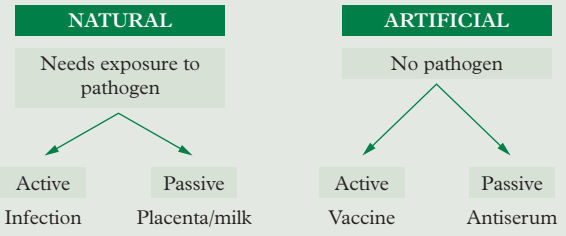


FIGURE 1 Immunity can be achieved by four different mechanisms.

Revision questions

Multiple choice

1 Which body system is responsible for transporting the cells and molecules of the immune system?
- **A** The lymphatic system
- **B** The skeletal system
- **C** The endocrine system
- **D** The respiratory system

2 Memory B- and T-cells are exposed to antigens in which type of tissue?
- **A** Appendix
- **B** Spleen
- **C** Tonsils
- **D** Lymph nodes

3 Which is not part of an antigen?
- **A** Antigen-binding site
- **B** Hinge region
- **C** Heavy chains
- **D** Extension arm

4 Which of the following is an example of an extracellular threat?
- **A** A virus
- **B** A helper T-lymphocyte
- **C** A bacterium
- **D** An antibody

5 The function of the thymus is to allow:
- **A** all lymphocytes to mature and differentiate into B- and T-lymphocytes.
- **B** B-lymphocytes to mature and differentiate into plasma cells.
- **C** T-lymphocytes to mature and to remove those that respond to self-antigens.
- **D** stem cells to produce lymphocytes and leukocytes.

6 A lymph node is:
- **A** a type of pathogen.
- **B** a cell in the adaptive immune system.
- **C** found at the end of a lymph vessel.
- **D** a place where antigen-presenting cells show the antigen to lymphocytes.

7 Which of the following are primary lymph structures?
- **I** Thymus
- **II** Spleen
- **III** Lymph node
- **A** I only
- **B** II and III
- **C** I, II and III
- **D** II only

8 Cytotoxic T-lymphocytes:
- **A** produce a specific antibody against the antigen.
- **B** produce cytotoxic granules that punch holes in a damaged cell.
- **C** phagocytose pathogenic cells.
- **D** are part of the innate immune response.

9 Which of the following statements about antibodies is incorrect?
- **A** Antibodies are quaternary proteins.
- **B** Antibodies have two sites that bind to a specific antigen.
- **C** Antibodies are present in snake antiserum.
- **D** Antibodies are part of the cell-mediated immune response.

10 B-lymphocytes:
- **A** produce antibodies.
- **B** can differentiate into plasma cells and memory B-lymphocytes.
- **C** produce cytotoxic granules.
- **D** can differentiate into phagocytic cells.

11 Which of the following is an example of natural passive immunity?
- **A** Vaccination
- **B** Antiserum
- **C** Breastfeeding
- **D** Infection with the pathogen

12 Which of the following is an example of artificial passive immunity?
- **A** Vaccination
- **B** Antiserum
- **C** Breastfeeding
- **D** Infection with the pathogen

13 Vaccinations prepare the body for a possible pathogen by:

A stimulating red blood cell production.

B inhibiting antigen production.

C stimulating antibody production.

D inhibiting leukocyte production.

FIGURE 2 Vaccinations protect the body from pathogens.

14 Which transplantation method would increase the chances of the transplanted tissue surviving?

A Using tissue cloned from the cells of the patient

B Using organs produced by genetic engineering to remove all proteins on the surface of the donor tissue

C Using organs from monkeys

D Using organs donated by a close relative because the MHCI will always be identical

Short answer

Describe and explain

15 Describe the structure and function of the lymphatic system.

16 Explain how T- and B-lymphocytes are able to recognise antigens.

17 Explain how the the adaptive immune system responds to both intracellular and extracellular threats.

18 Write the word ACTIVE or PASSIVE next to the following, to best describe their roles in initiating an immune response.

a Bacterial infection

b A mother's placenta

c Flu vaccine

d Snake antiserum

19 Explain how antiserum works to activate the immune system.

20 Explain how people who have been infected with measles are more susceptible to other infections in following years.

21 Draw an antibody and label its components.

22 Describe the role of antigen-presenting cells in the adaptive immune response.

23 Describe how a naive B-lymphocyte can become activated.

24 Define clonal proliferation.

Apply, analyse and compare

25 Elephantiasis is a disease caused by a parasitic worm that lives in the lymphatic system of its host. Infer how the presence of this parasite can result in the symptoms shown in Figure 3.

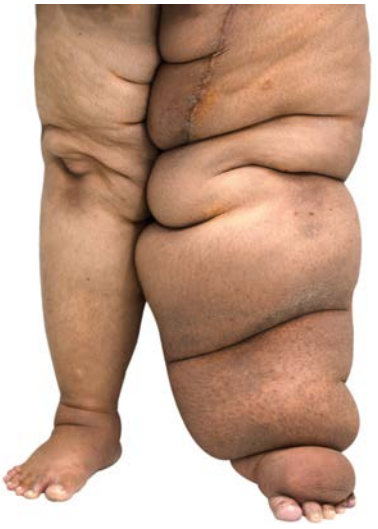


FIGURE 3 The symptoms of elephantiasis

26 Compare the functions of a helper T-lymphocyte and a cytotoxic T-lymphocyte.

27 Compare the impact that antibodies and cytotoxic T-lymphocytes will have on a targeted bacterial cell.

28 Describe the differences between active and passive immunity.

29 Read Case study 9.1 on page 222 and refer back to Figure 2 on the previous page to answer the following questions.

- **a** Compare lymphoedema to elephantiasis.
- **b** How is each condition related to the immune system?

30 A recent advertisement for baby formula states: 'Our formula provides more immunity for your baby than breast milk.'

- **a** Using your knowledge of natural passive immunity, analyse this statement.
- **b** Comment on the ethics of a company stating something like this.

31 Examine the graph in Figure 4.

- **a** Use your knowledge of the adaptive immune system to explain the trend shown.
- **b** Construct your own graph that shows what would happen if the body was exposed to the same antigen a third time.

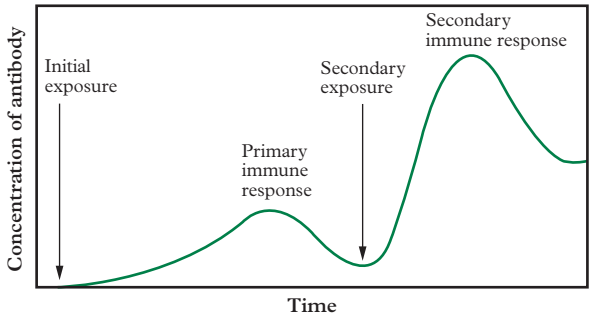


FIGURE 4 Immune responses graph

Design and discuss

32 In 2021, it was announced that the athletes on the Australian Olympic team would receive the AstraZeneca COVID-19 vaccine ahead of most of the Australian population so that they could attend the Olympics in Tokyo, Japan. Discuss the ethical implications of this decision.

33 Acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) attaching to helper T-lymphocytes and affecting their survival. Suggest how infection with this virus can cause the immune system to become deficient.

34 The virus that causes bird flu can attach to cells in the lower part of the human respiratory system, but not the upper respiratory system. Suggest a reason for this difference.

35 Explain why most vaccines need to be injected into muscle tissue rather than ingested orally.

36 Explain why skin transplanted from a patient's thigh to cover a burn on another part of their body will not be rejected.

37 Explain why immunologists recommend that newborn babies be breastfeed by their mothers for the first 6 months.

FIGURE 5 Mother breastfeeding a newborn baby

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Chapter quiz
Check your understanding of this chapter.

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Launch a quiz for your students on key concepts in this chapter.

Exam essentials

Responding to questions

In your exam, you may be expected to recognise a sequence of events and how they are related to other parts of a bigger system.

Break big ideas into smaller parts

The immune system has many parts. This can make it difficult to understand what information to give and how much detail to provide in an answer. Consider the following questions as a guide.

- Which part of the immune system is being discussed?
- Is it a normal immune response or an abnormal response?
- Has the person been exposed or vaccinated previously? Are memory cells involved?

The following question is taken from the 2016 VCE Biology Examination. Read the question carefully and consider the number of marks for each question and the key words or phrases that will be needed to gain full marks.

QUESTION 5 (2016 VCE Biology Written examination)

Yellow fever is a potentially fatal, mosquito-borne, viral disease that occurs in many countries in Africa, the Caribbean, and Central and South America. An effective and safe vaccine has been available since 1938.

a For the vaccine to be effective, it is recommended that travellers to these regions have the vaccination approximately two to four weeks before travelling.

Why is this time frame recommended? 2 marks

Response 1

Because it takes 2–4 weeks for the body to make memory B-cells that can become plasma cells which produce antibodies against the yellow fever.

Relates back to the question.

Identifies the type of cells that are important in vaccinations and which take time to undergo clonal selection.

The production of antibodies is important in fighting viruses.

This answer would receive 2 marks because it recognises that time is needed for memory B-cells to form, and that these can result in the production of the antibodies that are the main line of defence against viruses.

Response 2

So that your body can recognise the disease and produce enough immune cells before you travel. You may also get side effects of the vaccine that might make you sick and you will need time to recover before you travel.

Does not mention why 2–4 weeks is needed.

Not relevant to the question, which refers to why everyone needs to wait 2–4 weeks.

This response would not receive any marks since it does not provide any reference to memory cells or antibodies. It does not relate back to the question or give a reason for waiting to travel.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 5b (2020 Biology Written Examination)

5 b i State the role played by the lymphatic system in an immune response. 1 mark

To help macrophages break down bacteria

ii Describe the sequence of events that occurs in the secondary lymphoid tissue that results in the production of antibodies. 4 marks

1. The macrophage breaks down the bacteria.
2. The macrophage shows it to the T-cell.
3. The T-cell messages the B-cell.
4. The B-cell starts producing antibodies.

Source: 2020 Biology Written Examination Question 5b, Short answer, reproduced by permission © VCAA

Marking guide

Question 5 b i	- 1 mark for describing the role of: • lymph nodes in the storage of memory lymphocytes or carrying or trapping antigens • the lymphatic system in transporting lymphocytes/antibodies/immune cells or as the site of clonal expansion.
Question 5 b ii	- 1 mark for any four of the following: • an antigen-presenting cell presents the antigen • helper T-cell is activated • activates a B-cell • B-cell undergoes clonal selection • produces plasma cells • plasma cell produces antibodies.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

CHAPTER 10

Diseases

Infectious diseases are caused by the spread of pathogens from infected humans or other organisms. Infectious diseases can cause outbreaks such as widespread epidemics or even pandemics. Agencies such as the World Health Organization (WHO) and the Australian Peter Doherty Institute for Infection and Immunity are constantly fighting diseases and employing strategies to prevent the emergence of new diseases and the re-emergence of known diseases. They are also monitoring emerging diseases, where they need to identify the pathogens and the transmission and then decide how best to control the spread of the disease. This is evident from their response to the COVID-19 pandemic, where WHO is on the frontline of information regarding the virus. It was also the organisation to classify the virus outbreak as a global pandemic.

Vaccination programs are important in maintaining herd immunity in a population to indirectly protect those individuals who cannot be vaccinated. Some infectious pathogens that were once considered eliminated from the population are re-emerging because the herd immunity of the population is decreasing. Vaccines are considered effective in the prevention and control of diseases.

Immunotherapy is used for the treatment or prevention of a disease by promoting the immune system to initiate a response. Most immunotherapies are used in the prevention and treatment of different types of of cancer. Some immunotherapies are also used to treat allergies and autoimmune diseases.

KEY KNOWLEDGE

- → 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
- → 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
- → vaccination programs and their role in maintaining herd immunity for a specific disease in a human population
- → the development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer.

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FIGURE 1 Bacteria can cause emerging infectious diseases in humans by becoming resistant to treatments.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

10A Name two cellular and non-cellular pathogens that infect humans.

10A Groundwork resource
Cellular and non-cellular pathogens

10B How can vaccination make us immune to a pathogen?

10B Groundwork resource
Vaccines

10C What is the difference between self-antigens and non-self antigens?

10C Groundwork resource
Self and non-self antigens

PRACTICALS

PRACTICAL	**10.2** Testing the effectiveness of antibacterial substances

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

10.1 Emergence and re-emergence of pathogens

KEY IDEAS

In this topic, you will learn that:

- diseases can be classified into infectious and non-infectious diseases
- new pathogens can emerge and old pathogens can re-emerge
- European arrival to Australia brought foreign pathogens, negatively affecting Aboriginal and Torres Strait Islander Peoples.

epidemiology
a branch of medicine that investigates the incidence, distribution and control measures relating to disease and health-related events

Epidemiology is the scientific study of factors that affect the frequency and spread of disease in humans and other factors relating to human health. This can be infectious diseases caused by pathogens, or lifestyle diseases including heart disease or obesity. The results of epidemiological studies are used to evaluate health services and preventative programs or to identify individuals who are at high risk of contracting disease.

FIGURE 1 Epidemiology is the study of the distribution and cause of diseases.

Classifying diseases

non-infectious disease
a disease that is not caused by a pathogen

infectious disease
a disease caused by a pathogen that can be transferred between hosts

contagious
where a disease is able to spread from one individual to another individual

Diseases can be infectious or non-infectious. **Non-infectious diseases** are those that are not caused by pathogens or transferred between hosts.. These diseases could be caused by the environment, lifestyle or inherited from parents. Infectious diseases are those caused by pathogens (e.g. bacteria). Some **infectious diseases** can spread from one individual to another, and those that do are considered to be **contagious**.

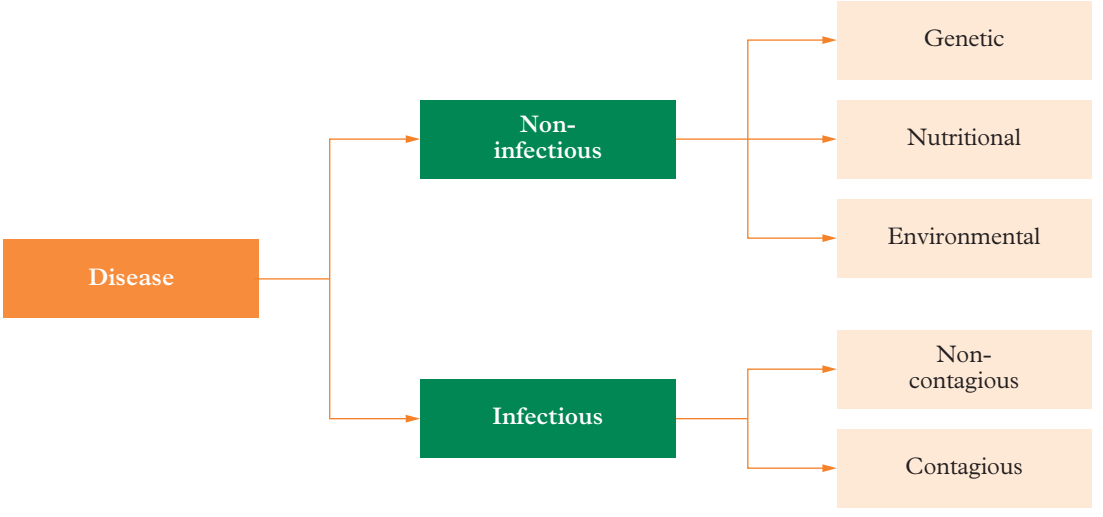


FIGURE 2 Diseases are classified as either infectious or non-infectious.

TABLE 1 Some types of human diseases and the pathogens that cause them

	Types of disease	Pathogen	Examples
Infectious	Disease caused by non-living organic particles	Prion or virus	Creutzfeldt-Jakob disease (CJD) Measles Rabies Influenza
	Diseases caused by microorganisms	Bacterium or protozoan	Cholera Tuberculosis Malaria Trypanosomiasis
	Diseases caused by multicellular organisms	Fungus, roundworm or flatworm	Ringworm Athlete's foot Acariasis Snail fever Tapeworm
Non-infectious	Nutritional deficiency diseases Metabolic disorders Degenerative diseases Cancer Inherited diseases Occupational or industrial diseases Mental health disorders	*Not caused by pathogens*	Diabetes Coronary heart disease Arthritis Cancer Sickle-cell anaemia Lung disease Depression Alcoholism

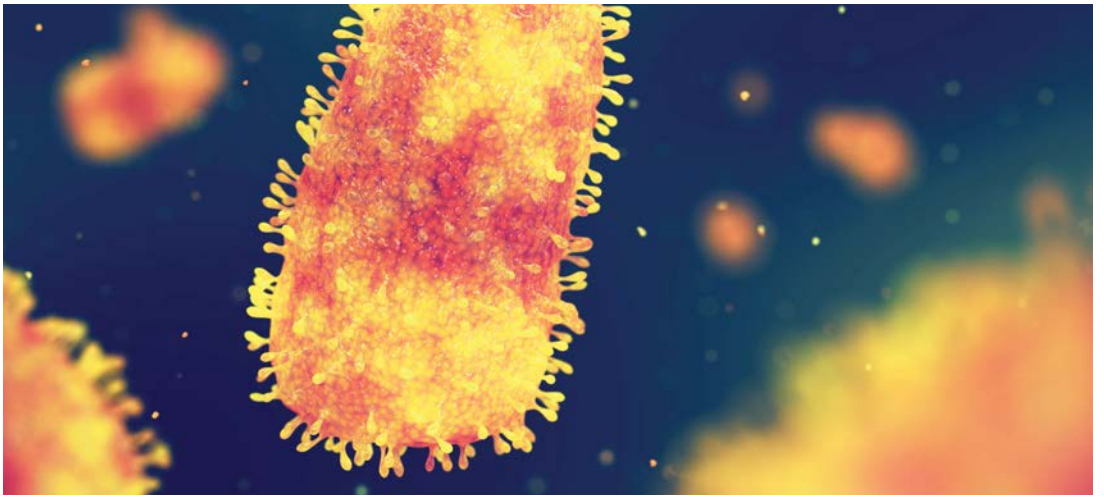


FIGURE 3 A 3D illustration of the rabies virus, a deadly disease of the nervous system

Infectious diseases

Many infectious diseases exist at low levels in a community at all times. For example, influenza (the flu) moves through a population regularly at relatively low levels. This pattern of infectious disease regularly found in a population is called **endemic** and these diseases are restricted to particular geographic areas. An **epidemic** of a particular disease occurs when a larger than normal number of individuals in a particular community are infected at a particular time. Epidemics are still contained within a geographic area.

endemic
a disease that is restricted to a certain place or community and which exists at low levels

epidemic
the rapid spread of an infectious disease in a community in a short period of time

pandemic
an outbreak of an infectious disease that crosses international borders

If the disease spreads across international borders and spreads within the populations of multiple countries, it is termed a **pandemic**. Some of the largest epidemic and pandemic outbreaks are caused by viruses, as shown in Table 2.

TABLE 2 Major epidemic and pandemic viral outbreaks

Disease	Location	Date	Estimated death toll
Influenza A(H2N1) – the Russian epidemic	Worldwide	1889–90	1 000 000
Spanish influenza A(H1N1)	Worldwide	1918–20	5 000 000
Asian influenza A(H2N2)	Worldwide	1957–58	1 100 000
Hong Kong influenza A(H3N2)	Worldwide	1968–69	1 000 000
SARS coronavirus (SARS-CoV)	Asia, Canada	2003	774
Swine influenza A(H1N1)pdm09	Worldwide	2009	12 469
Dengue fever	Pakistan	2011	350
Middle East respiratory syndrome	Worldwide	2012	449
Yellow fever	Darfur, Sudan	2012–13	847
Ebola	West Africa	2014–16	>11 300
Zika virus*	Worldwide	2015–16	
Yellow fever	Africa	2016	400
Measles	Worldwide	2018	140 000
HIV/AIDS	Worldwide	1981–present	>32 700 000
COVID-19 (SARS-CoV-2)	Worldwide	2019–present	3 497 026 (26/05/2021)

*Although not directly resulting in death, this virus has been linked to microcephaly in babies whose mothers contracted the disease during pregnancy. This has been reported only in South America.

Study tip

The prefix 'en' means *within/inside* and 'demic' refers to *population*; therefore 'endemic' means *inside a population*. The prefix 'epi' means *near*; therefore 'epidemic' means *near a population*. The prefix 'pan' means *all*; therefore a 'pandemic' is *all populations*.

Emerging 'new' and re-emerging 'old' pathogens

Today, infectious diseases are emerging at a higher rate than ever before. People are now travelling more frequently and further distances, spreading diseases across the world. People are also living in more densely populated areas, which allows infectious diseases to spread rapidly. Unfortunately, there are even some diseases that have been deliberately introduced for terrorist purposes (bioterrorism), including anthrax, smallpox and tularaemia.

Emerging diseases

emerging disease
a disease that has been recognised in a human host for the first time

Many **emerging diseases** come about due to a pathogen in animals being passed to humans (known as zoonosis). An example of this type of infectious disease is the avian flu, which originated in birds and spread to humans via close contact with infected birds. Other emerging diseases result from the evolution of a pathogen over time. For example, influenza (the flu) is known for its capacity to change its genetic information and become a 'new' virus that the immune system doesn't recognise, seasonally. In this way, it can emerge as a new infectious disease and cause epidemics and pandemics.

Re-emerging diseases

re-emerging disease
a disease that reappears in a population after apparent control or elimination

Some infectious diseases can re-emerge, even when thought to be under control. Common **re-emerging diseases** include malaria, tuberculosis, cholera, pertussis (whooping cough), influenza, pneumococcal disease and gonorrhoea. Several factors contribute to the re-emergence of known pathogens, including antimicrobial resistance. Bacteria, viruses and other microscopic pathogens can develop resistance to treatments, causing those treatments to become ineffective, allowing the disease to re-emerge. Another factor that causes re-emerging disease is a decrease in vaccination coverage, where people are choosing not to be vaccinated.

The measles virus was once thought to be eliminated from the high-income countries in 2016, but re-emerged not long after due to people opting out of vaccinations. Vaccines will be discussed in further detail in Section 10.3.

European arrival in Australia

Prior to the arrival of Europeans in Australia in 1788, Aboriginal and Torres Strait Islander Peoples inhabited the continent. There were over 500 indigenous groups with approximately 750 000 individuals inhabiting the land. Indigenous Peoples have a profound spiritual connection with the land and custody over the country. **Traditional medicines** are used to treat infections and other health concerns. One of the common plants used is the *Eremophila* species (emu bush), a type of desert plant. The leaves of the plant are used to treat headaches, backaches, sores and cuts, sore eyes and for general well-being. Traditional medication considers the practices, knowledges and beliefs of Aboriginal and Torres Strait Islander Peoples and is still in use today.

traditional medicine health practices, beliefs and knowledge incorporating plant-, animal- and mineral-based medications to prevent and treat disease and improve well-being

FIGURE 4 The leaves and flowers of an *Eremophila* species (*Eremophila alternifolia*) that was, and still is, used as traditional medicine by Indigenous Peoples

At the time of European arrival, the Aboriginal and Torres Strait Islander Peoples had been isolated from other populations for 60 000 years. This meant they had not been exposed to the pathogens that existed in the European populations. When Europeans arrived in Australia, they made contact with the Aboriginal and Torres Strait Islander Peoples, leading to the Australian Frontier wars, which resulted in many massacres and severe epidemics within the Indigenous population of Australia. In the 10 years that followed, the Indigenous population of Australia dramatically decreased by about 90%. This population decrease was due to many factors with one of the most significant being the introduction of new pathogens resulting in epidemics. These pathogens caused diseases such as smallpox (variola virus), tuberculosis (*Mycobacterium tuberculosis*), syphilis (*Treponema pallidum* bacterium), measles (rubeola virus) and influenza (influenza virus). Each of the diseases was responsible for excessive morbidity (rate of disease) and mortality (rate of death). It has been reported that smallpox killed more than half of the Indigenous Peoples in the Sydney region within 14 months of European settlement. In this region and other southern regions of Australia, there was further intentional spread of smallpox by way of possum skin cloaks being removed and replaced by infected blankets. The transmission of pathogens was further increased by the sexual abuse of Indigenous girls and women by European men. Transmission of pathogens is further discussed in Topic 10.2.

The Aboriginal and Torres Strait Islander Peoples did not have access to European medicine, and the traditional medicine that had worked well for over 60 000 years was not able to stop the spread of these new diseases. With no immunity against these new pathogens, the spread of the diseases had a tragic result.

CHECK YOUR LEARNING 10.1

Describe and explain

1 Define the term ‘epidemiology’.
2 Which type/s of pathogens that cause disease are non-living particles?
3 Why is a non-infectious disease not contagious?
4 Why do diseases re-emerge?
5 What does the term ‘traditional medicine’ mean in Australia?

Apply, analyse and compare

6 Compare the terms ‘endemic’, ‘epidemic’ and ‘pandemic’.
7 Why did European arrival result in severe epidemics in the Indigenous Peoples?

Design and discuss

8 Use Table 2 to identify a pandemic, and discuss why it would be considered a pandemic.

10.2 Control and prevention of pathogens

KEY IDEAS

In this topic, you will learn that:

- pathogens can be transmitted between host organisms
- different prevention methods can be used to avoid the spread of infectious pathogens
- to reduce the spread of infection, the pathogen must be identified and the host isolated
- social and scientific strategies can be used to control the spread of pathogens.

Transmission of infectious pathogens

transmission the passing of a pathogen from an infected individual to a non-infected individual

Transmission refers to the passing of a pathogen from an individual who is not infected. There are many ways in which a pathogen can be directly transmitted, including:

- physical contact by touching an infected individual
- physical contact with an object that is contaminated (this may be a door handle or another surface that was touched by an infected individual)
- contact with bodily fluids of an infected individual (this includes droplets containing the pathogen being released from the infected individual via coughing or sneezing)

airborne transported by air

- **airborne** transmission, which refers to pathogens that are able to remain suspended in the air for long periods of time, causing infection in individuals or groups of people who are exposed to the contaminated air (e.g. measles can survive in the air for up to 2 hours)
- faecal-oral transmission, which occurs primarily because of poor personal hygiene or contaminated food and water due to lack of sanitation (this is a particular problem in low-income countries where there are often few or no sewage systems in place).

Diseases can also be indirectly transmitted via another organism known as a vector.

vector an organism that carries a disease-causing pathogen from one organism to another

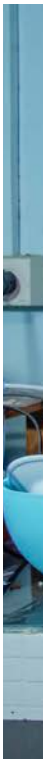
A **vector** is an organism that spreads a disease by penetrating the primary defence of the skin. Typically, a vector will be a biting insect, tick or parasite that transmits a disease from one animal or plant to another.

Generally, people get sick at school, around sick friends or at home around sick family members. This happens because the spread of pathogens is facilitated by close contact with infected individuals. The longer a pathogen is able to survive in a host before it is detected by the immune system, the greater the chance it has to spread to more new hosts. A successful pathogen is able to spread from one host to many new host organisms. The transmission of the disease can increase in dense populations with highly mobile individuals. Limited healthcare or support and low education levels will also contribute to the spread of the pathogen (and therefore the disease) across a population.

Prevention strategies

World Health Organization (WHO) an agency of the United Nations concerned with public health

Diseases, whether infectious or non-infectious, are monitored by health authorities and agricultural departments around the world. In Australia, for example, it is mandatory to report certain identified infectious diseases to local, state and federal authorities as well as to the **World Health Organization (WHO)**.



PRACTICAL
10.2 Testing the effectiveness of antibacterial substances
Go to pages **421–423**

In order to control diseases, prevention measures may be put in place. If a disease does emerge, the pathogen must be identified, treatments developed for infected individuals and controls put in place to limit the further spread of the pathogen.

Personal hygiene

Personal hygiene is important in decreasing the risk of contracting pathogens. Regular washing of hands can significantly reduce the transfer of pathogens. Many microorganisms stick to small droplets of oils released by skin glands. Water alone will not remove these. Soap affects the surface tension of water and allows the oil, and hence the pathogen, to be washed away.

Correct food storage and handling

Both contagious and non-contagious diseases can result from contaminated food. Food poisoning often occurs because food is kept too long or stored incorrectly. Some decomposing bacteria in the food release toxins, which then affect the person eating the spoilt food. Several contagious diseases can be transferred from people or animals in the food preparation area since either can be carriers of disease without displaying any of the symptoms.

Transportation of food

Meat bought in Australia and in most other Western countries is subject to strict inspection procedures that check for parasites. It is still important that meat such as pork is thoroughly cooked. The heat generated in the cooking process kills the larvae of tapeworms and other flatworm parasites that can be passed on to humans. Few pathogens can survive or reproduce in temperatures below 4°C or above 65°C.

Maintaining clean water supplies

Many diseases are spread by water, either directly or via a vector. In Australia, clean drinking water is taken for granted. All major towns and cities have strict treatment and quality control of household water supplies. This is not the case in many parts of the world, particularly in low-income countries, where people must use local streams or wells for water. Upstream of a town's water supply is often another town's waste supply where the water is frequently contaminated by humans and domesticated animals.

FIGURE 1 Having a clean place to prepare food minimises the risk of food poisoning.

Vaccination programs

To limit the re-emergence of known pathogens, it is important to have vaccination programs in place to build up the level of immunity in the population. If the majority of individuals are vaccinated against a disease, then those individuals who are unable to be immunised (elderly, newborns and immune compromised) are better protected from the disease since it is much more difficult for a disease to spread. Vaccination is, therefore, considered an excellent population-wide prevention measure and will be further discussed in Topic 10.3.

FIGURE 2 People in many parts of the world do not have access to clean drinking water.

Biosecurity

To prevent the spread of pathogens carried by exotic plants and animals, there are limitations on what may be taken into Australia from other countries and between states or even regions of Australia. For example, as planes arrive in Australia from overseas, the cabins are sprayed in an attempt to kill any airborne pathogens before people can disembark. Many airports also screen arriving passengers for fever using a thermal camera. There are strict regulations as to what passengers can and cannot bring into Australia, and this is monitored by customs. All living plants and their parts (such as fruit and seeds) are restricted from entering Australia in order to avoid any pathogens being brought in on the plant tissue.

susceptibility test test used to determine the sensitivity of a pathogen to particular factors

Identifying pathogens

Once an outbreak has occurred, it is important to identify the pathogen causing the disease in the host, so specific control measures can be put in place and treatments can be delivered or developed as necessary. As you have learnt in Chapter 8, pathogens can be cellular or non-cellular, and there are different techniques used to identify each.

- **Structural analysis** – cellular pathogens (e.g. bacteria, protozoa, fungi and worms) can be identified by their structure. For example, bacterial species can be grown in culture and differentiated by their shape as coccus (i.e. spherical), bacillus (i.e. rod-like) or other intermediate shapes.
- **Chemical analysis** – bacterial cells can also be classified by the chemical components of the cell wall via a procedure known as Gram-staining. Some bacteria have a thick layer of peptidoglycan in their cell wall and will stain purple, whereas bacteria that do not contain large amounts of peptidoglycan will stain pink.
- **Susceptibility tests** – cellular pathogens will have different conditions of growth, which can be identified by susceptibility tests. Susceptibility tests subject the cultured cellular pathogen to various factors such as the presence or absence of oxygen or other nutrients, and their response is monitored.

All these techniques are used to detect the type of pathogen, but each method has limitations and can be very time-consuming. This presents challenges when a disease needs to be quickly identified to prevent rapid spread.

Non-cellular pathogens (viruses and prions) cannot reproduce without a host cell; therefore, it can be difficult to culture the pathogen for testing. The type of host cell required for the survival of the non-cellular pathogen is a

FIGURE 3 Bacteria can be Gram-stained to identify the components of their cellular wall and their shape observed in order to identify the species.

key identification factor. Once the host cell is discovered, the pathogen can be replicated for further investigation.

- **Genetic analysis** – is the most reliable form of pathogen identification. This is based on the idea that pathogens have unique sequences of DNA or RNA, and when the genetic material is replicated by PCR and sequenced, it can be compared to a database of known pathogen sequences.

Controlling the spread of pathogens

The control of many diseases can be achieved by minimising the availability of vector organisms, quarantining infected individuals, social distancing (reducing close contact between people), tracing the point of origin of an outbreak, vaccinations programs and other public health educational programs.

Minimising contact with vectors

Minimising contact with vectors of disease is the best means of controlling the impact of an infectious disease. Suggested measures include:

- personal insect repellents in places and times where vector insects are most likely to be active
- clothing that minimises being bitten by insects – long, loose trousers and shirts
- insect screens in windows and doors of houses
- checking your body regularly for ticks in tick-prone regions
- mosquito nets to cover beds
- avoiding contact with blood
- strict hygiene for food and avoiding unpasteurised milk in tick-borne encephalitis areas
- building materials that do not harbour insect pests
- avoidance of wading or swimming in waters suspected to be contaminated by pathogens and/or their hosts.

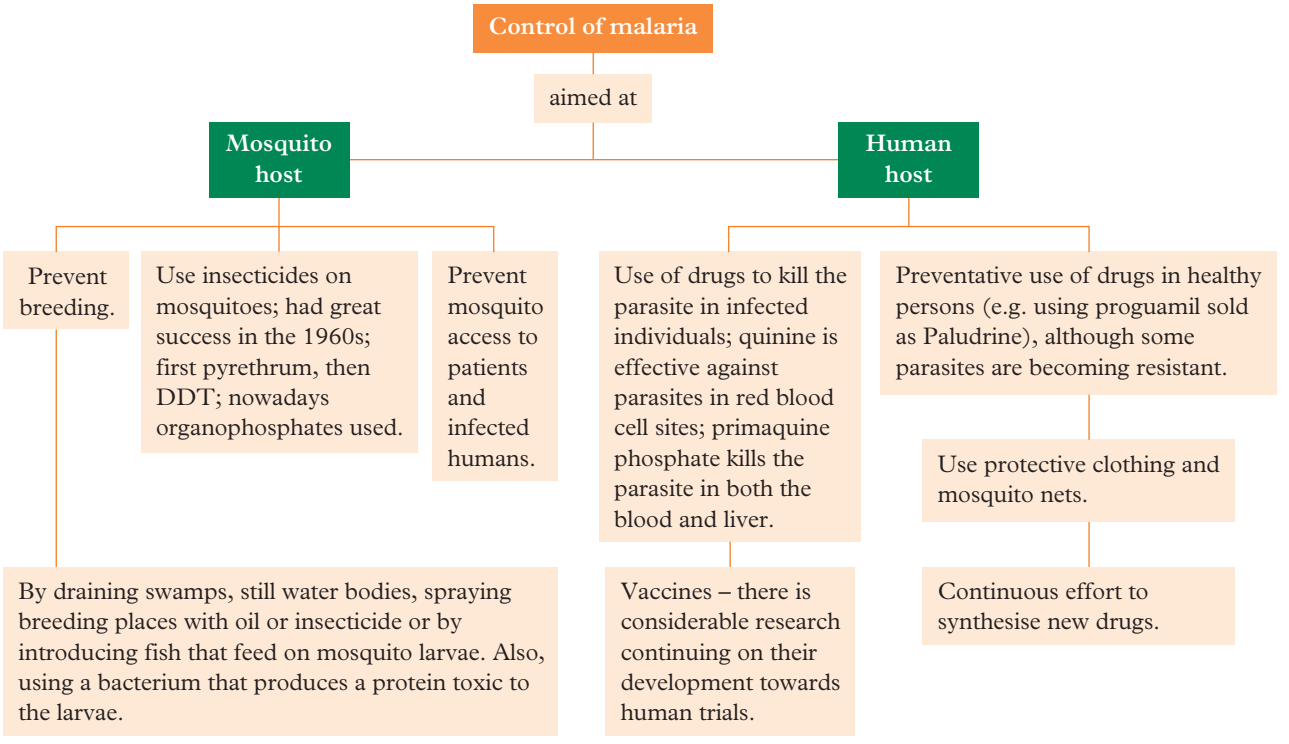


FIGURE 4 Mechanisms to control malaria, a vector disease

Quarantine

quarantine
a period of isolation for infected individuals

Quarantine is the isolation of infected individuals (humans or animals) to minimise the number of people who come into contact with the infected host and therefore reduce the spread of disease. The period of isolation depends upon the incubation period of the pathogen as well as the length of time of infection. Quarantine can be applied locally, nationally and internationally.

Study tip

The word 'quarantine' comes from a phrase meaning 40 days, which for many diseases is the time required for the disease to pass and treatment be completed.

In serious outbreaks within a community, further action is taken. Schools, aged-care facilities, public places and other large assemblies of people may be closed. For example, in 2009 during the swine flu outbreak, five schools in Victoria were temporarily closed due to several students and teachers showing signs of the flu virus. In 2020, large and small gatherings of people were banned in an attempt to control the spread of the COVID-19 coronavirus (SARS-CoV-2).

There are limitations to quarantine as a control measure. If community facilities are shut down, it is challenging to keep essential services running. There is also the question as to whether the healthcare workers who are required to look after the individuals in quarantine should also be in quarantine. Quarantine is only a physical barrier for the spread of diseases, and may not work to contain diseases that spread via migrating animals and insects. For example, avian flu spreads via wild birds that naturally migrate, and so quarantine is not an effective control measure. Sentinel animals, like birds, are those animals that are used to monitor the spread of diseases. For this reason, regular blood tests are performed on sentinel animals, to monitor particular diseases that may have come from South-East Asia or the Pacific region via migration.

FIGURE 5 Quarantine is based on physically separating an infected or potentially infected person and healthy people. Quarantine can range from hospital isolation to at-home quarantine.

Contact tracing

If a disease outbreak occurs, health officials will attempt to trace all contacts of the original infected person (patient zero). This has occurred repeatedly throughout 2020 during the COVID-19 pandemic. People in Australia were linked to particular buildings, workplaces, pubs, cruise ships, events, aged care homes, and so on where they came in contact with the original infected person. All these people were tested for the virus and told to self-isolate to decrease any further spread of the virus.

Public health programs

Public health programs are important in delivering information and implementing control measures. Programs that encourage all children to be vaccinated before starting childcare or school dramatically improve herd immunity. Needle-exchange centres for drug users have helped prevent the spread of HIV and hepatitis that is usually associated with the shared use of needles.

FIGURE 6 In Victoria, the 'No Jab, No Play' legislation requires all children to be fully vaccinated (unless they have a medical exemption) before they can be enrolled in childcare.

CASE STUDY 10.2

Ebola

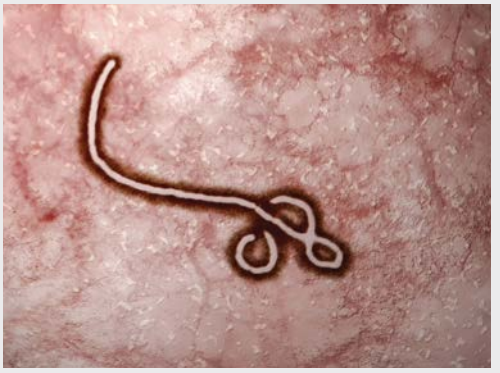


FIGURE 7 Electron microscope image of the Ebola virus

The Ebola virus disease (EVD) caused an outbreak in 2014–2016 in West Africa. The disease has a high risk of death, with approximately 50% of infected individuals dying from the virus. Symptoms are flu-like at the beginning and progress to internal and external bleeding, resulting in low blood pressure. The virus originated in West Africa and spread between countries, starting in the rural region of south-eastern Guinea and moving into Sierra Leone and Liberia. The virus is thought to have originated in fruit bats since they are the natural Ebola virus hosts. The virus was transmitted to people and then spread through the human population via bodily fluids, mainly by blood from infected individuals or animals.

There were several factors that assisted this virus to become an epidemic. Initially, it took almost 3 months to identify the pathogen, by which time the virus had already spread beyond the original infected community. Other cases were not identified quickly enough in Liberia and Sierra Leone and therefore not reported to WHO. The healthcare system is weaker in West Africa and neighbouring areas, which are some of the poorest areas in the world. Health infrastructures were damaged after many years of civil war and unrest. Road systems and communications were slow, particularly in the rural settings.

Transport of infected individuals to treatment centres and identification of individual cases were difficult. Dead bodies remain infectious for several days, so people handling the remains and those involved in traditional burial rituals were at a greater risk of infection. These factors contributed to this outbreak becoming an epidemic.

CHECK YOUR LEARNING 10.2

Describe and explain

1 Explain airborne transmission.
2 Describe three prevention strategies to reduce the re-emergence of known diseases or the pread of new diseases.
3 Describe direct and indirect transmission methods for pathogens.

Apply, analyse and compare

4 Analyse how quarantine reduces the spread of an infectious pathogen.
5 Compare techniques to identify cellular and non-cellular pathogens.
6 Compare the terms ‘quarantine’ and ‘contact tracing’.

Design and discuss

7 Discuss how vaccinations can be considered a prevention and control measure.
8 Discuss the ways in which the Ebola virus described in Case study 10.2 developed into an epidemic.

10.3 Vaccination programs

KEY IDEAS

In this topic, you will learn that:

- vaccination programs involve providing a planned series of vaccinations or booster vaccinations for individuals
- herd immunity is the ability of an immunised population to prevent the spread of infection to vulnerable individuals.

Australia has a National Immunisation Program (NIP) that provides free vaccines to help reduce the spread of preventable diseases through the population.

Some vaccinations require a series of booster shots to increase the number of memory cells in an individual to a level that they will be able to respond quickly enough to an infection. Because the average life expectancy of a person in Australia is 82 years, it is not expected that the memory cells will remain active for that length of time. Instead, it is recommended that most adults receive a booster during their lifetime for particular immunisation schedules.

virulence the ability of a pathogen to cause disease

Different pathogens have different levels of **virulence**. A harmless pathogen is described as avirulent, while a pathogen that rapidly causes death is highly virulent. The level of virulence of a disease is directly related to how easily the disease is able to spread. A pathogen that is not virulent enables its host to freely move around. This causes the pathogen to spread more easily. In contrast, a highly virulent pathogen makes its host so sick they cannot travel far. This limits the spread of the disease. Many pathogens are able to spread to other people during the **incubation period** (the period after the pathogen has entered but before the symptoms start). For example, measles has an incubation period of 7–14 days, but patients are contagious (able to pass on the pathogen) for 1–4 days before the appearance of the distinctive rash.

incubation period the time between exposure to a pathogen and the first symptoms

R_0 value

The level of contagion of an infection can be determined mathematically. This R_0 value provides a prediction of the number of people who will be infected by a single contagious person. An R_0 of 1 suggests that each infected person will pass the infection on to one other person.

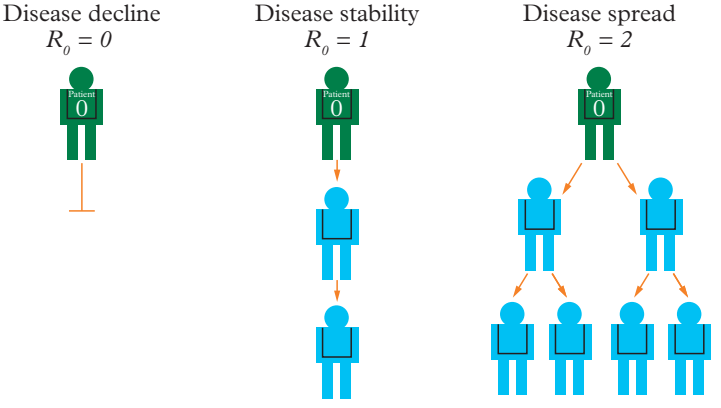


FIGURE 1 R_0 is a measure of how contagious a disease is.

R0 values tell us the average number of people who will contract a contagious disease from one infected person:

- If $R_0 = 1$, then each infection causes only one infection. The spread of disease is considered stable.
- If $R_0 > 1$, then each infection causes more than one new infection. The spread of the disease is growing.
- If $R_0 < 1$, then each infection causes less than one new infection. The disease is considered to be declining.

TABLE 1 Infection level of contagion

Disease	Transmission	R_0	Percentage vaccination rate for herd immunity
Measles	Airborne	12–18	94%
Whooping cough	Airborne droplet	12–17	94%
Smallpox	Social contact	5–7	85%
Influenza A(H1N1)	Airborne droplet	2–3	?
Ebola	Fluid contact	2	?
COVID-19 (SARS-CoV-2)	Airborne droplet	Average: 3.48	?

The calculation of R_0 values can range quite a lot and depend not only on vaccinations, but whether people are practising 'social distancing' or not. For example, the range of the R_0 values for COVID-19 kept changing depending on what level of lockdown a country was enforcing. Originally it was thought that the R_0 value in Wuhan in 2019 was ~2.2–2.7, though other Chinese mathematical models calculated an R_0 value of >6. The R_0 value for New Zealand, which implemented level 4 lockdown for 33 days, became <1. An R_0 value above 1 means an infection is still growing throughout a population and an R_0 value lower than 1 means it is decreasing in a population.

FIGURE 2 Melbourne's The Block Arcade was almost empty on a typically busy March 2020 weekend, because shoppers were practising social distancing at home in level 3 lockdown.

Herd immunity

Some people in the population are more vulnerable to a disease than others. These people may be too young to be vaccinated or immunodeficient due to HIV/AIDS, leukaemia or other blood cancers or treatments. These individuals rely on their friends and social contacts (their herd) to protect them and to provide a barrier between them and the disease. If these individuals are surrounded by vaccinated people who are immune to infection, the chain of infection is stopped, and they are indirectly protected from the disease. This ability to indirectly protect vulnerable individuals from a disease due to a larger percentage of the population having immunity is called **herd immunity**.

herd immunity when the majority of the population are immunised against an infectious disease, indirectly protecting those who are not immunised

The herd immunity threshold is the proportion of a population that needs to be immunised to prevent the spread of an infection. A high level of vaccination in one age group can protect another age group from infection. An example of this is whooping cough, a disease that causes an extensive cough, and which can lead to severe respiratory problems in a newborn baby. Newborn babies cannot be vaccinated until they are 2 months old. Vaccinating all the adults and older children around the baby will prevent the infection from being passed on to the newborn infant.

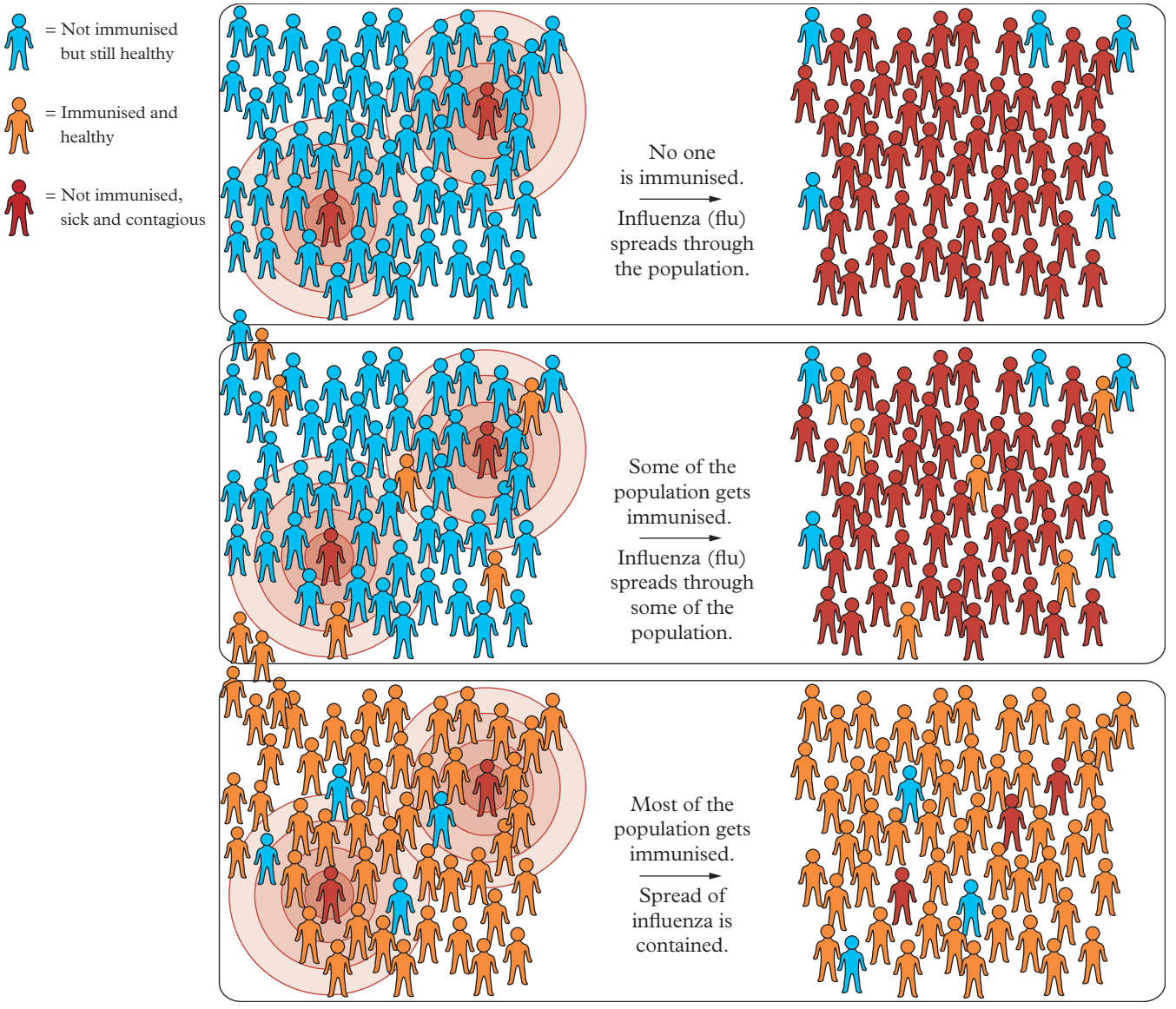


FIGURE 3 Comparison of the proportion of people in a population contracting a disease relative to those that have been immunised

CHALLENGE 10.3

No vax, no visit

'No vax, no visit! Our baby boy is due in one month. We can't wait to meet him! If you would like to meet our son, we ask that you ask your doctor for a whooping cough booster this week. Our son will not be able to receive his first vaccination until he is 8 weeks old, so he relies on us to keep him safe from germs.'

Many new parents post social media messages similar to this one before their babies are born. They will often refuse a visitor if that person doesn't agree with their views on vaccination.

1 Why might the parents post such a message?

2 Would you receive a booster for whooping cough before visiting the new parents? Explain the reasoning for your decision.

CHECK YOUR LEARNING 10.3

Describe and explain

1 Why are some people unable to be vaccinated?

2 What does the R_0 value for a disease represent?

3 Explain one other way the R_0 value can change in a community, excluding vaccinations.

4 Define herd immunity.

Apply, analyse and compare

5 Explain how herd immunity can protect newborn babies from whooping cough.

6 Compare the R_0 value of Ebola and measles. Which disease is more contagious? Explain your reasoning.

7 Discuss the difference between virulence and the R_0 value of a pathogen.

Design and discuss

8 In 1998, it was falsely claimed that there was a link between the measles vaccine and autism. This resulted in a lower proportion of children being vaccinated for measles. Why did it take 10 years before the levels of measles infections rose?

9 Discuss why herd immunity through exposure to the virus (not vaccination) is not a preferred method of protecting a community from COVID-19.

10 At the beginning of the COVID-19 pandemic, Sweden was one of the few countries that chose to aim for herd immunity during the COVID-19 pandemic. This means individuals did not have to self-isolate and were still able to see friends and family in public places. Other countries chose the suppression or elimination method of trying to stop people from contracting the virus. This has included implementation of social distancing and nation-wide lockdowns.

The virus has infected people of all ages, but is particularly dangerous for elderly people who contract it. However, harsh lockdowns have caused a spike in mental health difficulties and had a substantial impact on economies.

Discuss the pros and cons of herd immunity versus suppression/elimination and consider the ethical issues associated with both approaches.

10.4 Immunotherapy

KEY IDEAS

In this topic, you will learn that:

- immunotherapies are used to treat cancer and some autoimmune diseases
- monoclonal antibodies can be used to treat cancer and autoimmune diseases.

immunotherapy
a treatment that uses substances to either suppress or activate the immune system to help fight cancer and other diseases

Immunotherapy is the treatment or the prevention of a disease by promoting the immune system to initiate a response. Most immunotherapies are for the prevention and treatment of different types of cancer, but there are also immunotherapies to treat allergies and autoimmune diseases.

Allergen immunotherapy

In Chapter 8 you learnt that allergens are non-harmful substances that initiate an immune response. Antihistamines are the most commonly used medications to treat allergies by reducing the immune response. Although this treatment of allergies is usually effective in reducing symptoms, antihistamines are not a cure. Allergen immunotherapy, or **desensitisation**, alters the immune response to allergens so that the allergen becomes tolerated over time. The patient is injected with a small dosage of the allergen. This injection is normally given monthly and the dosage is slowly increased. This process continues until the number of regulatory T-lymphocytes are increased. These cells regulate and control the rest of the immune system, allowing it to tolerate the allergen, which could take several years. The benefits of this allergen immunotherapy should last for 5–8 years; however, maintenance injections may be required during that time. A less invasive oral drop method is also available as an alternative to the injection. This involves the same theory as the injection, but can be administered by the patient themselves.

desensitisation
a treatment process that gradually reduces the immune response to allergens

Cancer

cancer
a disease in which abnormal cells divide uncontrollably

tumour
a growth of abnormal cell tissue that can be benign or malignant

benign
not spreading throughout the organism

malignant
cancerous; able to invade other tissues

Cancer is a disease where cells fail to apoptose and multiply abnormally. There are many different types of cancer, affecting different cells in the body. Healthy cells have genes to regulate the rate of cell division and apoptosis so there is a balance between producing new cells and removing old or damaged cells. Mutations can occur in these genes, which may cause a cell to divide uncontrollably. If these cells continue to divide and are not stopped by the immune system, a **tumour** may start to develop.

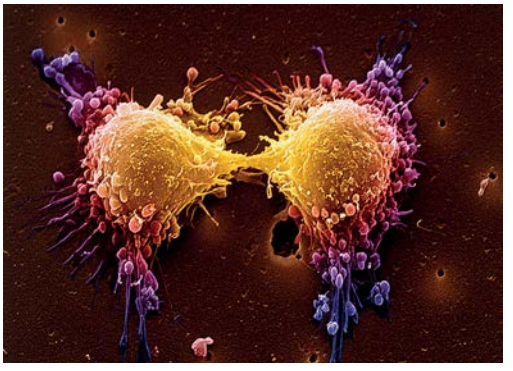


FIGURE 1 A cancer cell dividing, beginning the formation of a tumour

A tumour may be **benign** (does not invade other tissue) or **malignant** (invasive). Benign tumours do not spread throughout the body, but can press on nerves or blood vessels. Cells within malignant tumours can break off and invade other tissues, spreading throughout the body. The process of cells breaking off the original tumour (primary tumour) and travelling through the body to form a secondary tumour is known as **metastasis**.

metastasis
the spread of cancer cells from a primary tumour to another area of the body

People at risk of developing cancer include the elderly, individuals who use immunosuppressive medications for a long period, individuals with immunodeficiency diseases, those with weak immune systems, and those whose lifestyle or environment exposes them to carcinogens (cancer-causing substances). However, even individuals with efficient immune systems can develop cancer if the cancerous cells evade the cells of the immune system. Cancer cells may evade the immune system by mutating genetic material to change surface proteins, avoiding cytotoxic T-cells from detecting them, or by releasing cytokines and/or enzymes that suppress the T-cell response.

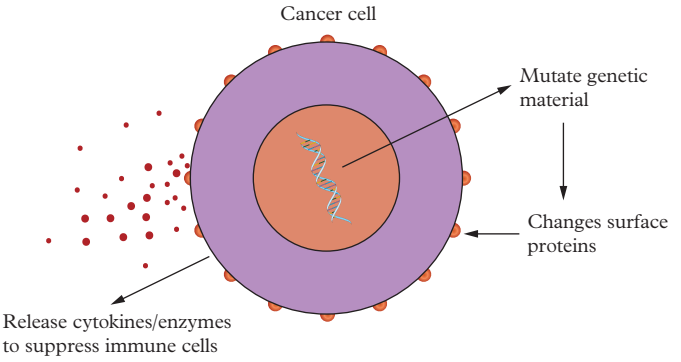


FIGURE 2 Cancer cells have various mechanisms to avoid destruction by T-cells.

Cancer treatment

The immune system can prevent or slow cancer growth. Cancer immunotherapy is a type of cancer treatment that is usually specific to the patient, and some types are personalised treatments. Traditional cancer treatments such as surgery, chemotherapy and radiotherapy are still the most common cancer treatments, as described below.

- **Surgery** – procedures with the aim to remove all cancerous tissue. This treatment is most effective for solid tumours located in a single area.
- **Chemotherapy** – a type of treatment that uses drugs to destroy or slow the growth of cancerous cells. The drugs target cells that are undergoing cell division. This means healthy cells that are reproducing are also affected (e.g. hair follicles or cells lining the intestines). Chemotherapy is usually used as part of a treatment plan and its effectiveness depends on the dosage given and the type of cancer it is used against.
- **Radiotherapy** (radiation therapy) – involves exposing the patient to high levels of radiation, either externally or internally to the cell. The radiation damages DNA, causing cells to apoptose. This affects cancerous cells, but it can also damage neighbouring healthy cells.

These traditional treatments are still used to treat cancer, though immunotherapy can provide a more targeted approach. There are several different types of immunotherapy treatments for cancer, but these are only effective against a select few cancers so far.

Types of cancer immunotherapy

There are several types of immunotherapies currently being used in Australia. They are either non-specific, boosting the immune response to cancerous cells, or specific, where they target a particular antigen on the cancer cell.

Checkpoint inhibitors

There are surface receptors on cytotoxic T-cells known as checkpoint proteins that cause the cytotoxic T-lymphocytes to go to sleep (senescence). This prevents them from attacking the cancer cells. **Checkpoint inhibitors** are drugs designed to block these surface receptors, preventing the cancer cells from inactivating the T-cells (Figure 3). This allows the cytotoxic T-cells to remain active and destroy the cancer cells. This is non-specific immunotherapy since it stimulates the immune system for a better overall immune response.

checkpoint inhibitor
a drug that promotes an immune system response to cancer cells

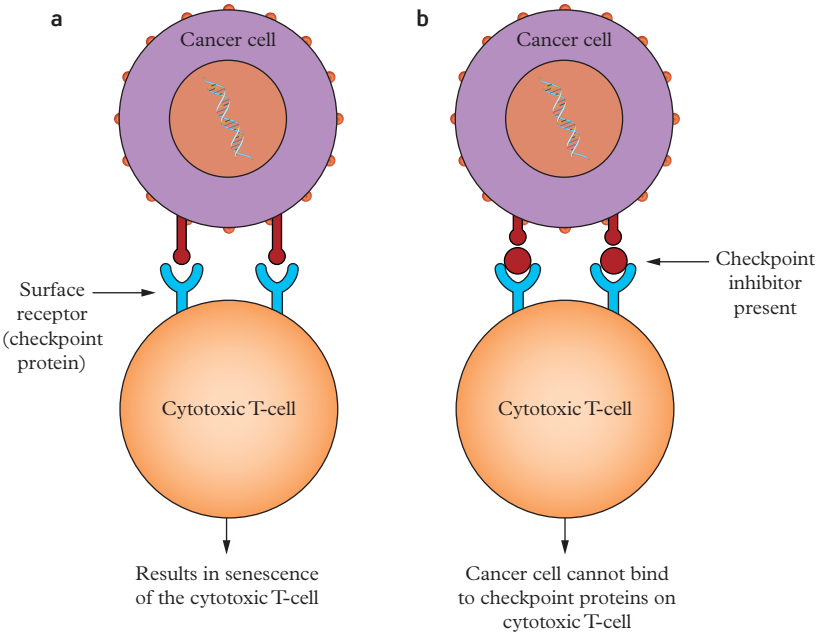


FIGURE 3 Using checkpoint inhibitors. **a** Cancer cells can inhibit cytotoxic T-cells, destroying them by binding to checkpoint proteins. **b** The cancer cell no longer binds to the cytotoxic T-cell checkpoint proteins because the checkpoint inhibitors block the binding sites, and the cytotoxic T-cell is then able to destroy the cancer cell.

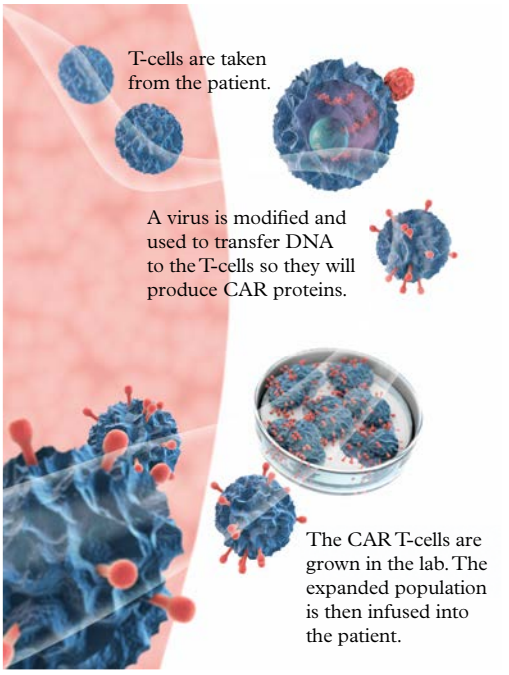


FIGURE 4 The production of chimeric antigen receptor (CAR) T-cells can be used as a specific immunotherapy.

T-cell transfer therapy

T-cell transfer therapy
a type of immunotherapy where T-cells are given to a patient to target cancerous cells

As you learnt in Chapter 3, Case study 3.2 (Using CRISPR to fight cancer), CAR T-cells can be produced to fight cancer (Figure 4). **T-cell transfer therapy** is a type of cancer immunotherapy where T-cells are taken from the tumour via a biopsy (removing tissue for testing). These cells are tested and those that are most active against the tumour are selected. They are then genetically engineered using CRISPR-Cas9 to produce special protein receptors – chimeric antigen receptor (CAR) – that gives the T-cells the ability to target a specific antigen on the cancer cells. Scientists grow large numbers of these chimeric antigen receptor T-cells (CAR T-cells) in the laboratory. They are then injected back into the patient who has the cancer, boosting the amount of T-cells that target the specific cancer. This is considered a specific immunotherapy because it targets particular cancerous cells.

Cancer vaccines

cancer vaccine
a vaccine that can prevent the development of a cancer or treat an existing cancer

Cancer vaccines are vaccines that boost the immune response to particular cancer-causing viruses. These viruses enter a cell and embed their DNA into the chromosomes. This may interrupt the normal functioning of the genes, resulting in a cell becoming cancerous. The cancer vaccine contains viral antigens, and once given to an individual, they will stimulate an immune response that is specific for that particular antigen. These vaccines have no side effects, and they can be preventative (i.e. against HPV) or therapeutic (i.e. against melanoma). This is a similar approach to vaccines designed to prevent pathogen infection, as you learnt in Chapter 9.

CASE STUDY 10.4

HPV preventative vaccine

The human papillomavirus (HPV) is the most common sexually transmitted infection (STI) and is associated with the cause of 5% of all cancers. The most common cancers caused by HPV are cervical cancer and genital warts. Transmission of the virus occurs through skin-to-skin contact, in particular during sexual activity. The viral infection is most common in sexually active young women between the ages of 18 and 30.

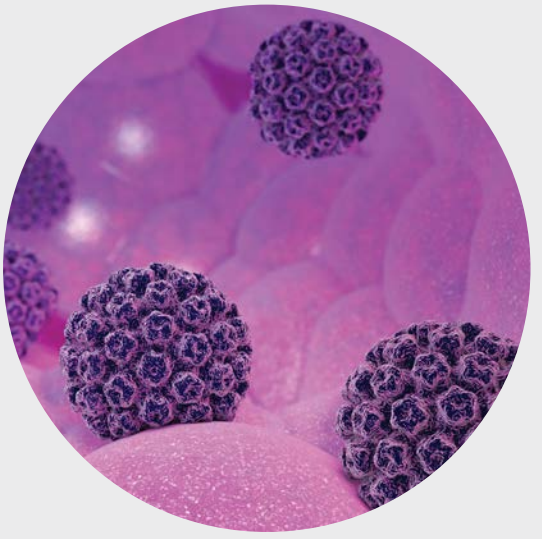


FIGURE 5 Digital illustration of the human papillomavirus (HPV)

A preventative vaccine to target HPV was developed by an Australian-based team at the University of Queensland and led by Ian Frazer. The surface proteins of the virus were identified and grown using yeast cells, and then purified. These surface proteins form the vaccine, which when injected, stimulates the immune system to produce antibodies against the HPV antigen (surface protein). This also produces memory cells for long-lasting immunity. The vaccine needs to be delivered before an individual is exposed to HPV, therefore typically before they are sexually active.

Australia was the first country in the world to publicly fund a HPV vaccination program targeting teenage girls, which began in 2007. Since 2018, the vaccination program has expanded and is now administered to boys and girls, aged 12–13. Cervical cancer may be the most common form of cancer caused by HPV, but the virus can also develop penile, anal, vaginal, vulval and throat cancer. Since the vaccine program was launched in Australia, there has been a reduction in the diagnosis of HPV-associated cancers, making the vaccine a huge success.

Monoclonal antibodies (mAbs)

Monoclonal antibodies are antibodies that are produced in a laboratory to bind to a specific cancer antigen. Large numbers of the same antibody are produced from a single clone of a B plasma cell grown in a cell culture. These antibodies are injected into the patient and target the specific antigens present on the patient's cancer. This is considered a personalised treatment for each individual.

monoclonal antibodies therapeutic antibodies produced from a cloned B plasma cell targeting a specific cancer antigen

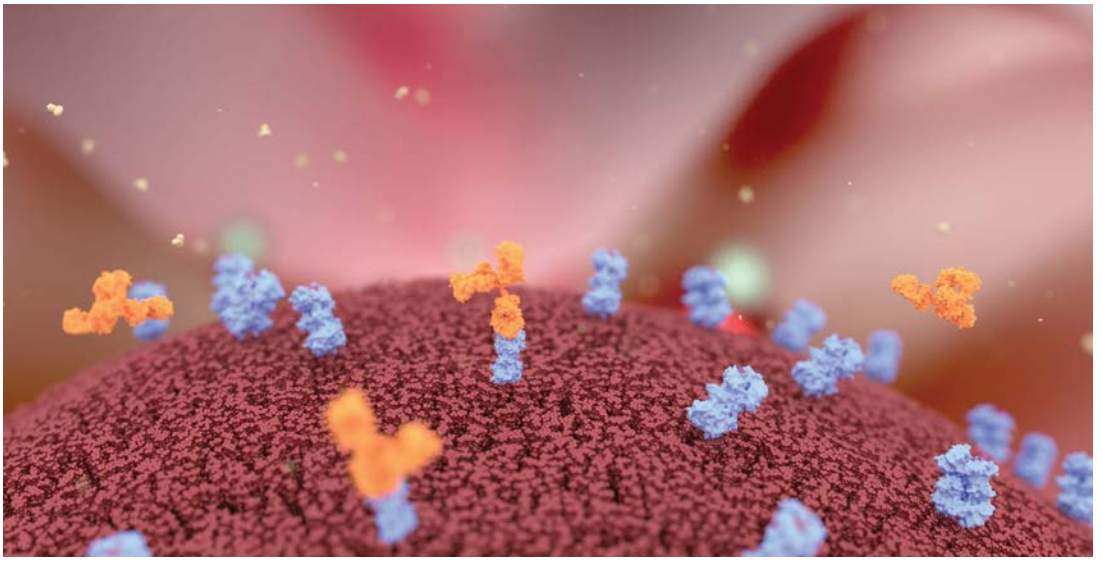


FIGURE 6 Monoclonal antibodies binding to antigens on the surface of a cancer cell

Video
Monoclonal antibodies

Monoclonal antibodies are produced via the following steps (as seen in Figure 7).

1. A patient's cancer is identified and an antigen for the cancer cells is injected into a mouse.
2. The mouse's immune system produces specific antibodies against the antigen that was injected.
3. The activated B plasma cells are isolated from the mouse spleen and fused with **myeloma cells**, which will continue to divide without mutation. This is done because B plasma cells only have a limited lifespan and will not produce enough antibodies without first being fused with myeloma cells.
4. The fused B plasma cells and myeloma cells result in a hybridised cell known as a **hybridoma**.
5. Hybridomas are quite stable in cell culture and will continue to make antibody clones to be harvested.

myeloma cells
cancerous cells that develop from B plasma cells in the bone marrow

hybridoma
a hybridised cell that is a fusion of a plasma cell and a tumour cell

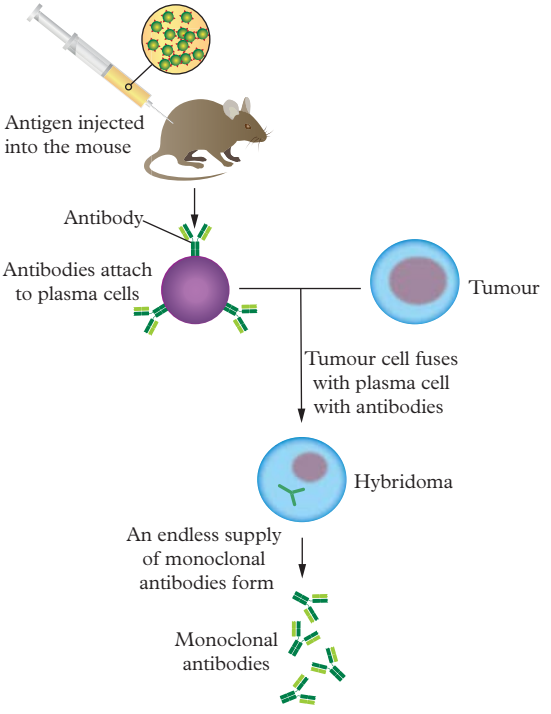


FIGURE 7 Monoclonal antibodies are produced via a series of steps.

Autoimmune disease

The cell membrane of all cells in your body contains glycoproteins called self-markers to help your immune system recognise your cells from other foreign pathogens. When your body tissues are not recognised as 'self', an immune response is initiated against your own cells. This is known as an **autoimmune disease**. The cause of autoimmune diseases is usually difficult to determine. Common autoimmune diseases include type 1 diabetes, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and lupus.

autoimmune disease
when the immune system attacks the body's own cells

The cells involved in autoimmune diseases include the following.

- Cytotoxic T-cells of the adaptive immune response – these directly attack body tissue by inducing apoptosis of 'self' cells.
- B-lymphocytes become plasma cells that secrete specific antibodies to target self-antigens, and therefore act indirectly. These antibodies are called **autoantibodies**.
- Mast cells are also activated due to the activation of T- and B-lymphocytes, releasing histamine and resulting in an inflammatory response.

autoantibodies antibodies produced by B plasma cells that target 'self' markers on body cells

Causes of autoimmune disease may include environmental factors, genetic predisposition, or a combination of particular environmental triggers and inheritable susceptibility.

Autoimmune immunotherapy

Autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease can be treated with immunotherapy. The immunotherapy in these cases is an inhibitor of a cytokine called tumour necrosis factor alpha (TNF-α). This factor contributes the inflammation response. The immunotherapy that binds and blocks this factor prevents the inflammation that causes the disease.

As you just learnt, CAR T-cells can target specific antigens on cancer cells to treat cancer. The same method can be used for some autoimmune diseases where CAR T-cells can target autoantigens to suppress the immune response causing the autoimmune disease.

Monoclonal antibodies and autoimmune disease

Monoclonal antibodies are also used to treat several types of autoimmune diseases, including rheumatoid arthritis and multiple sclerosis. With monoclonal antibodies, it is possible to suppress the production of autoantibodies causing autoimmune diseases. Monoclonal antibodies are produced to block the antigens on the surface of B plasma cells that are activating the autoantibodies. Monoclonal antibodies can also target antigen-presenting cells that would initiate a response to produce autoantibodies. In this way, the production of the autoantibodies can be suppressed, which would minimise the effects of the autoimmune disease.

CHECK YOUR LEARNING 10.4

Describe and explain

1 Define the term 'immunotherapy'.
2 Describe how a vaccine can be considered a form of cancer immunotherapy.
3 How are monoclonal antibodies produced?

Apply, analyse and compare

4 Describe two ways a cancer cell can avoid detection by the immune system.
5 Produce a table to summarise the similarities and differences between the use of the checkpoint inhibitors and T-cell transfer immunotherapy.
6 Explain how monoclonal antibodies can be used to treat:
   a cancer
   b autoimmune diseases.

Design and discuss

7 Discuss the advantage of a preventative cancer vaccine, such as the HPV vaccine described in Case study 10.4, over other cancer treatments that are not preventative.
8 Investigate and describe two different ethical issues related to the production and use of monoclonal antibodies.

CHAPTER 10

Review

Chapter summary

10.1
- Diseases can be classified into infectious and non-infectious diseases.
- New pathogens can emerge and old pathogens can re-emerge.
- European arrival to Australia brought foreign pathogens, negatively affecting Aboriginal and Torres Strait Islander Peoples.

10.2
- Transmission of infectious pathogens can be through physical contact, contact with bodily fluids, airborne transmission and faecal-oral transmission.
- Prevention measures to avoid the spread of infectious pathogens include good personal hygiene; proper food storage, handling and transportation; maintaining clean water; vaccinations; and biosecurity.
- Strategies for prevention of the spread of pathogens include identifying the cause of an infectious disease and using suitable control measures.

10.3
- Vaccination programs involve providing a planned series of vaccinations and booster vaccinations for individuals.
- Herd immunity is the ability of an immunised population to prevent the spread of infection to vulnerable individuals.
- R_0 values calculate the number of people that can be infected from one infected individual. This can change due to social distancing and vaccinations.

10.4
- Types of cancer immunotherapy treatments include checkpoint inhibitors, T-cell transfer therapy, cancer vaccines and monoclonal antibodies (mAbs).
- Autoimmune diseases can be treated with immunotherapy and monoclonal antibodies.

Revision questions

Multiple choice

1 A contagious disease is a disease that:
- **A** is able to spread from one organism to another organism.
- **B** is able to be transmitted through the environment.
- **C** is unable to be spread through the environment.
- **D** is able to spread only from one person to another person.

2 Which of the following was a significant cause of death for many Aboriginal and Torres Strait Islander Peoples within months of the arrival of Europeans to Australia?
- **A** Aboriginal and Torres Strait Islander Peoples weren't able to access the traditional medicines due to Europeans building towns and roads, in the process destroying Australian flora.
- **B** Aboriginal and Torres Strait Islander Peoples had not been exposed to the contagious diseases that Europeans bought with them, so therefore they had no immunity.
- **C** Aboriginal and Torres Strait Islander Peoples had already been exposed to the diseases the Europeans brought with them; however, the Europeans did not allow access to Western medication needed to treat such diseases.
- **D** The Europeans came in large groups and destroyed Indigenous Peoples' infrastructure, exposing them to arid climates.

3 If a disease is passed on through genetic inheritance and is not contagious, it would be considered:
- **A** an environmental non-infectious disease.
- **B** a non-contagious infectious disease.
- **C** an infectious disease.
- **D** a genetic non-infectious disease.

4 In 2009, there was an outbreak of the H1N1 influenza virus derived from human, swine and avian strains. It was initially reported in Mexico but then spread worldwide. This kind of outbreak would be considered:
- **A** an epidemic.
- **B** a pandemic.
- **C** an endemic.
- **D** an eradication.

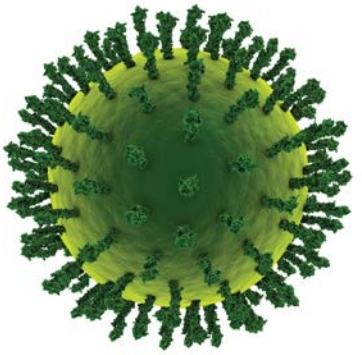


FIGURE 1 An illustration of the H1N1 influenza virus

5 Which of the following would not be considered a transmission of an infectious pathogen?
- **A** The pathogen is able to remain suspended in the air for long periods of time and can be inhaled by unaffected individuals.
- **B** The pathogen is found in sewage and contaminates a town's water supply.
- **C** The bodily fluids of an infected person contain the pathogen and can be released when the individual coughs or sneezes.
- **D** The pathogen is inherited from one of the parents, leading to a genetic disease.

6 Which of the following best describes a vector of a disease?
- **A** A mosquito carrying the pathogen and transmitting the disease to a human
- **B** The transmission of the pathogen via water
- **C** The disease-causing pathogen
- **D** An organism that is infected by a disease

7 Where are mouse plasma cells isolated from?

A Spleen

B Thymus

C Bone marrow

D Blood

8 Monoclonal antibodies are an important immunotherapy treatment for several types of cancer and autoimmune diseases. Monoclonal antibodies are produced via a series of steps. Consider the following steps.

1 – Fused B plasma cells and myeloma cells form a hybridoma and the antibodies produced are harvested.

2 – A patient's antigen is identified and it is injected into a mouse.

3 – B plasma cells are isolated from the mouse spleen and fused with myeloma cells so they continue to replicate.

4 – The mouse's immune system produces antibodies against the injected antigen.

Select the correct order below.

A 2, 4, 3, 1

B 1, 4, 3, 2

C 3, 4, 2, 1

D 4, 2, 1, 3

9 Which cancer immunotherapy is a drug designed to inhibit cancer cells from inactivating cytotoxic T-cells?

A Checkpoint inhibitors

B T-cell transfer therapy

C Cancer vaccine

D Monoclonal antibodies

Short answer

Describe and explain

10 What are the different ways you can classify a disease?

11 Explain why correct food storage and handling can be considered a prevention strategy for infectious diseases.

12 Describe the impact of European arrival on Aboriginal and Torres Strait Islander Peoples, in terms of infectious disease.

13 Define the term 'emerging disease'.

14 How does a vector transmit infectious pathogens?

15 Describe the ways in which a pathogen can be directly transmitted from an infected individual to a non-infected individual.

16 Why is sanitation important in preventing the spread of disease?

17 Explain how checkpoint inhibitors are used as a cancer treatment.

Apply, analyse and compare

18 Why is social distancing effective at slowing the spread of COVID-19?

19 Analyse how public health programs can reduce the spread of an infectious pathogen.

20 Analyse how a cancer vaccine produces immunity against a cancer-causing virus. Refer to memory B-cells and/or T-cells in your response.

21 In what ways are monoclonal antibodies and normal immune antibodies similar?

22 Effective immunotherapies in treating cancer can have advantages over traditional cancer treatments such as radiotherapy, chemotherapy and surgery. Describe these advantages.

23 Why would it be essential to identify the pathogen causing a disease before a treatment can be developed?

24 Nine people who ate chicken at the same restaurant on the same day became infected with salmonella bacteria, which can cause diarrhoea, fever and abdominal pain. Outline two strategies that could have been used by the restaurant to:

a identify the pathogen

b control the transmission.

Design and discuss

25 Discuss the statement 'Vaccines will eliminate infectious diseases.'

26 An outbreak of a disease was reported to the World Health Organization (WHO) and the pathogen identified was the yellow tongue fever virus. The disease was spread via a mosquito vector. Discuss control measures that could be put in place to limit the spread of the disease.

27 Diphtheria is a contagious disease caused by *Corynebacterium diphtheriae*. It is transmitted via the transfer of bodily fluids through sneezing, coughing or open wounds. Symptoms of diphtheria are cold-like symptoms and difficulty breathing, and this disease is potentially fatal. Although it can affect people of any age, it was commonly diagnosed in children, and about 10% of infected individuals die from the disease. In the 1920s, the pathogen was identified and a vaccine was developed. By 1932, vaccination programs began in schools. In 1953, a combination vaccine (DTP) was introduced to vaccinate against diphtheria as well as whooping cough and tetanus.

In the last 10 years, there have been few cases of diphtheria reported in Australia. Most of the cases are in people who were infected due to overseas travel. Although this disease is under control in Australia, it is still a major health issue in some countries.

a Why might diphtheria still be a major health concern in countries other than Australia?

b By referring to Figure 2, describe the effectiveness of the vaccination program in Australian schools.

c Explain what could happen if vaccination rates for DTP fell too low.

d Besides vaccination as a preventative measure, describe other preventative measures individuals could take to reduce the risk of contracting diseases such as diphtheria.

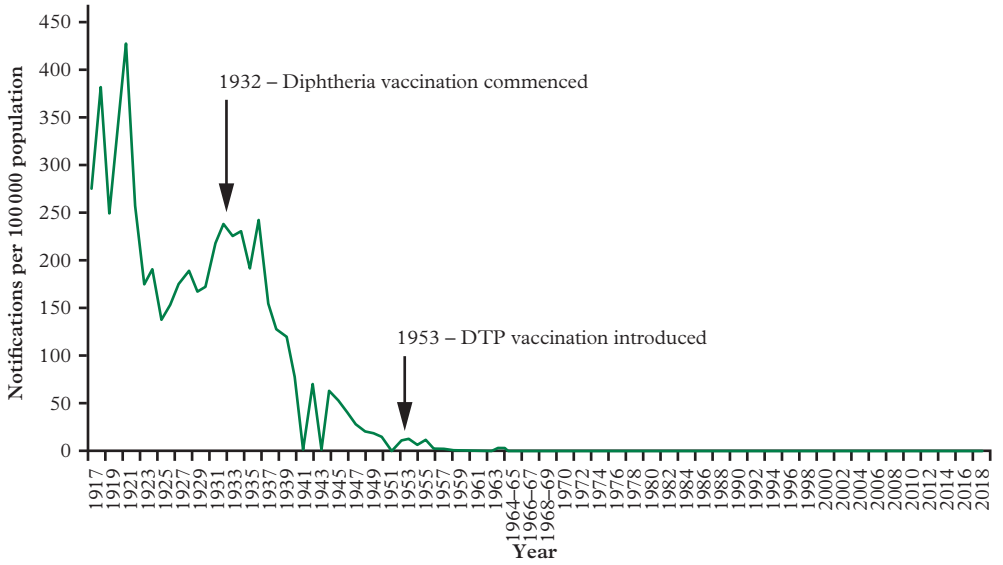


FIGURE 2 Diphtheria outbreaks in Australia from 1917–2018.

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Exam essentials

Responding to questions

In your exam, you may be expected to provide a definition when explaining a process.

Be specific when defining terms

When defining terms, you need to show a clear understanding of the term and how it is applied in biology. While it sometimes seems easier to memorise a definition, it is also important to understand why some words are included in the definition. For example, a monoclonal antibody needs a reference to a 'single clone of a cell' that produces 'identical' antibodies.

The following question is taken from the 2018 VCE Biology Examination. Read the question carefully, then consider whether the responses show an adequate understanding of key terms.

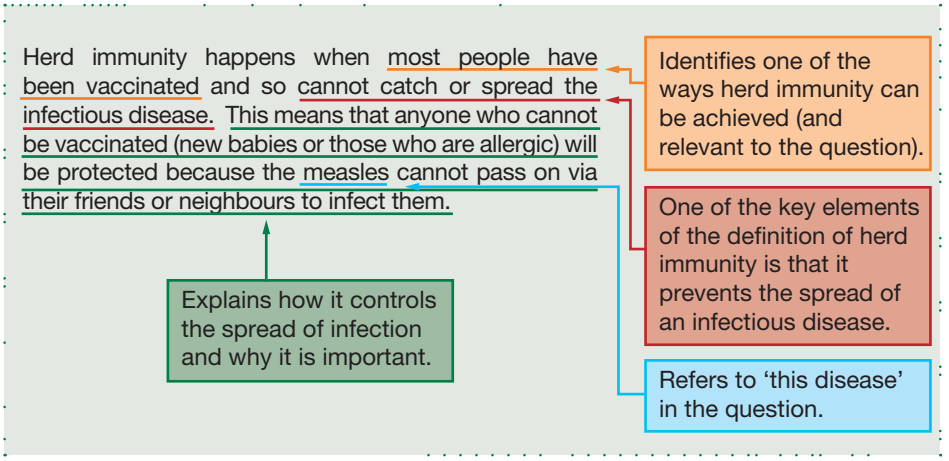
QUESTION 5b (2018 VCE Biology Written examination)

b Controlling the number of measles cases in a population relies on herd immunity.

What is herd immunity and how does it help control the number of cases of this disease? 2 marks

Source: 2018 Biology Written Examination Question 5, Short answer, reproduced by permission © VCAA

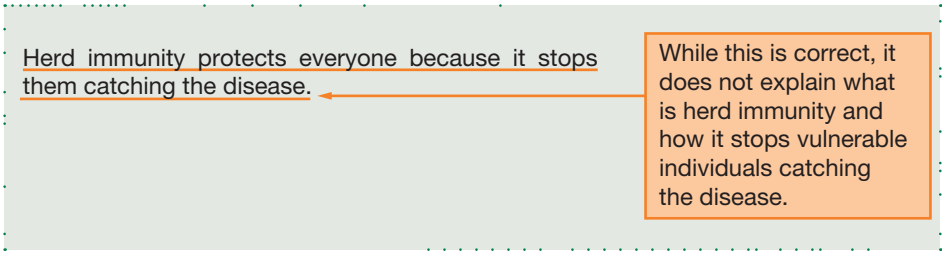
Response 1



This response would receive 2 marks since it describes:

- how most of the community become immune to the disease
- how the unvaccinated are protected by preventing the disease being passed between hosts.

Response 2



This response would not receive any marks since it does not provide any new information from the stem of the question.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 10d (2020 Biology Written Examination)

d Describe **two** strategies, other than vaccination, that could reduce the transmission of measles. 2 marks

No sport

using disinfectant

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Marking guide

Question 10 d	- 1 mark for one strategy that also included a description of how it reduced transmission. - 1 mark for a second strategy that also included a description of how it reduced transmission.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

Check your Student obook pro for these digital resources and more: pro

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CHAPTER 11

Evolving population genetics

The gene pool is constantly being changed by natural selection preferring one phenotype over others. Migration of individuals can change the frequency of an allele in a group of interbreeding individuals, and new alleles can be introduced through mutations. As the gene pool changes, the population evolves, resulting in permanent changes to the phenotypic appearances. If the gene flow is reduced between populations, or small populations become isolated or experience a catastrophic event, the genetic diversity of the population will decrease, threatening the survival of all members of the species. Humans can also manipulate the allele frequencies of gene pools through selective breeding programs of plants and animals. Natural selection of bacteria due to the use and misuse of antibiotics, and antigenic shift in viruses, leads to continual challenges in the treatment of human pathogens.

KEY KNOWLEDGE

- 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
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity
- manipulation of gene pools through selective breeding programs
- consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens

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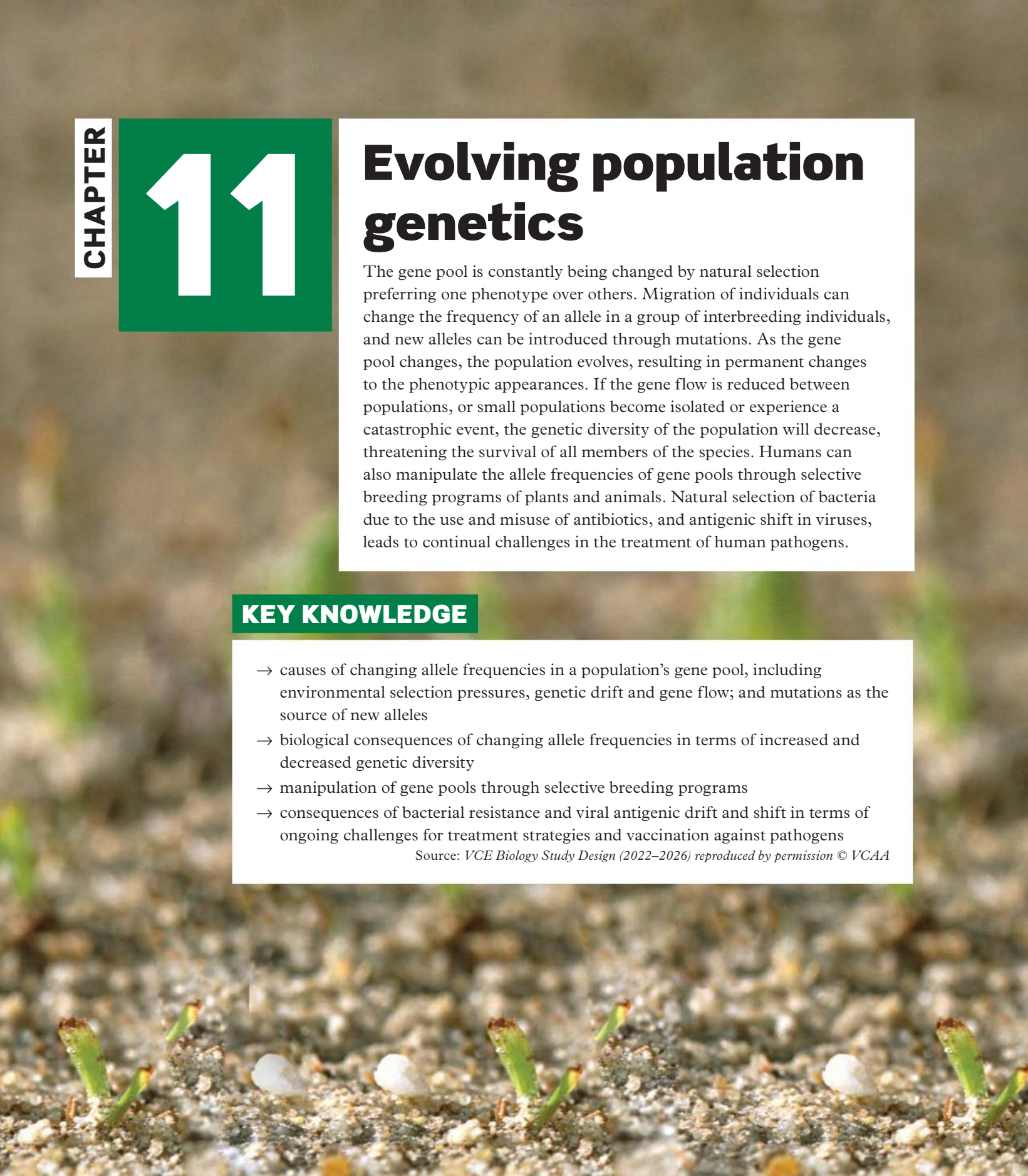

FIGURE 1 Mutations can come in many shapes and forms, such as this two-headed turtle.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

11A What is an allele?

11A Groundwork resource
Alleles

11B Define the term 'natural selection.

11B Groundwork resource
Natural selection

11C How can humans artificially select desirable traits so that these can increase within a population over time?

11C Groundwork resource
Selecting traits

PRACTICALS

NO-TECH PRACTICAL	**11.1** Genetic changes over time

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

11.1 Changing allele frequencies

KEY IDEAS

In this topic, you will learn that:

+ environmental selection pressures, mutations, genetic drift and gene flow are sources of new alleles.

gene pool
the entire set of genes and their allele combinations in any given population

A **gene pool** is the entire set of genes, including all the different allele combinations of an interbreeding population. The gene pool of a population is continually changing due to mutation, migration of individuals, catastrophic disaster (e.g. volcanic eruptions) and natural selection. Population genetics investigates how these different factors can lead to evolutionary changes within a population over time.

Alleles

diploid
having two complete sets of chromosomes within a cell; somatic cells contain a diploid set of chromosomes

allele
an alternative form of a gene

genotype
the genetic make -up of an individual

phenotype
the physical expression of a genotype

genetic equilibrium
when the allele frequencies of a population remain stable over time

Humans are **diploid** organisms, which means they have two complete sets of chromosomes in each somatic cell, one inherited from each parent. Humans have two copies of each gene, each found at the same location or locus across the two chromosomes. Most genes have slight variations within their nucleotide base sequences, which can be expressed as differences in a particular characteristic or trait. These different versions of a gene are called **alleles**.

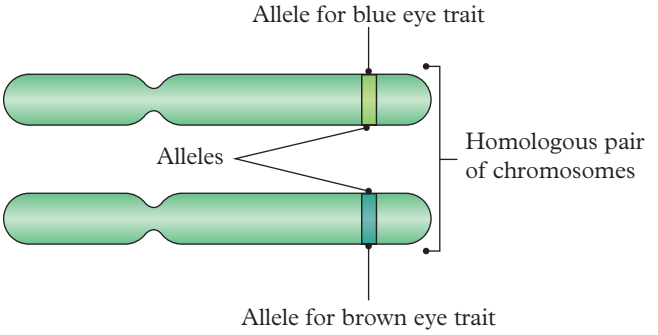

FIGURE 1 An allele is a different form of the same gene.

The **genotype** is the different combinations of alleles for each particular gene, whereas the **phenotype** is the way in which the gene is chemically or physically expressed. The phenotype is determined by the genotype as well as the environmental conditions of the individual. For example, a person may have the genotype for tall stature, though if they experience low nutrition levels they may never achieve their full height.

It is the interaction of different genes, or a variety of different alleles (with varying nucleotide sequences), for particular genes that produces the phenotypic range within a species. While each individual only possesses two alleles per gene, there can be many more alleles within a population.

Study tip

The term 'allele' is often confused with the term 'gene'. Remember that alleles are a type of gene that express variations of a trait (e.g. colour on a petal is the work of genes, but the different colours are because of different alleles).

Allele frequencies

The allele frequency is the proportion of a population that has a particular allele. When calculated, allele frequencies always add up to 1.0 or 100.0%. If the frequency of an allele is 1.0, this means there is only one type of allele in the gene pool. This makes the population vulnerable since one allele for a gene indicates there is only one phenotype for the gene. The genetic diversity is low, and the organism has no way to adapt or evolve if the environment changes.

By analysing the frequencies of alleles for any particular gene in a population over a period of time, changes in the gene pool can be determined. If there are no changes, the population is said to be in **genetic equilibrium** for those alleles.

Genetic equilibrium

Change in allele frequencies (i.e. microevolution) occurs only when something alters the genetic equilibrium. For example, a large migration of individuals in or out of a population may change the frequencies of specific alleles. To be in genetic equilibrium, a population must satisfy five conditions:

1 The population must be large enough to make it unlikely that chance alone could significantly alter the allele frequencies.
2 Mutations must not occur, or if they do, the number of harmful mutations that develop in a population must be equal to the number of harmful mutations that are eliminated (through the death of the individual) so as to not affect the allele frequency.
3 There must be no **gene flow** by immigration and emigration of individuals or gametes into or out of the population. For example, plant pollen can be blown away and seeds can be carried in a bird's digestive tract, or the gametes of aquatic animals can be carried in water currents.
4 Reproduction must be completely random with no selective mating.
5 There must be no natural selection.

Given these five strict conditions for genetic equilibrium, it is not surprising that equilibrium is not met and evolution occurs.

gene flow
the movement of genes between populations of a species due to migration of individuals

Mutations

Mutations are permanent changes in DNA that occur spontaneously or due to **mutagens** such as UV light and X-rays. Mutations can affect the nucleotide sequence of a gene. These are called **gene mutations**. Mutations can also affect large sections of chromosomes or an entire chromosome. These are called **chromosomal mutations**.

mutagen
a factor that increases the rate of mutation above the usual spontaneous rate

gene mutation
mutation that occurs within the DNA sequence of genes within a chromosome

chromosomal mutation
mutation that alters entire sections of chromosomes

FIGURE 2 Radiation is a mutagen that can cause mutations in skin cells. For this reason, radiologists wear protective clothing and typically leave the room when performing X-rays.

The type of cell a mutation occurs in determines how it affects a gene pool. If the mutation occurs in a **somatic** (body) cell it will only affect the individual. This is because the mutation is not able to be passed on to a subsequent offspring. This means that the effect on the population tends to be temporary. In contrast, **gametic** mutations, those that occur in the **germline cells** that produce gametes (e.g. egg, sperm, ovules, pollen grains), are able to be passed onto offspring. This can result in changes to the genetic make-up of a population over time through the introduction of new alleles.

somatic
a diploid body cell

gametic
involving gametes, or germline cells with half the usual number of chromosomes

germline cell
a cell in the gonads that gives rise to gametes (ovum and sperm)

CASE STUDY 11.1

Chernobyl – genetic mutations or coincidence?

The 1986 nuclear power plant accident in Chernobyl, Ukraine released significant amounts of radiation, a known mutagen for all living organisms. The size of the area affected by radiation is still under debate today, but an area was deemed too dangerous for humans – an exclusion zone of 2600 km^2. There are numerous accounts of somatic cell mutations occurring in humans after the Chernobyl disaster, eventuating in the development of cancer in the affected population. The United Nations Scientific Committee reported that more than 6000 adolescents developed thyroid cancer after exposure to the Chernobyl radiation; however, these findings are still challenged by other experts.

Investigations into gametic cell mutations (mutations that can be passed down from the affected parents to their offspring) after the 1986 event continue. A study conducted in 2021 suggested that the number of mutations in children in the area was no larger than normal. It is thought that exposure over a longer period (rather than a short burst) allow the DNA time to repair. More studies need to be conducted to confirm whether these defects are the result of genetic mutations that can be tracked back to the radiation from the Chernobyl accident in 1986.

FIGURE 3 Masks and suits were used by people in the affected areas of Chernobyl in the attempts to decrease exposure to radiation.

Video
Point mutations

Point mutations

point mutation
mutation affecting the nucleotide sequence of a gene by the substitution, insertion or deletion of a nucleotide

Point mutations are gene mutations that occur within a specific DNA nucleotide triplet where one nucleotide is substituted by another, or the order within the triplet is changed. There are two types of point mutations:

- substitution mutations
- frameshift mutations.

Substitution mutations

substitution mutation
the mutation where a nucleotide is swapped for a different nucleotide

Substitution mutations occur when a single nucleotide in either DNA or RNA is exchanged for another. This results in a different triplet or codon being transcribed and translated during protein synthesis. The following are types of substitution mutations:

- **silent mutation:** a mutation that causes no change to the amino acids in the polypeptide (see Figure 4)
- **missense mutation:** a mutation that causes a change to one amino acid in the polypeptide (see Figure 5a)
- **nonsense mutation:** a mutation that causes stop codon and results in a dysfunctional protein (see Figure 5b).

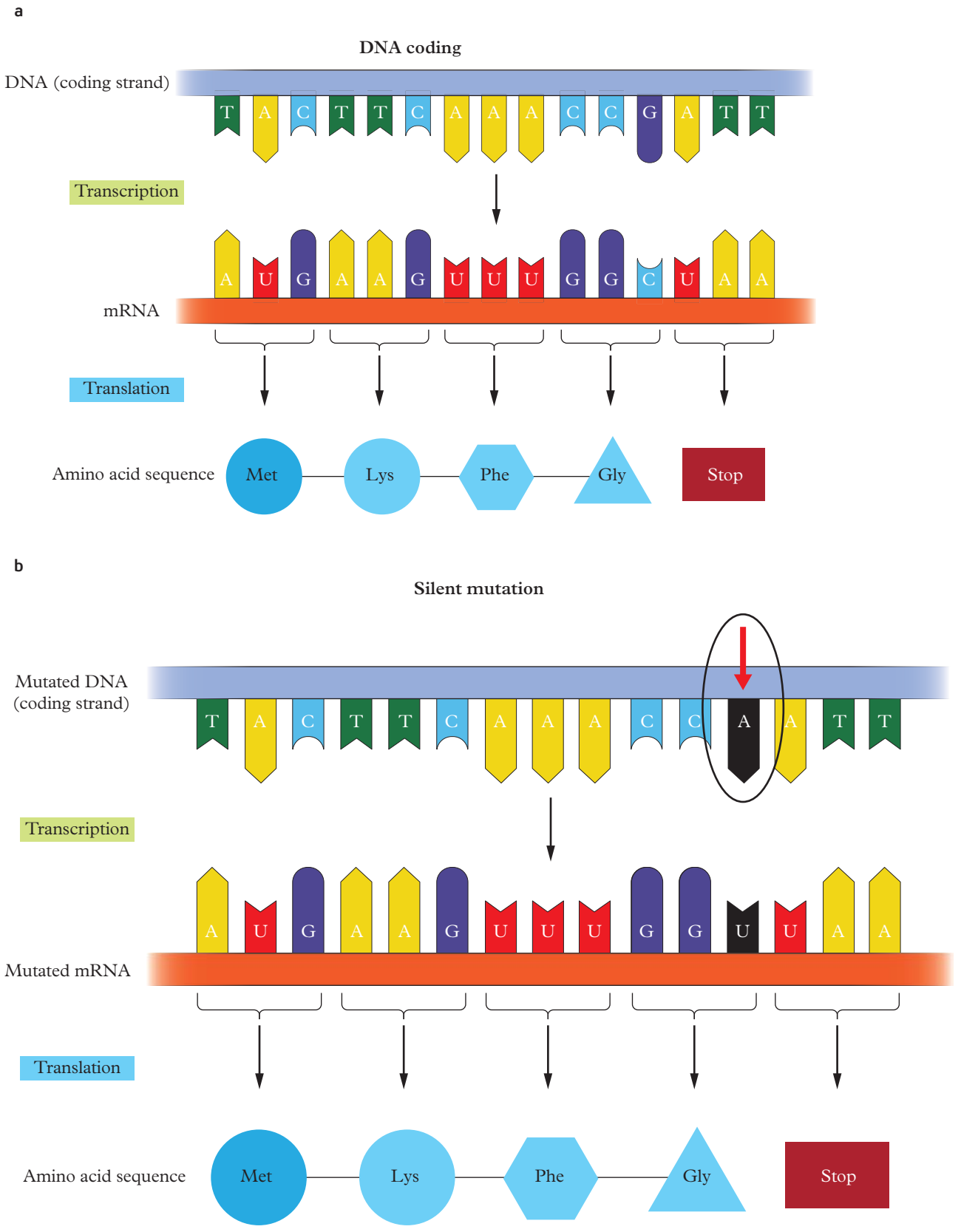

FIGURE 4 **a** The normal DNA sequence codes for the correct amino acid sequence. **b** The silent mutation has substituted the guanine in the fourth triplet to adenine. The new triplet still codes for the amino acid glycine, and therefore there is no change in the amino acid sequence.

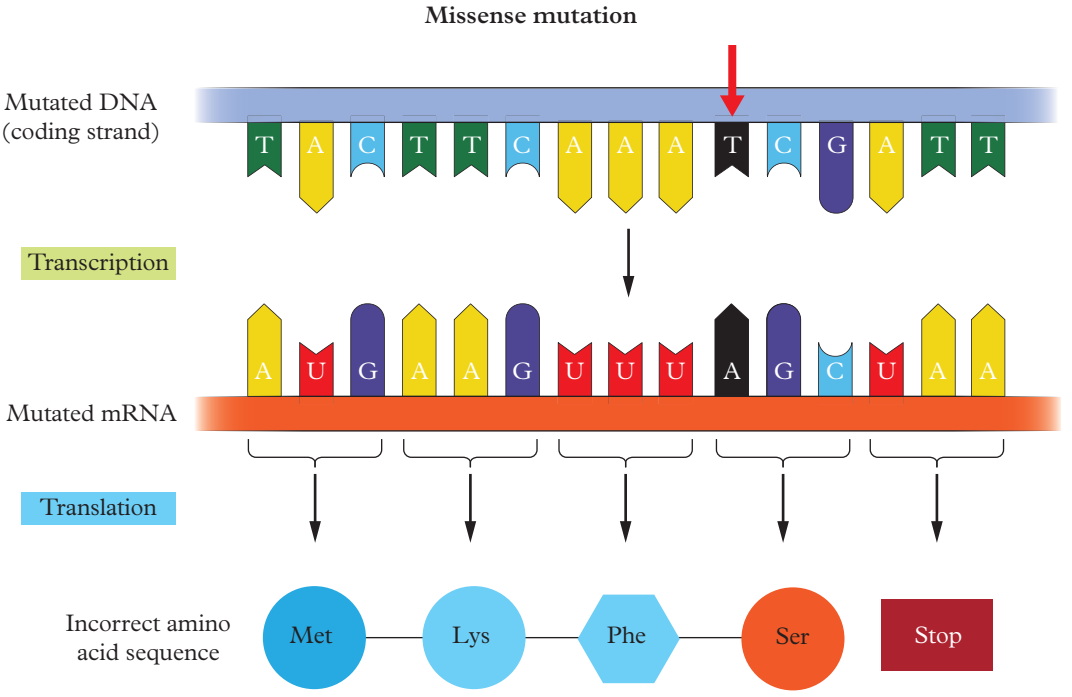

FIGURE 5 The missense mutation has substituted the cytosine of the fourth triplet to thymine. This new triplet codes for the amino acid serine instead of glycine, resulting in a different amino acid sequence.

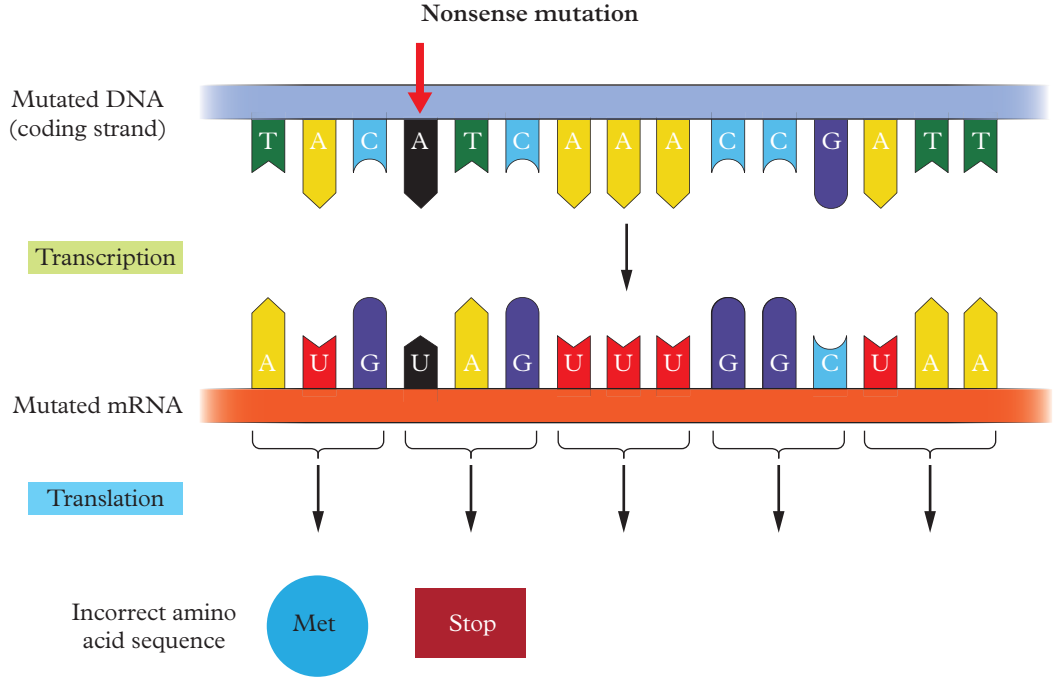

FIGURE 6 The nonsense mutation has substituted adenine for thymine in the second triplet. This new triplet is a stop codon and the end of translation.

frameshift point mutation the addition or deletion of a nucleotide that alters the entire sequence of amino acids from the point of mutation onwards

Frameshift point mutations

Sometimes nucleotides are inserted or deleted from a DNA sequence, resulting in an entirely new sequence of amino acids from the point of mutation onwards. These **frameshift point mutations** involve either insertion (addition of a new nucleotide) or deletion (removal of a nucleotide). In framing errors, there is an increase or decrease in

the number of nucleotides in the DNA strand. Each codon is read together in a **reading frame** as the ribosome moves along the mRNA strand. All triplet codes, and therefore the codons, following the frameshift will be altered. This results in a completely different sequence of amino acids being translated, and the production of a different protein that may not be functional.

(normal sequence)	THE CAT ATE THE RAT AND RAN FAR
(point mutation)	THE CAR ATE THE RAT AND RAN FAR
(frameshift deletion)	THE CAA TET HER ATA NDR ANF AR
(frameshift addition)	THE CAT TAT ETH ERA TAN DRA NFA R

reading frame the sequence of ordered triplets within a gene that are translated into mRNA codons that result in a specific sequence of ordered amino acids that comprise a protein

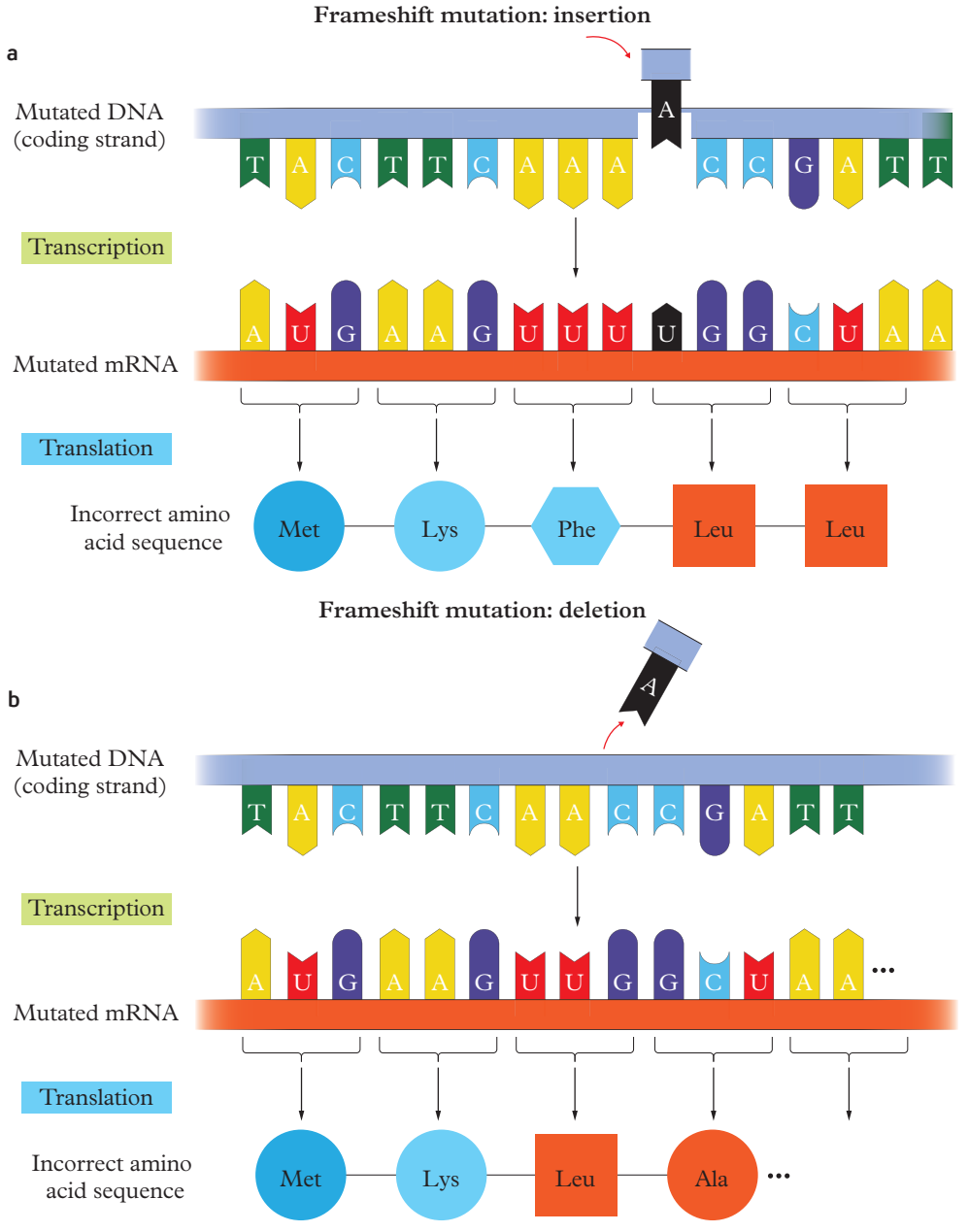

FIGURE 7 Frameshift mutations: **a** The mutated DNA has an adenine inserted after the third triplet. **b** The adenine in the mutated DNA has been deleted from the third triplet. In both cases, the DNA reading frame has been 'shifted' – **a** right and **b** left.

Block mutations

block mutation
a chromosomal mutation that alters large sections of a chromosome

A **block mutation** is a type of chromosome mutation where large sections of a chromosome are permanently altered. This often occurs during crossing over in meiosis I where the homologous pairs of chromosomes swap sections incorrectly. Because these mutations involve many genes being altered, chromosomal mutations are often severe and result in faulty gamete production and developmental defects during embryo development. If sections are deleted, the resulting chromosome will not contain all the genetic information required for a phenotype to be expressed correctly. If sections are duplicated, the resulting chromosome will contain double the genetic information required and excess protein is produced by the duplicated genes. This can provide raw material for new alleles and phenotypes to arise in a population if additional mutation occurs within these duplicated regions.

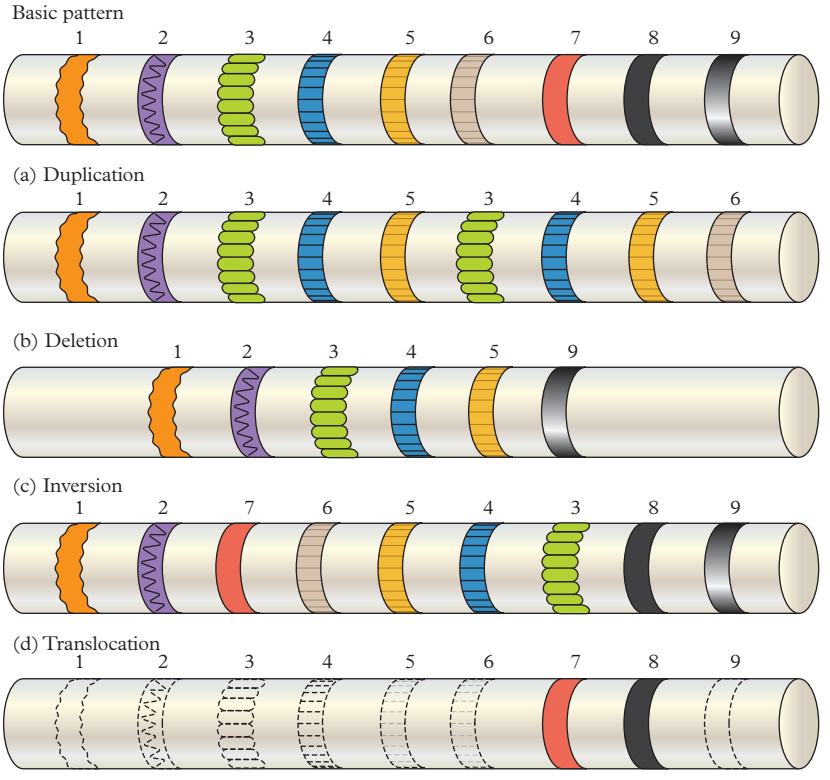

FIGURE 8 Block mutations involve permanently altering large sections of chromosomes.

Study tip

Make sure you understand how mutations are a source of new variation for natural selection to act on in a population.

Selection pressures

natural selection
the differential survival and reproduction of individuals due to differences in phenotype that ultimately lead to the evolution of a species

Selection pressures are environmental factors that affect allele frequencies of a population and ultimately the survival and reproduction of organisms. For example, an insect that is brightly coloured will be easily seen by a hungry bird. This means that the bird will see, and eat all the brightly colour insects and ignore the camouflaged insects. In this case, the hungry bird and colour of the insects were the selection pressures. The only insects that survived to reproduce were the camouflaged insects. This changed the frequency of the (bright colour) alleles, and therefore the phenotypes in the insect population. This process for changing allelic frequencies is called **natural selection**.

Other selection pressures include:

- extreme changes in temperature
- availability of resources such as water and mates
- competition for resources such as water and food
- presence of predators.

Natural selection

Natural selection causes gradual changes in a species over time. As selection pressures change in a population, the survival of individuals better suited to these selective forces increases. Some phenotypes are naturally selected because they have a survival advantage in the changing environment.

In the process of natural selection:

1 There is phenotypic variation in the population caused by mutation.
2 Selection pressures allow some phenotypes to die and others to survive.
3 Those that survive have fertile offspring that inherit the parental phenotypes.
4 This selects and increases the desired allele frequencies in the population.

Gene flow

The movement of genetic material into and out of a population as individuals immigrate and emigrate is called **gene flow**. This causes changes in the gene pool of a population and reduces differences between populations of a species. As long as there is continual movement of individuals between two populations, they will share genetic information when they reproduce and pass on their unique allele combinations. If there is a reduction in the amount of gene flow due to a barrier that prevents individuals from entering or exiting, the populations will slowly accumulate differences, and this can lead to the formation of new species. Smaller populations are affected greatly by gene flow, whereas large populations act as a buffer, preventing total loss of particular alleles and allele combinations if an individual emigrates.

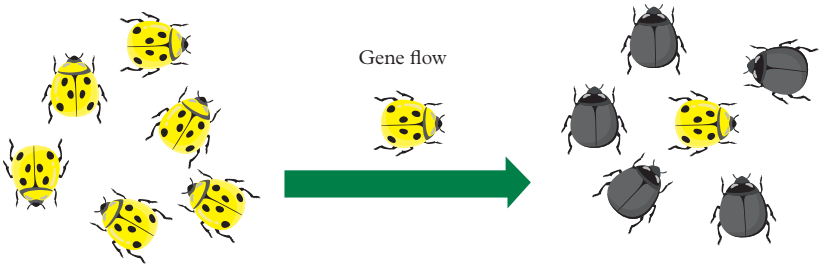

FIGURE 9 Gene flow

Video
Genetic drift and gene flow

Genetic drift

In small, isolated populations there is a greater susceptibility to random fluctuations in the gene pool, which can easily lead to the loss of an allele from the population. This can occur even when that allele is advantageous to survival. These random fluctuations can also result in the fixation of alleles, where all individuals are homozygous (identical) for a particular allele. This means chance events (catastrophes or colonisation) can cause evolutionary change in small populations in a relatively short period of time. This is called **genetic drift** and has an equal chance of increasing or decreasing the survival advantage of a population.

Unlike natural selection, genetic drift occurs regardless of the effect an allele has on the individual.

There are two causes of genetic drift: the founder effect and the bottleneck effect.

genetic drift
random changes in the allele frequencies of small isolated populations

The founder effect

The **founder effect** is a cause of genetic drift that results from a small subgroup of a 'parent' population colonising a new area. The founders of the subgroup are completely isolated from the original population and gene flow is instantly prevented due to the distance between them. This is called **genetic isolation**.

The alleles and allele combinations of the founding members of the subgroup become the source of variation for natural selection. Because this subgroup may not be genetically representative of the original parent population from which it was derived, it will have a limited number of different alleles in the population. As the small population increases in size, there will be continued drift that is different from that of the parent population.

founder effect
where a small number individuals become isolated from an original population to become the founding members of a new population with reduced genetic diversity

genetic isolation
when a population is completely isolated and there is no gene flow

Zoos are an example of founder populations. They contain extremely small populations of organisms that are not genetically representative of the original populations. As such, care must be taken when zoos carry out captive breeding programs to ensure that genetic diversity is maximised as much as possible. Zoos work together to deliberately relocate animals to other zoos in order to maximise the genetic variability in the captive organism. This ensures they breed with other small populations, which increases gene flow and decreases the founder effect in each zoo.

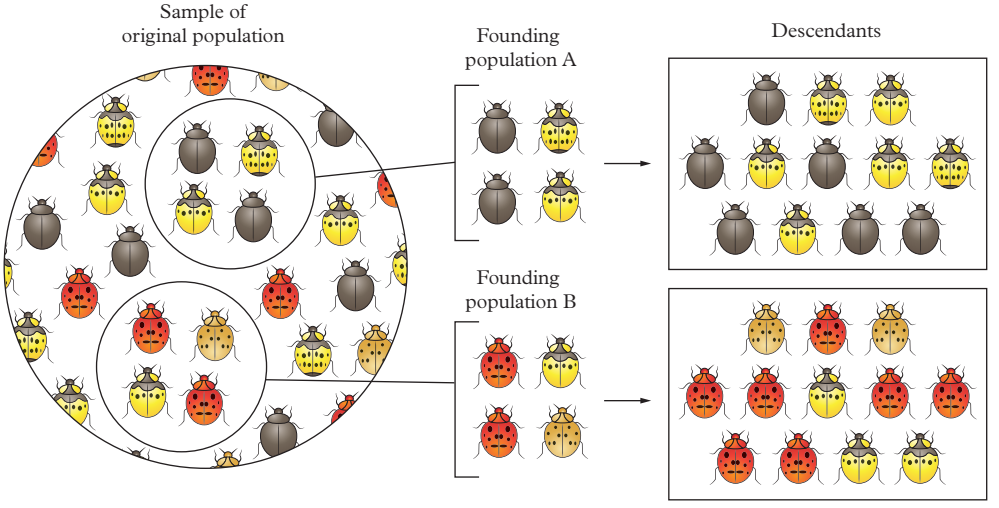

FIGURE 10 Genetic drift occurs in small founder populations. Individuals carrying particular alleles may, by chance, produce more offspring than those with other alleles. Over time, this alters the allele frequency in the gene pool. In this diagram, populations A and B represent two possible outcomes of the founder effect.

The bottleneck effect

bottleneck effect where a small number of individuals survive a catastrophic event, leading to a small population with reduced genetic diversity

Occasionally the size of a population will undergo a drastic reduction that is completely unrelated to natural selection. This is called the **bottleneck effect**. This type of genetic drift may be caused by a disease that affects a large percentage of a population or the effect of a predator (such as hunting by humans) that affects one or more generations. As a result, some alleles are eliminated from the population, causing an over-representation of a small number of remaining alleles. Following this population bottleneck, the population may once again increase in size, despite the overall decrease in the variation of alleles available. In some instances, this can make the population vulnerable to any further changes in the environment since the limited remaining alleles are not as equipped to handle a new threat to the population.

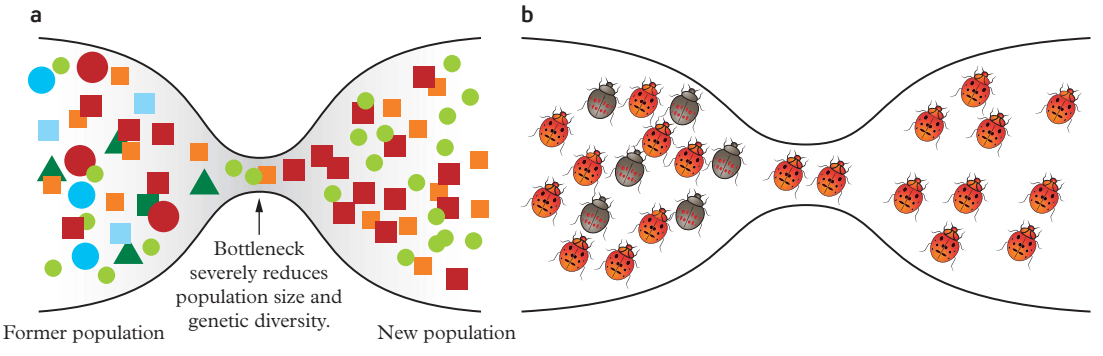

FIGURE 11 The bottleneck effect **a** showing the effect on alleles and **b** showing the effect on phenotype.

It is thought that the population of Tasmanian devils underwent a population bottleneck millions of years ago. Although the size of the population was able to recover, only a limited variety of alleles remain. This means that most of the current devil population have similar self-markers on the surface of their somatic cells. When a cancer cell develops in the mouth of one devil, it is able to spread to other devils by breaking off during the biting that occurs during mating. Because the markers on the body cells of all devils are similar, the immune system cannot recognise the foreign cancer cell, resulting in the spread of the disease (known as devil facial tumour disease).

FIGURE 12 Tasmanian devil facial tumour disease affects the devil's ability to eat, ultimately causing death.

WORKED EXAMPLE 11.1

CALCULATING ALLELE FREQUENCY

In a population of 50 organisms, 35 individuals have two copies of allele A, 5 individuals have two copies of allele B, and 10 individuals have a copy of both allele A and B. Calculate the allele frequencies of A and B.

SOLUTION

50 individuals have 100 alleles in the gene pool.

35 individuals have 2 × allele A

$$2 \times 35 = 70 \text{ allele A}$$

10 individuals have 1 × allele A

$$1 \times 10 = 10 \text{ allele A}$$

$$\text{Total number of allele A} = 70 + 10$$

$$= 80 \text{ allele A}$$

$$\text{Allele frequency (allele A)} = \frac{80}{100}$$

$$= 0.8$$

$$\text{If total allele frequency} = \text{allele A} + \text{allele B}$$

$$= 1$$

$$\text{Allele frequency (allele B)} = 1 - \text{allele A}$$

$$= 1 - 0.8$$

$$= 0.2$$

Worked example 11.1 Calculating allele frequency

CHECK YOUR LEARNING 11.1

Describe and explain

1 In your own words, describe how new variation can arise in a population.
2 Describe the difference between point mutations and chromosomal mutations.
3 Explain what is meant by the term 'genetic drift' using an example.

Apply, analyse and compare

4 In a turtle population, there are 180 individuals. Forty-two turtles are heterozygous for the speckled shell pattern (one allele for the speckled pattern and one allele for the striped pattern) and the other 138 have the striped shell pattern that requires two alleles from the striped pattern. Calculate the allele frequency for the striped allele.

Design and discuss

5 Discuss the significance of different block mutations that alter the expression of phenotypes in a population.
6 Explain the significance of a silent gene mutation to a gene pool.

11.2 Selective breeding

KEY IDEAS

In this topic, you will learn that:

+ selective breeding programs can manipulate gene pools.

Throughout history, humans have manipulated gene pools by artificially selecting specific individuals (i.e. plants or animals) with desirable phenotypes and breeding them to produce offspring with those desirable traits. Humans, in this case, act as the selection pressure within the population, changing the allele frequencies of particular genes within the gene pool of a population and consequently altering the evolution of a species.

Selective breeding of phenotypes

selective breeding when humans select organisms based on particular traits and interbreed them to increase the desired phenotype in the population

artificial selection another term used to describe selective breeding

Selective breeding involves selecting parents with a desirable phenotypic trait and interbreeding them to produce the maximum possible number of offspring with that same desirable trait. Continual interbreeding of individuals with a particular characteristic leads to an increase in allele frequency of those phenotypic traits within the gene pool. The population has undergone **artificial selection** by selective breeding by humans. This is an advantage to agricultural industries because they can maximise productivity and therefore increase profit.

Currently, agricultural industries use a combination of desirable traits that are selected for rather than a single characteristic. For example, in wheat crops, individual plants would be selected for based on their height, straw strength, disease and drought resistance as well as yield and bread-making qualities. This is much more difficult than selecting a single trait and can take many years to produce a population of individuals with all the required characteristics.

FIGURE 1 Wheat may be selected based on height, straw strength, disease and drought resistance or yield and bread-making qualities.

Selective breeding is much faster than natural selection. Both result in gradual changes; however, selective breeding by humans provides a constant pressure in a particular direction, while natural selection can vary as the environment varies.

Selective breeding of mutations

Sometimes a desirable trait in an individual may be the result of a harmful gene mutation. Selective breeding can increase the allele frequency of a mutated gene that would otherwise be lost from a gene pool via natural selection.

Belgian Blue is a beef cattle breed that has a gene mutation that prevents the production of myostatin – a protein that regulates muscle development. The absence of myostatin leads to a condition known as double muscling, whereby Belgian Blues have excess lean muscle production. This also leads to other harmful conditions such as heart defects and difficulty giving birth. Cattle farmers have selected the Belgian Blue for this desirable trait, enabling them to produce large quantities of lean meat in a relatively short amount of time. Because of selective breeding, this harmful allele has increased within populations around the world.

Disadvantages of selective breeding

Often one gene is linked to many other genes on a chromosome and these are inherited together. Selective breeding for one trait can often result in less desirable features also being increased in a population over time. For example, purebred dogs often have detrimental traits, such as hip dysplasia in labradors and German shepherds, or deafness in dalmatians, due to selective breeding. These undesirable characteristics have been present in individuals with desirable traits and therefore been passed onto offspring. These detrimental traits are often seen in domesticated breeds yet are rare in wild dog populations.

FIGURE 2 Excess muscle production in selectively bred Belgian Blue cattle is a result of a mutation that prevents muscle production being regulated.

Selective breeding reduces variation between individuals of a population, increasing susceptibility to diseases. Lower genetic variation reduces the ability of a population to persist when selection pressures within the environment change.

This type of breeding can often reduce biodiversity since artificially selected organisms replace varieties in the wild. This is because the human-selected breeds are often more vigorous, reaching reproductive age more rapidly, and can have traits such as drought resistance, enabling them to have higher survival rates. This could have detrimental effects on ecosystems because if one species is removed from a food chain it will impact all other species in the chain and ultimately an entire food web.

FIGURE 3 Purebred English bulldogs are prone to a range of health issues including skin allergies, and mobility and breathing issues related to their short face and snout.

Selective breeding in animals

Animals have been selectively bred by humans to improve the performance of livestock since ancient times when animals were first domesticated. For example, Darwin interbred pigeons while developing his theory of natural selection. He kept records and observed how selective breeding could produce new pigeon breeds. Animals are selectively bred for companionship, meat quality, yield and other animal products such as wool, eggs and milk.

Some traits are not entirely due to genetics alone and partially due to the environment. Desirable traits are often interbred over a few generations before the level of heritability is identified. For example, variation in milk production in dairy cattle is approximately 30% genetic variation, while all other variation is due to environmental factors such as feed quality and quantity, animal care, and weather.

It is often difficult for breeders to source individuals that contain every desirable trait required, because some traits are recessive and rarely appear in the offspring. For example, dominant traits such as absence of leg and head fur in sheep is an easily identifiable trait; however, an absence of stomach fur is recessive and difficult to find in combination with other desirable traits in these individuals.

CHALLENGE 11.2

Selective breeding of pets

Adopting a puppy from a breeder is still a common method of acquiring a new family pet. Many people search for breeds that do not shed fur, such as the poodle. Others search for the breed that matches their childhood dog.

While this idea is a nice thought, many pets are purchased from large-scale breeders who continue to breed animals with long-term health defects.

1 Discuss the ethical issues around purchasing your next pet from a breeder. Make sure to include the pros and cons of your argument and your final conclusion.

Selective breeding in plants

Selective breeding in plants is primarily carried out to produce food crops of high quality and yield. Seeds are collected from individual plants with particular characteristics such as large fruit size, resistance to disease or high yield. In agriculture, superior strains of corn, wheat and soybeans have resulted from careful breeding. The species *Brassica oleracea* includes many varieties such as cabbage, broccoli, cauliflower, Brussels sprouts, collards and kale – these were all selectively bred from an original wild type.

Ancient farmers began selectively breeding an ancient corn, teosinte (*Euchlaena mexicana*), more than 6000 years ago by selecting the largest number of corn kernels (seeds) and kernel rows to plant for the next year's crops. Over time, the gene pool contained a higher frequency of these favoured alleles. Genetic diversity in corn has decreased over time because kernels have been selected for by these qualities alone. These desirable phenotypes are often linked to other genes (e.g. the alleles for both kernel colour and size are located on the same chromosome). A farmer may select a kernel that is the desirable size, but with that particular trait may also come a negative trait such as susceptibility to disease or infertility.

FIGURE 4 **a** Corn was selectively bred from **b** teosinte more than 6000 years ago.

Study tip

Questions are often asked on selective breeding in relation to mutations or as comparisons to natural selection. Changes in allele frequencies should always be mentioned in your responses to such questions.

CASE STUDY 11.2

A2 milk production

Milk is a mixture of water, proteins, sugars, fats and minerals. There are six main proteins in cows' milk, one of which is called beta-casein (β-casein) and makes up approximately 30% of the protein content in cows' milk. There are two alleles of the β-casein gene: A1 β-casein and A2 β-casein. This is due to a single nucleotide difference in the DNA sequence of the β-casein gene. This gives them a slightly different protein structure due to a change in a single amino acid. Any cow will have one of three genotypes for beta-casein: A1A1, A1A2 or A2A2.

Researchers believe that A1 β-casein was the result of a substitution mutation that occurred when cattle were first domesticated. Some studies have suggested that when susceptible individuals drink milk without the A1 protein they may suffer less from bloating and indigestion. Those who are not susceptible will not be affected.

Farmers have selectively bred cows that only produce the A2 milk protein to meet the human demand for A2 milk. By interbreeding cattle that only produce the A2 β-casein alleles, entire populations of cattle have been produced that contain only the A2 β-casein allele.

FIGURE 5 Gene mutation of β-casein

CHECK YOUR LEARNING 11.2

Describe and explain

1 Define the term 'artificial selection'.
2 Describe how a desirable trait can be increased in a population by selective breeding.
3 Explain why genetic conditions such as overbite in boxer dogs are often more common in populations that have been selectively bred than in wild populations.

Apply, analyse and compare

4 Compare and contrast the process of artificial selection with natural selection.
5 Apply your understanding of selective breeding to explain how this process can lead to reduced variation over time.

Design and discuss

6 Discuss how advances in biotechnology have changed how selective breeding is carried out now compared to ancient times.
7 The CSIRO Centre for Plant Biodiversity Research is involved in collecting indigenous wild plant varieties. They are screened for particular genes that may have been lost from selectively bred modern varieties. Suggest why this research is essential to Australian plant conservation and the culture of Aboriginal and Torres Strait Islander Peoples.

11.3 Consequences of overusing antibiotics

KEY IDEAS

In this topic, you will learn that:

- overuse of antibiotics can lead to natural selection of resistance in bacteria
- viruses such as influenza mutate regularly, resulting in antigenic drift
- recombination of viral genetic material can lead to antigenic shift and the spread of viruses to new species.

Like multicellular organisms, single-celled bacteria are subject to the selection pressures of their environment. In any population of bacteria, there will be some variation due to DNA mutation. This may mean that some bacteria will be more suited to their environment than others. The bacteria that are more suited to their environment will survive and reproduce, passing on their genetic information to their offspring. As a result, the next generation of bacteria will carry the same physical characteristics and be able to thrive in their environment. In this way, bacteria can develop resistance to antibiotics.

Antibiotics

The first antibiotic (penicillin) was discovered by Alexander Fleming in 1928 and purified by Ernest Chain, Howard Florey and Normal Heatley in 1940. Penicillin is a molecule produced by a group of fungi (genus *Penicillium*) that prevents molecules in the bacterial cell wall from linking together. This means the bacteria are unable to repair any cell wall damage, grow or reproduce. As a result, the bacterial cells will die.

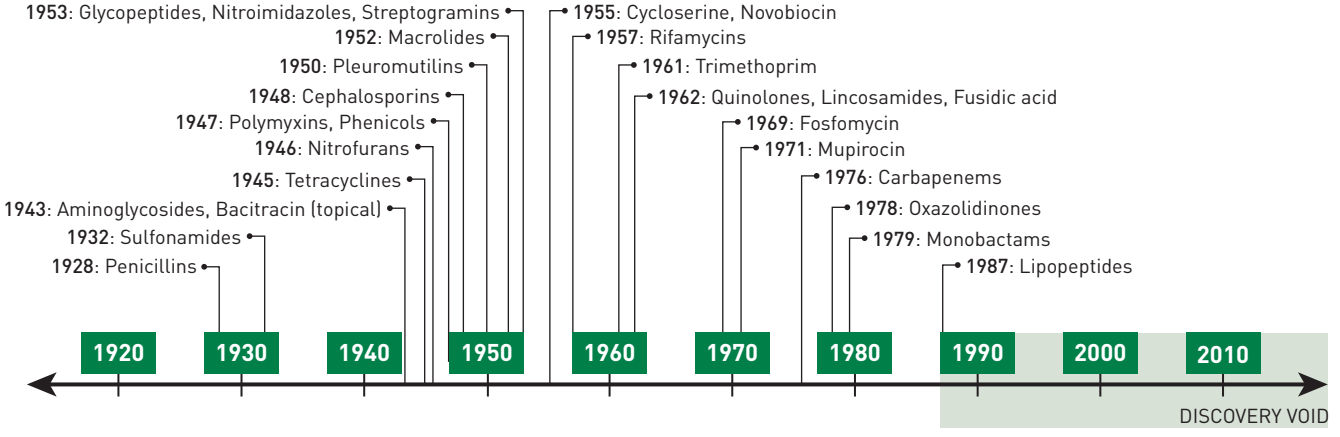

FIGURE 1 A timeline for the discovery of antibiotics. The last antibiotic was discovered in 1987.

Antibiotics are grouped according to the way in which they affect bacterial growth. These include:

- inhibition of protein synthesis
- inhibition of DNA synthesis
- inhibition of cell wall repair and synthesis
- disruption of the bacterial membrane function
- inhibition of bacterial enzymes.

Antibiotic resistance

antibiotic resistance when bacteria develop the ability to survive in the presence of an antibiotic

Antibiotic resistance may be inherent in some bacteria due to the structure of their cell. For example, Gram-negative bacteria have two cell membranes either side of the cell wall. This outer membrane prevents some antibiotics such as penicillin from entering the cell.

Video
Antibiotic resistance

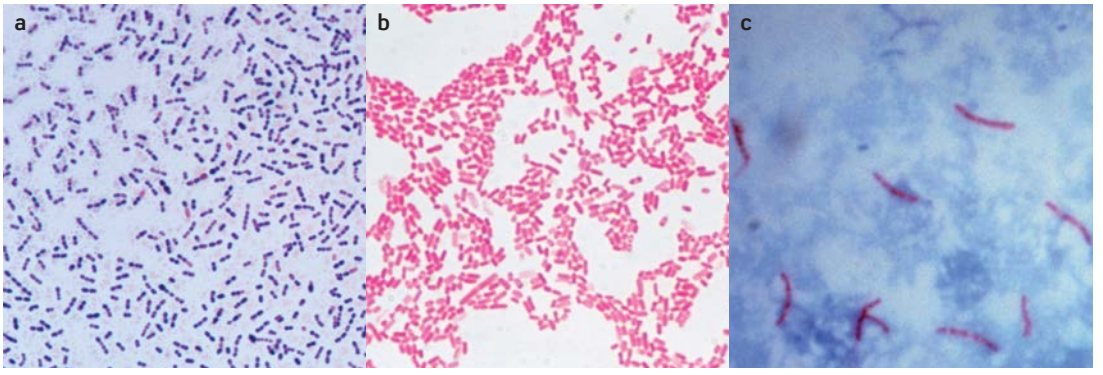

FIGURE 2 **a** The cell wall of Gram-positive bacteria is able to bind to the purple crystal violet dye in the Gram staining process; **b** and **c** Gram-negative bacteria do not retain the purple dye and are stained pink.

Bacterial cells can also acquire resistance through random mutation. These mutations occur naturally and can result in a modified or new gene that allows a single bacterial cell to survive long enough to reproduce in the presence of the antibiotic. The offspring of this bacterial cell will also be able to reproduce, increasing the population. Further mutations in the rapidly reproducing organism will eventually lead to full resistance to the antibiotic.

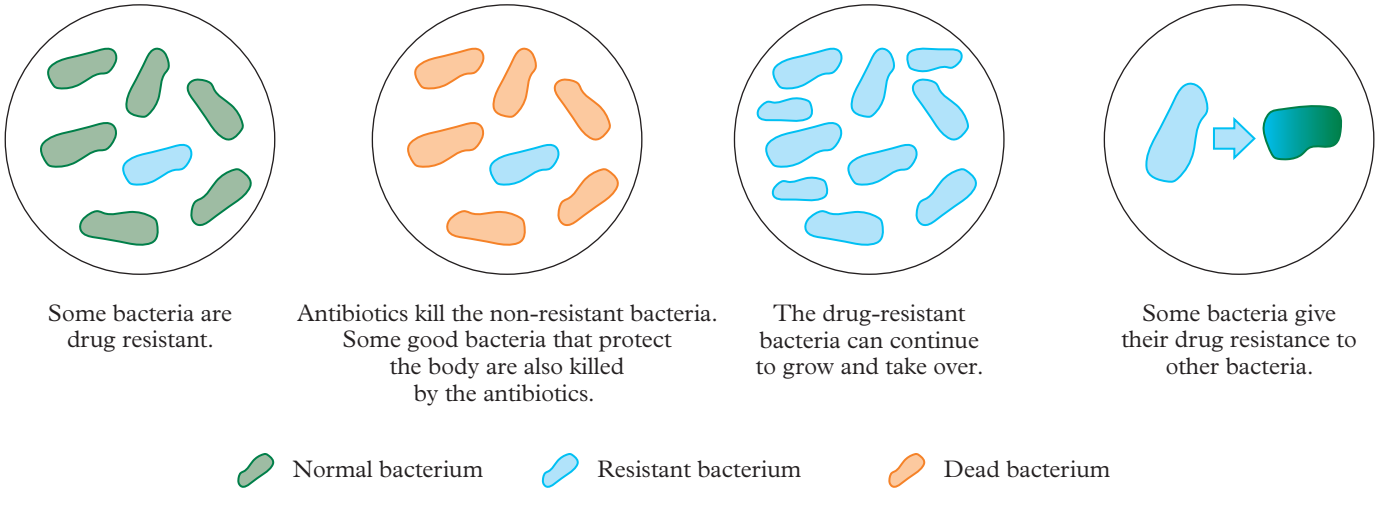

FIGURE 3 Some bacteria may become resistant to antibiotics.

The way the bacterial cells resist the antibiotic will vary according to the type of mutation the bacteria have. Some bacterial cells will modify the proteins in the cell wall to prevent the entry of the antibiotic, while others will actively pump it out of the cell. Some cells will use enzymes to modify the antibiotic, or modify the shape of the protein that the antibiotic binds to.

Passing on resistance

Natural selection allows bacteria to pass on the ability to resist antibiotics to their daughter cells (vertical gene transfer). This is not the only means bacterial cells can use to transfer antibiotic resistance. Horizontal or lateral gene transfer involves the gene for antibiotic resistance being passed between bacterial cells that are not parent/daughter cells. This occurs when a plasmid carrying the resistance gene is transferred between cells through direct contact (conjugation), via a virus, or when a cell ruptures and the DNA is taken up by a neighbouring bacterial cell.

Consequences of resistance

The use of antibiotics to treat common infections means that people no longer die from an infected scratch. Elective surgeries are now possible, such as joint replacements, open heart surgery and the delivery of babies by caesarean. Unfortunately, as levels of antibiotic-resistant bacteria increase, the ability to fight bacterial infection decreases. An increase in antibiotic-resistant bacteria could result in some elective surgeries becoming too risky to attempt.

CASE STUDY 11.3

Antimicrobial resistance in the food chain

With bacteria evolving to become resistant to an increasing number of antibiotics, the last line of defence is vancomycin. Doctors rarely use this antibiotic since it is one of the few that is still effective against Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or 'golden staph'.

In contrast, a vancomycin-like glycoprotein called avoparcin has been used to promote animal growth and sprayed over fruit trees to prevent and treat infections in many emerging economies across the world. The prevalence in using the antibiotic-like molecules has led to the emergence of vancomycin-resistant enterococci (VRE) bacteria. These bacteria have been able to make their way into the intestines of the human population. When patients are given antibiotics for other illnesses, the VRE bacteria are selected for, while the other intestinal flora are killed. The high population of VRE can lead to an increased risk of infections in the bloodstream, urinary tract and abdomen.

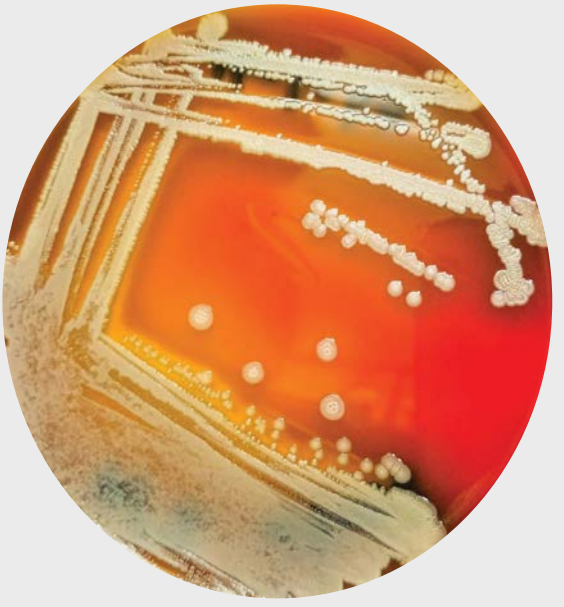

FIGURE 4 *Staphylococcus aureus*

In 2017, the World Health Organization provided guidelines suggesting that antibiotics should not be used as a preventative tool in the absence of disease in animals.

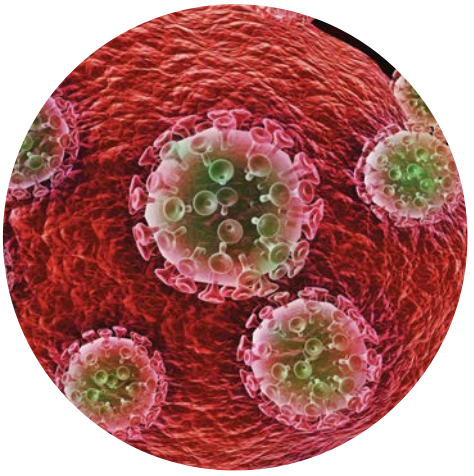

FIGURE 5 A virus must attach to a complementary molecule on its host cell before it can penetrate the cell and replicate itself.

Viral infections

Viruses are non-cellular pathogens that consist of a nucleic acid (either RNA or DNA) surrounded by a protein coat. They are not able to reproduce on their own. Instead, they must invade a living cell and use the ribosomes to reproduce the nucleic acid and proteins necessary for replication. There are five stages for this process.

1 **Attachment:** the viral particle binds to a complementary molecule on the cell's membrane.
2 **Penetration:** the nucleic acid penetrates through the membrane and enters the cell.
3 **Replication:** the viral particles are replicated.
4 **Assembly:** the viral particles are assembled into a new virus.
5 **Release:** the replicated viruses are released by the cell and may result in cell lysis. These new viruses can then go on to invade other cells in the host.

Each virus is specific to its host. This is due to the specificity of the attachment. Each type of virus is capable of binding to one or two complementary molecules on a specific host cell.

The host's immune system will often have difficulty detecting a virus since it will spend most of its time inside the host's cells. The most effective form of protection against a virus is the rapid production of antibodies by plasma cells. This may be a result of previous exposure (natural active immunity) or through vaccination (artificial active immunity). The antibodies are able to bind to the proteins on the surface of the virus, preventing it from attaching to the host's cells. If it cannot enter the cell, then it cannot replicate, and it will then be vulnerable to the phagocytic cells of the immune system.

Some viruses have evolved ways to change so that a host's immune system will no longer recognise it.

Antigenic drift

The influenza virus is able to regularly mutate its DNA so that two of its surface proteins (haemagglutinin (H) and neuraminidase (N)) change their structure. These proteins often act as antigens to a host's immune system. A change in the shape of one or more of these proteins can result in the previously produced antibodies not being able to bind. This means the host's immune system will not be activated, and the influenza virus will be able to enter the host's cells and replicate. These small regular mutations that result in gradual changes in the antigen particles on the virus is called **antigenic drift**. The small changes that occur from antigenic drift will usually produce viruses that are closely related to each other. Although the influenza virus may have only changed the structure of its surface molecules a small amount, your immune system may not recognise it, and you will develop the 'flu'. This is the reason a new influenza vaccination needs to be developed every year. Scientists constantly review how the virus changes over the previous season and try to predict its form in the coming year.

antigenic drift
small regular mutations that result in gradual changes in the antigen particles on a virus

Antigenic shift

Not all changes in a virus are gradual. **Antigenic shift** can occur when two different viruses inhabit the same host cell. The viral molecules of both are replicated in the cell; however, the assembly can result in a viral particle that contains nucleic acid and proteins from both viruses. This occurred in 2009 when the influenza A(H1N1)pdm09 'swine flu' contained genes from North American swine/pigs, Eurasian swine, humans, and bird viruses.

antigenic shift
a major change in the structure of a virus that can result from a reassortment of two different viruses in a single host cell

The genes from human flu allowed it to enter human cells, and to spread quickly through the population. This resulted in a pandemic.

Antigenic shift can also occur when a significant change in an animal virus allows it to invade human cells. This is one hypothesis for the development of the 2019–2020 COVID-19 (SARS-CoV-2) virus, when a bat virus is thought to have infected a pangolin, mixing with a similar coronavirus already in that population. This potential mixing of genes allowed the virus to change so that it could attach to a molecule on the surface of human lung cells. Because the virus was new to humans, few immune systems were able to recognise the virus and therefore kill it. Its ability to replicate in the lung cells meant it was able to transfer through coughing and sneezing. As a result, the virus was able to quickly spread throughout human populations, driving a global pandemic.

Study tip

If a virus undergoes an antigenic shift to invade a new species, it may no longer be able to attach to its original host species. This means the 2020 SARS-CoV-2 virus that caused the COVID-19 disease has difficulty infecting other animals.

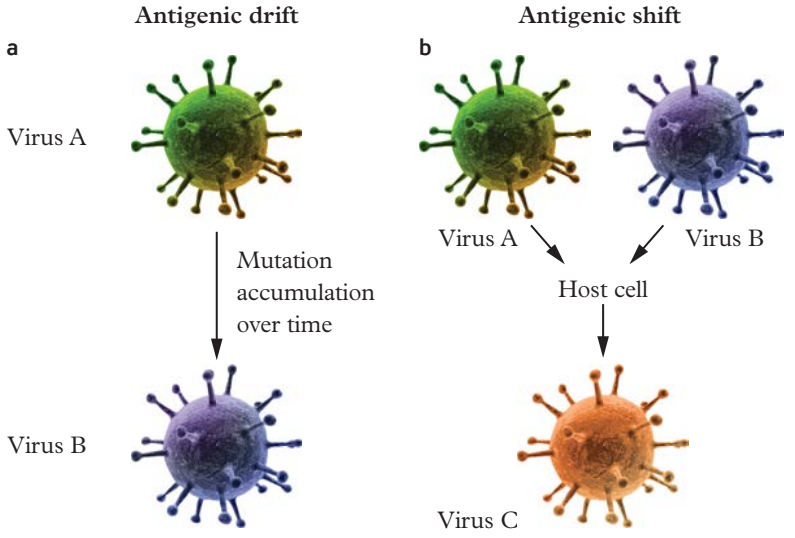

FIGURE 6 **a** Antigenic drift results from small regular mutations; **b** antigenic shift is often caused by a reassembly of viral molecules from two viruses.

CHECK YOUR LEARNING 11.3

Describe and explain

1 Define 'antibiotic resistance'.
2 Describe how a bacterial cell can evolve to have antibiotic resistance.
3 Explain four ways a bacterial cell can avoid being damaged by antibiotics.
4 Describe the five stages of viral replication.

Apply, analyse and compare

5 Compare antigenic shift and antigenic drift.
6 Suggest why a failure to follow instructions for taking antibiotics can contribute to antibiotic resistance.
7 Explain what is meant by host specificity.
8 Compare antibiotics and vaccinations as methods for treating pathogens.
9 Read Case study 11.3. Explain how spraying avoparcin on crops has led to the emergence of VRE in humans.

Design and discuss

10 Discuss why the World Health Organization (WHO) recommended that antibiotics should not be used in animal feed.
11 Medical tourism involves people travelling overseas to obtain cheap medical procedures. Explain how this could result in an increase in antibiotic resistance in Australia.

CHAPTER 11

Review

Chapter summary

11.1
- Mutations often become a new source of alleles that can increase variation within a population.
- Point mutations and block mutations cause changes in the allele frequencies in a population's gene pool.
- Natural selection changes a species gradually over time due to environmental selection pressures that act on particular phenotypes and changes the allele frequencies of genes over time.
- Increased gene flow leads to increased variation within a population as individuals exchange alleles between populations.
- Populations are also affected by chance events – such as the founder effect and bottleneck effect – that reduce populations to small sizes and which decrease the genetic diversity of the affected population.

11.2
- Humans manipulate the gene pools of plants and animals by carrying out selective breeding programs.

11.3
- The overuse of antibiotics can lead to natural selection of antibiotic resistance in bacteria.
- Antigenic drift is small regular mutations of a virus that results in gradual changes in its antigen particles.
- Recombination of viral genetic material can lead to antigenic shift and the mutation of viruses.
- The rate at which the genome of different pathogens changes leads to continual challenges in the treatment of human pathogens.

Revision questions

Multiple choice

1 Selective breeding:
- **A** decreases biodiversity.
- **B** increases resistance to environmental changes.
- **C** reduces genetic abnormalities in a population.
- **D** is another term for natural selection.

2 Antigenic shift is:
- **A** an example of genetic drift.
- **B** a type of gene mutation.
- **C** the reassortment of two viruses in a single host.
- **D** the duplication of sections of a chromosome.

3 Which of the following is a source of new variation within a population?
- **A** The founder effect
- **B** Mutation
- **C** The bottleneck effect
- **D** Natural selection

4 What type of mutation is shown in the DNA sequence below?

ATT CGC GGG AAA
↓
ATT CGC GG**T** AAA

- **A** A block mutation
- **B** A deletion mutation
- **C** A point mutation
- **D** A frameshift mutation

5 Natural selection in bacterial species of human pathogens often occurs as a result of:
- **A** antibiotic resistance.
- **B** antigenic shift.
- **C** antigenic drift.
- **D** reading-frame shifts.

6 Genetic isolation occurs because:
- **A** a small number of individuals become the founders of a new region.
- **B** a large population is reduced to a few individuals.
- **C** a catastrophic event reduces a population to a few random individuals.
- **D** two populations become isolated from each other and there is no gene flow.

7 For natural selection to occur, there needs to be:
- **A** unequal survival of individuals.
- **B** reproduction.
- **C** variation within the population.
- **D** all of the above.

8 The predator and prey relationship is an example of what type of mechanism that affects allele frequencies?
- **A** Selection pressure
- **B** Genetic drift
- **C** Natural selection
- **D** Selective breeding

9 The radiation released from the Chernobyl nuclear power plant accident could have caused what type of change in allele frequencies in humans?
- **A** Genetic mutation
- **B** The founder effect
- **C** Gene flow
- **D** The bottleneck effect

10 The Tasmanian devil's facial tumours are an example of what?
- **A** Frameshift mutation
- **B** Substitution mutation
- **C** The bottleneck effect
- **D** The founder effect

11 Wild mustard has evolved into broccoli, Brussels sprouts and cauliflower.

FIGURE 1 **a** Wild mustard; **b** broccoli

This is an example of:

A artificial selection.

B antibiotic resistance.

C point mutation.

D gene flow.

Short answer

Describe and explain

12 Name one type of genetic mutation and how it works.

13 Explain why the World Health Organization (WHO) has suggested that antibiotics should not be used as a preventative tool.

14 Humans are diploid organisms. Explain what diploid means.

15 Explain what it means if the allele frequency equals 1.0 or 100%.

16 To be in genetic equilibrium, a population needs to satisfy five conditions. Describe these five conditions and explain why they are hard to achieve.

17 Define the term 'selection pressure'.

18 Explain how selective breeding can manipulate gene pools.

19 Give an example of gene flow in plants and explain how this can increase genetic variation in a population.

20 Explain, using examples, how the environment contributes to the phenotype of an organism.

21 Explain a biological consequence of decreased allele diversity in a population.

Apply, analyse and compare

22 In a kitten population, there are 100 individuals. Twelve kittens are heterozygous for the striped fur pattern (one allele for the striped pattern and one allele for the patched pattern) and the other 88 have the patched fur pattern that requires two alleles from the striped pattern. Calculate the allele frequency for the striped allele.

23 Use a Venn diagram to compare and contrast the two types of genetic drift.

24 Suggest why phenotypic variation is important for natural selection.

25 Suggest why mutations that alter the sequence of amino acids are usually harmful.

26 Contrast natural selection and artifical selection.

27 Analyse the point mutation shown in the nucleotide sequence below. Discuss how this specific type of mutation affects protein production and compare this to other types of point mutations.

AAC GGC GGT TCT TAG

↓

AAG GG**G** CGG TTC TTA G

28 Explain why antibiotics can be effective in the treatment of bacteria but not viruses.

29 Draw diagrams to demonstrate the different types of block mutations that can occur and compare how each of these affects phenotypic expression in an individual.

30 The graph in Figure 2 shows the population of a species of bird before and after a bottleneck event.

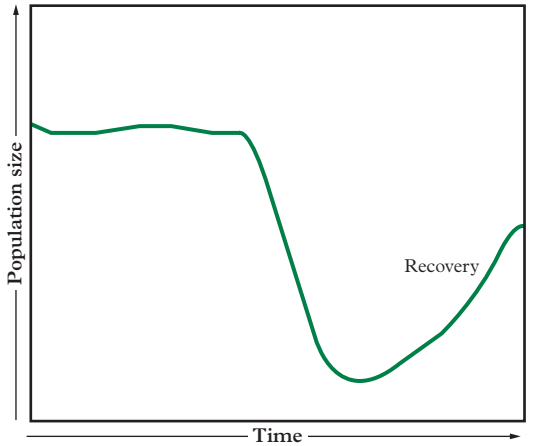

FIGURE 2 Graph of bird population

a Identify the point on the graph at which the bottleneck event occurs.

b Explain what has occurred to the allele frequencies in the population after the bottleneck event occurs.

c Explain how the population size is able to recover after the bottleneck event.

Design and discuss

31 New mutations that arise in geographically isolated populations are important sources of variation for the process of natural selection. Discuss how different types of mutations could be of benefit to evolving populations.

32 Humans are continually producing vaccinations for viruses such as influenza. Discuss why the same influenza vaccination is unable to be used year after year and why it is essential that these vaccines are produced rapidly.

33 The blowfly (*Lucilia cuprina*) lays eggs in the wounds of sheep. The larvae that hatch from these eggs burrow into the sheep's skin and can cause harm, reduced wool production and sometimes death. Farmers used pesticides to stop blowflies from laying eggs in their sheep, though the blowfly became resistant and these chemicals became less effective over time.

FIGURE 3 The Australian sheep blowfly *Lucilia cuprina*

Discuss how the process of natural selection can lead to blowfly populations that are resistant to these pesticides.

34 Figure 4 shows changes in allele frequencies of a particular characteristic within a population of mountain pygmy possum (*Burramys parvus*) over one generation. The population was reduced to a few surviving individuals because of bushfires and habitat loss due to land clearing around Mt Buller, Victoria.

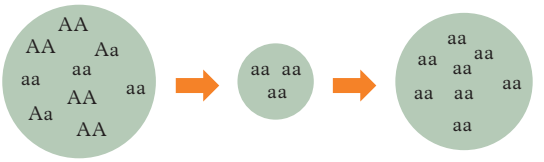

FIGURE 4 Mountain pygmy possum population

Discuss how catastrophic events can lead to changes in allele frequencies and reduce genetic variation in a population over time.

35 Whooping cough is a bacterial respiratory disease. There is a high vaccination rate for whooping cough in Australia. Suggest a reason why whooping cough rates are increasing in Australia, despite the high vaccination rate.

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Exam essentials

Responding to questions

In your exam, you may be expected to explain a process.

Explain the steps in a process

When describing a process (or how an event occurs) it is important to include all the steps. A good general rule is to write the first step and the final step from the question, and then at least two steps in-between. Each step should refer to the information in the stem of the question.

The following question is taken from the 2017 VCE Biology Examination. Read the question carefully, then consider whether enough steps are used to explain the process in the responses.

QUESTION 5b (2017 VCE Biology Written examination)

The rufous bristlebird (*Dasyornis broadbenti*) is a ground-dwelling songbird. The rufous bristlebird is found in gardens near thick, natural vegetation and builds nests in shrubs close to the ground. The rufous bristlebird feeds on ground-dwelling invertebrates. It is a weak flyer and is slow to go back to areas from which it has been previously eliminated. Two distinct populations of rufous bristlebird exist in Victoria. The distribution of each population is shown on the map of Victoria below. The distance between Population A and Population B is over 200 km.

Source: *Flora & Fauna Guarantee Action Statement*, 1993, no. 49; © The State of Victoria, Department of Sustainability and Environment, 2003

Both of the rufous bristlebird populations in Victoria are small.

b Referring to the theory of natural selection, explain why the rufous bristlebird is at risk of extinction. 3 marks

Source: 2017 Biology Written Examination Question 5b, Short answer, reproduced by permission © VCAA

Response 1

Small population size means a limited gene pool for the birds. Inbreeding can make all members vulnerable to the same diseases. If there was a change in the environment, this could lead to new disease for the birds. Sick ground-dwelling birds will die or be killed by predators. If one bird is affected, they will all be affected, making them at risk of extinction (permanent loss of all members of species).

New introduced selection pressure.

This is the starting information.

Information relevant to question and linked to natural selection.

Final step from stem of question, including definition of extinction.

This response would receive 3 marks since it links the small population size to a limited gene pool and outlines each step in the process.

Response 2

Small populations are more likely to die because they do not have much variation. Natural selection says that some animals survive and some die. Those that live are able to pass on the genes to the next generation. Those that die can become extinct.

Individuals do not become extinct.

General information is not modified to include information about the question.

Does not include an example of a new selection pressure.

This response would only receive 1 mark since it links small population size to a lack of variation.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 7c (2018 Biology Written Examination)

Populations of the lizard species *Anolis sagrei* are found on the many islands of the Bahamas. There is natural variation between the phenotypes of individuals within each population.

In 2004 a hurricane killed all populations of *A. sagrei* lizards on seven of the smaller islands. Scientists randomly chose seven males and seven females from a remaining population on a large island. They introduced one male and one female to each of the seven smaller islands. Over the next three years, the scientists noted that the size of the populations increased on each of the seven smaller islands. The scientists measured the genetic diversity within each of the populations and found there was lower genetic diversity in each new population compared with the population on the large island.

c The scientists noted that after three years there was a significant decrease in the average length of the hind legs of the lizards living on the smaller islands compared with those on the large island.

Explain what may have happened on the smaller islands to produce this decrease in the average length of hind legs. 2 marks

The lizards didn't need to use their hind legs to survive and so passed their short leg trait on to the next generation.

Source: 2018 Biology Written Examination Question 7c, Short answer, reproduced by permission © VCAA

Marking guide

Question 5 a	- 1 mark for describing a selection pressure that may have provided an advantage to having shorter legs. - 1 mark for describing how surviving short-legged lizards passed their traits onto the next generation.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own response to the same question to receive full marks from an examiner.

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CHAPTER 12

Evolving species

Evolution is the process of organisms developing new characteristics from their ancestors over time. Evidence of evolution is observed through fossils – the preserved remains, impression or trace of once living organisms – and through the fossil record, showing the succession of life forms on Earth over millions of years. The fossil record is just one form of evidence that supports the divergence of a single ancestral species into different species. This divergence can result from a population being separated by a permanent barrier, or by the availability of a new niche in the environment. New mutations and selection pressures can contribute to the reproductive isolation of a population so that it becomes a separate species.

KEY KNOWLEDGE

→ changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transition fossils, relative and absolute dating of fossils

→ 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

Source: *VCE Biology Study Design (2021–2025) reproduced by permission © VCAA*

FIGURE 1 Fossils do not need to be the bones and teeth of past species; they can also be impressions of species left behind. This image shows three-toed dinosaur footprints at Gantheaume Point in Broome.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

12A What geographical barrier could prevent two populations from mating with each other?

12A Groundwork resource
Evidence for evolution

12B Use natural selection to explain how traffic noises may cause changes in the mating call of a bird.

12B Groundwork resource
Natural selection

12C How long ago was Australia joined with any other landmass?

12C Groundwork resource
Continental drift

PRACTICALS

NO-TECH PRACTICAL	**12.1** Absolute age

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

12.1 Species change over geological time

KEY IDEAS

In this topic, you will learn that:

- fossils are evidence of species change over geological time.
- the geological timescale provides the sequence of events in Earth's history.
- relative dating and absolute dating are two methods of dating fossils.

The continual change in allele frequencies that occurs as a result of changing selection pressures (as discussed in Chapter 11) can result in phenotypic variations in a population. In time, these phenotypic variations can result in different populations no longer being able to reproduce with each other. This reproductive isolation is the key determining factor in the two populations becoming different species. These sorts of changes have been occurring since the beginning of life on Earth.

There is a large body of evidence that supports changes in species over geological time. This includes studies of living species with similar features, fossil evidence and dating techniques. Although comparisons of the structural and biochemical features of living organisms can only give a glimpse of shared common ancestors, it is supported by the fossil record and dating techniques that are evidence of the **geological timescale**.

geological timescale
the sequence of events in Earth's history based on the geological rock record

Fossils

The term **fossil** is a broad umbrella term that covers the preserved remains of organisms or traces of organisms such as footprints, coprolites (fossilised faeces) or other impressions.

fossil
the preserved remains, impression or trace of a once-living organism

Since few fossils can survive the high temperatures at which igneous and metamorphic rocks form, almost all fossils are found in sedimentary rock. The structures that are most likely to remain as fossils are 'hard' shells, bones, teeth, woody tissues and leaves. Soft tissues decay quickly and rarely fossilise. In all cases, the formation of fossils requires:

- quick burial
- a lack of oxygen
- a lack of disruption to the remains.

This is why fossils are such a rare occurrence.

FIGURE 1 A fossil impression of a leaf. The leaf itself is not preserved, just an imprint of the leaf on the rock surface.

Fossils originate when organisms become encased in sand or mud sediments, usually at the bottoms of seas, lakes or marshes. New layers of sediment build up to cover the older layers, sealing the traces or remains from rapid decay due to bacteria. Dissolved minerals wash through the fossils and are left behind in the gaps between tissue. The hardened minerals eventually form the rock-like structure of some fossils. At a much later time, the sedimentary rocks covering the fossils may become exposed and be susceptible to erosion, revealing the preserved fossils.

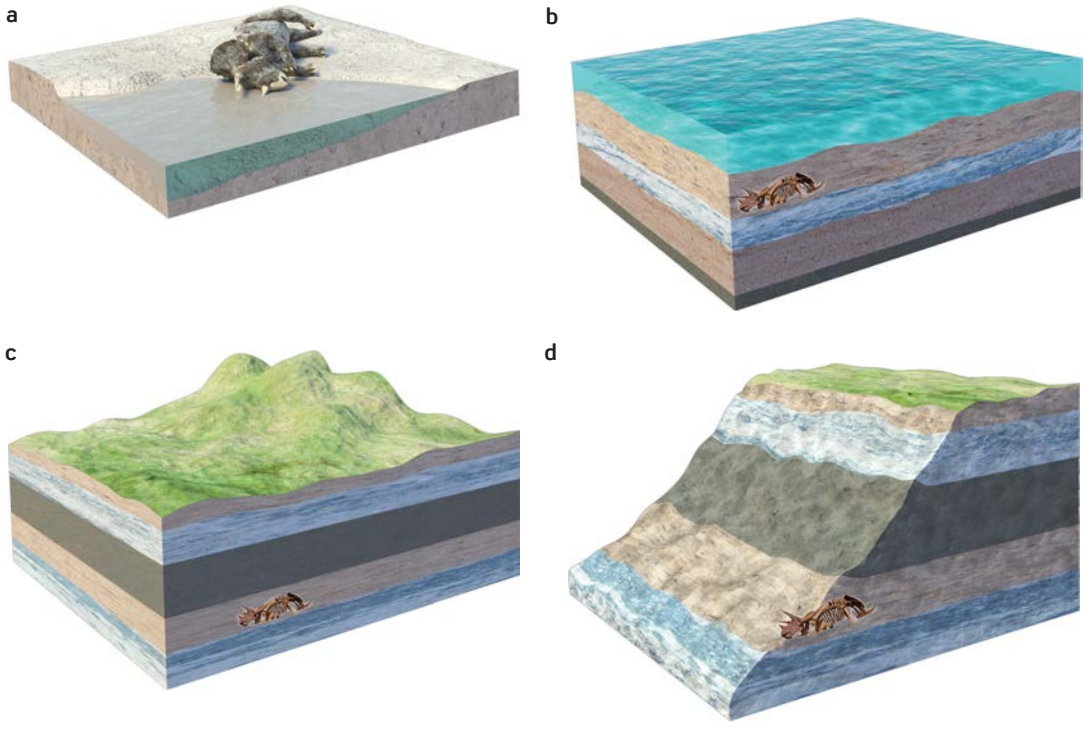

FIGURE 2 The formation of a fossil: **a** an organism dies and is **b** buried under fine-grained sediment and water. **c** Over millions of years more sediment covers the fossil and minerals start to preserve the fossil. **d** The fossil is exposed through erosion and uplift.

Fossil record and dating techniques

The **fossil record** refers to the history of life on Earth documented in fossils. It shows that there has been a succession of different life forms on Earth over millions of years. The law of **faunal (fossil) succession** says that the types of organisms found in the different layers of rocks are in a consistent order. Over time, new fossils form on top of the old fossils. This means that the deeper the fossils are found in the Earth, the older the fossil is thought to be.

The law of faunal succession can be extended to determine the **relative age** of the fossil. If the same fossil is found in rocks from different places, it can be assumed that the organism from which the fossil came from lived at the same time, and therefore that the rock is the same age. This process is called **relative dating**.

Absolute dating is a dating technique that uses the rate of isotope decay to determine the age of the fossil.

Some fossils show characteristics of both an ancestral species and more recent species. These fossils are classified as **transitional fossils**. Transitional fossils can be said to offer a 'missing link', providing evidence of species progression over time.

There are many examples of transitional fossils in the fossil record. One of the most common examples is the fossil of *Archaeopteryx*, which has characteristics of both reptiles and modern birds. With a blend of both avian features (feathers, wings, wishbone and reduced fingers) and reptile features (a complete set of teeth, flat sternum, long bony tail and three claws on the wing), *Archaeopteryx* has been dated to about 150 million years ago in the late Jurassic period.

fossil record
the record of organisms over geological time as inferred from fossil evidence

faunal (fossil) succession
the observation that fossils found in sedimentary rock strata succeed each other vertically in an orderly manner

relative age
the age of a rock determined by the ages of surrounding rocks, events and organisms; this is an estimation age or age range

relative dating
a technique to determine the geological age of a fossil or rock strata, relative to other organisms, rocks, features or events without expressing absolute age

absolute dating
a technique to determine the age of a fossil based on the decay of radioactive isotopes

transitional fossil
a fossil that exhibits traits that are common to both an ancestral group and its descendent group

Video
Relative and absolute dating

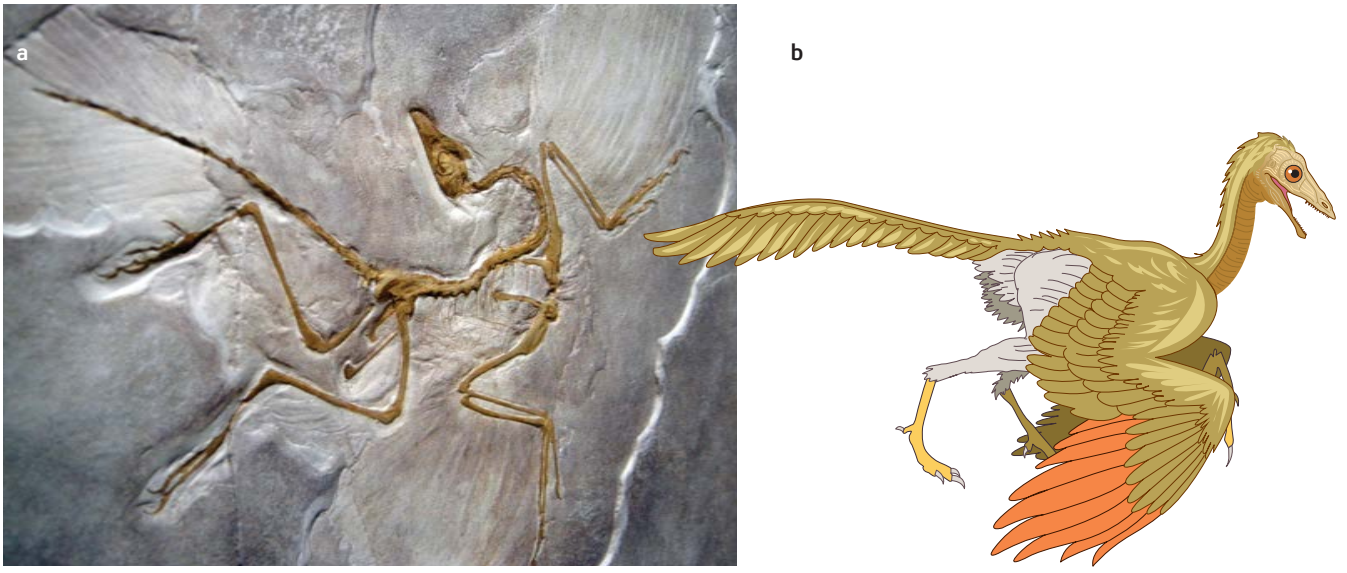

FIGURE 3 **a** A fossil of *Archaeopteryx* and **b** a 3D rendering, showing both avian and reptile characteristics

Relative dating

strata
the layers of sedimentary rock

Fossils that are found in ancient, deeper **strata** tend to have simpler structures than younger, upper strata fossils. Each strata layer of rock has unique fossil groupings. Fossils from deeper, older strata contain similar types of organisms across the world. Relative dating is a dating technique that uses the understanding that fossils found in deep layers of strata are older than those from more recent layers of rock.

index fossil
a distinctive, abundant fossil with a wide geographic distribution over a relatively short geological period of time

Occasionally, some fossilised species completely disappear from the upper levels of rock. As a result, palaeontologists devised methods of identifying different rock layers that contain similar fossil forms; the fossils in these rocks are defined as **index fossils**. Rock sections with index fossils have a lower boundary and an upper boundary, where the index fossil first and last appeared in the rock record. The order of geological events is able to be determined using the lower and upper boundaries of rock strata.

For example, trilobites were the first hard-shelled invertebrates and were constantly evolving to inhabit new areas for around 270 million years. Some species lived in sediment, while others crawled or swam in open water. The formation of large ice sheets over the ocean caused most trilobite species to die within a relatively short geographical time period. This means trilobite fossils can be found in rock aged between 540 million and 252 million years old.

FIGURE 4 Fossilised remains of a trilobite, the first hard-shelled invertebrate on Earth

Study tip

Remember that there are three different types of rock:
- igneous
- sedimentary
- metamorphic.

Igneous rocks are formed through the cooling of magma. Sedimentary rocks are formed from sediment due to of weathering and erosion, and metamorphic rocks are igneous or sedimentary rocks altered through temperature and pressure.

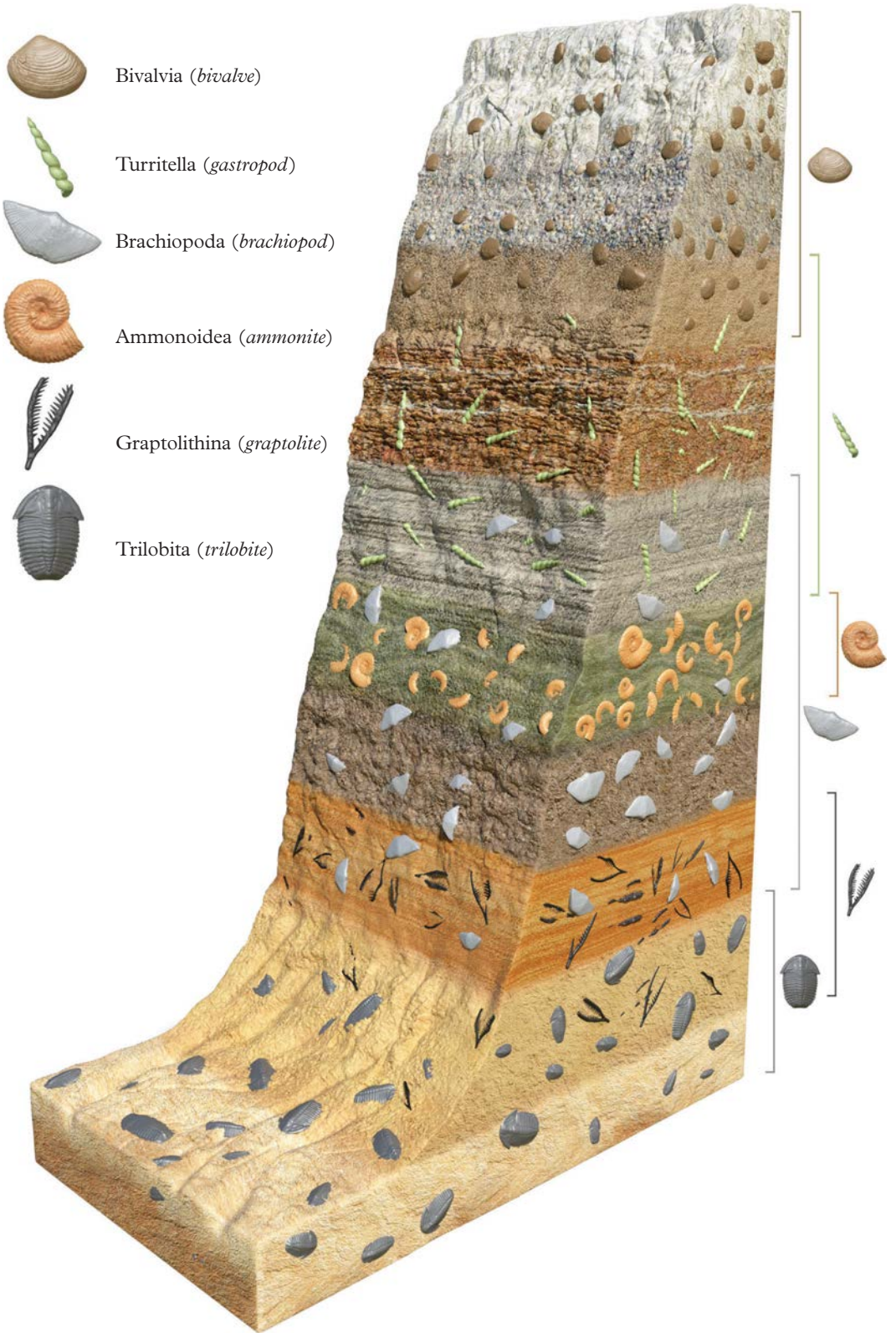

FIGURE 5 The law of fossil succession. Each fossil has a limited range in a succession of strata. Widespread fossils with a short range are index fossils.

Absolute dating

isotope
variations of an element that differ in the number of neutrons within their nuclei; many isotopes are radioactive forms of an element

The atmosphere surrounding Earth is constantly being bombarded by cosmic rays. This causes some atoms to form **isotopes** (i.e. elements that have a different number of neutrons in the nuclei to their standard amount). Although they have the same atomic number (i.e. number of protons), their atomic masses differ (i.e. protons and neutrons). Some isotopes are radioactive and release energy (e.g. carbon-14), whereas others are stable (e.g. carbon-12). Living organisms absorb background levels of radioactive material, such as carbon-14, through uptake of gas and nutrients.

half-life
the time taken for a quantity of a radioactive isotope to decay to half of its initial value

While an organism is alive, the amount of radioactive isotopes present in their body remains constant. However, once an organism dies, the isotopes change to a more stable state (i.e. they are said to decay). This change decreases the amount of radioactive isotope. How fast this happens is called the rate of decay. The length of time it takes for half the remaining isotope to become stable is called the **half-life**. For example, 1 kg of carbon-14 will take 5730 years for half (0.5 kg) to become stable. After another 5730 years, half the remaining mass of carbon-14 (0.25 kg) becomes stable. This means after 11 460 years, only a quarter of the original radioactive carbon-14 will remain.

TABLE 1 The half-life of some elements used to date rocks

Radioactive element	Approximate half-life (years)	Product of decay	Appropriate age of fossil for dating (years)
Carbon-14	5730	Nitrogen-14	Less than 50 000
Uranium-235	0.7 billion	Lead-207	More than 50 000
Potassium-40	1.3 billion	Argon-40	More than 50 000
Uranium-238	4.5 billion	Lead-206	More than 50 000
Thorium-232	14 billion	Lead-208	More than 50 000

Video
Worked example 12.1: Absolute dating

WORKED EXAMPLE 12.1

ABSOLUTE DATING

Potassium-40 has a half-life of 1.25 billion years. In igneous rocks closely associated with a fossil layer, potassium-40 has a 1:3 ratio with its radioactive breakdown product, argon-40. What will the age of the fossils in the fossil layer be?

SOLUTION

1 Calculate the percentage of potassium-40 remaining (in this case, use the ratio given):

$$\%\ \text{potassium-40} = \frac{1}{4} \times 100 = 25\%$$

2 Calculate the number of half-lives by producing a flowchart, starting from the initial amount (100%) and halving each time until the end amount (25%):

$$100\% \rightarrow 50\% \rightarrow 25\%$$

Therefore, there have been two half-lives (determined by counting the number of arrows in the flowchart).

3 Calculate the age of the fossil by multiplying the half-life by the number of half-lives passed:

$$\text{Age of fossil} = \text{number of half-lives passed} \times \text{half-life}$$

$$\text{Age of fossil} = 2 \text{ half-lives} \times 1.25 \text{ billion years}$$

$$2 \text{ half-lives} \times 1.25 \text{ billion years} = 2.5 \text{ billion years}$$

The fossils in this fossil layer containing 25% potassium-40 will be approximately 2.5 billion years old.

Some **radioactive elements** have such a long half-life that they are not useful for dating very young rocks because their products of decay are too small to measure accurately. Similarly, elements like carbon-14 decay so rapidly that their quantities in old rocks and fossils (>50 000 years old) are too small to measure. The age of fossils >50 000 years can be determined by the comparative decay rates of different radioactive elements such as uranium-235 and potassium-40 in the rocks surrounding the fossil. By using the decay of other radioactive elements, geologists have been able to determine the age of Earth at 4.6 billion years old.

radioactive element
an element that emits radiation as a result of the spontaneous degeneration of its nucleus

History of life on Earth

It has been estimated that the first life forms (i.e. prokaryotes) originated about 4 billion years ago. Each population of prokaryotes would have become specialised to their conditions through natural selection. Evidence of changes in populations over time can be seen in the fossil record. Some of the earliest fossils, stromatolites (e.g. sediment trapping cyanobacteria), can be found across the world, including in Shark Bay in Western Australia (Figure 6). These have been dated back to 3.5 billion years ago.

Video
What is geological timescale?

Figure 7 shows a diagrammatic summary of the history of life on Earth. The timescale is divided into eras, from the Precambrian, which hosted only prokaryotes and some jellyfish, to the Cenozoic, which includes all life until the present. Each era is subdivided into periods, and each period is subdivided into epochs. The divisions are all characterised by specific index fossils.

FIGURE 6 The Shark Bay stromatolites in Western Australia are thought to be some of the earliest fossils on Earth.

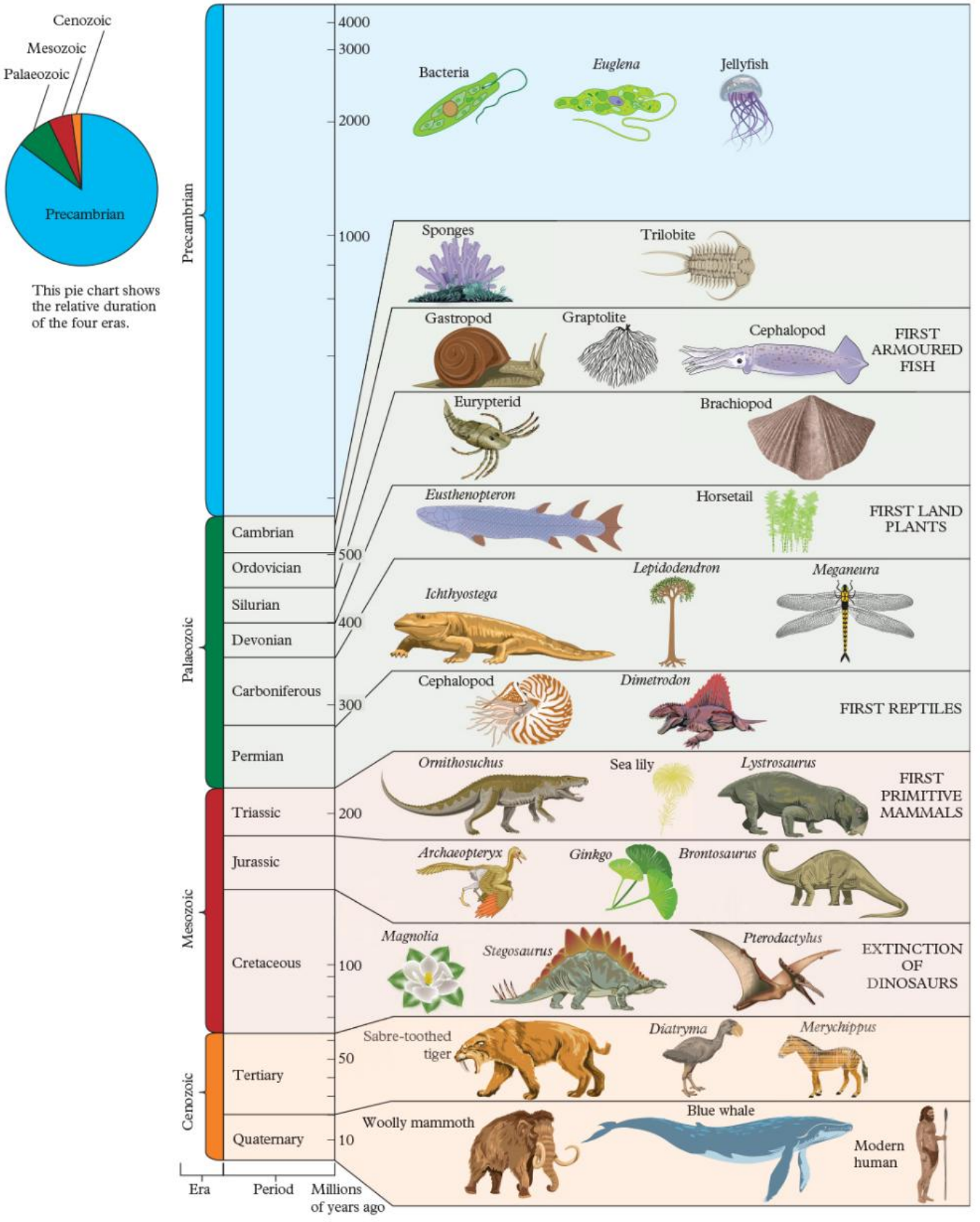

FIGURE 7 Possible history of life on Earth, illustrating the emergence of key species in different periods of geological time

CHECK YOUR LEARNING 12.1

Describe and explain

1 Explain how fossils provide evidence of evolution.

2 Define the term 'index fossil'.

3 Describe two techniques used to date fossils or rock strata.

4 Identify reasons why scientists may want to date a fossil.

5 Identify the most appropriate absolute dating method for a lizard fossil aged 680 years old.

6 Explain why a fish bone found on the beach is unlikely to be a fossil.

Apply, analyse and compare

7 Why are transitional fossils important in the fossil record?

8 A giant penguin that stood as tall as a person has been identified from fossil leg bones discovered by an amateur palaeontologist on New Zealand's South Island. At 1.6 m and 80 kg, the new fossil species, *Crossvallia waiparensis*, would have been four times as heavy and 40 cm taller than the emperor penguin (*Aptenodytes forsteri)*, the largest living penguin today.

- **a** Describe the appropriate technique to date *Crossvallia waiparensis*, considering the fossil is predicted to be approximately 60 million years old.
- **b** Which geological period would this species have existed in?

9 When animals die, bacteria decay the remains. This requires warmth, moisture and oxygen. In northern Russia, whole remains of the woolly mammoth (*Mammuthus primigenius*) have been found preserved in frozen soil. Why did they not decay?

10 A mud layer contained a leaf that was 4800 years old. Below that was a fossilised tree trunk in sandstone. Below the fossilised tree was a fish skull dated back to 300 million years old.

- **a** What age could the fossilised tree be?
- **b** Is this an example of relative dating or absolute dating?

11 A volcanic eruption destroyed a hillside of vegetation and scientists want to know when the event occurred. There are fossils of burnt trees in the ash layer indicating they died at the time of the eruption. A 1:1 ratio of uranium-235 and lead-207 has been identified in the ash layer. Calculate when the volcanic eruption destroyed the trees.

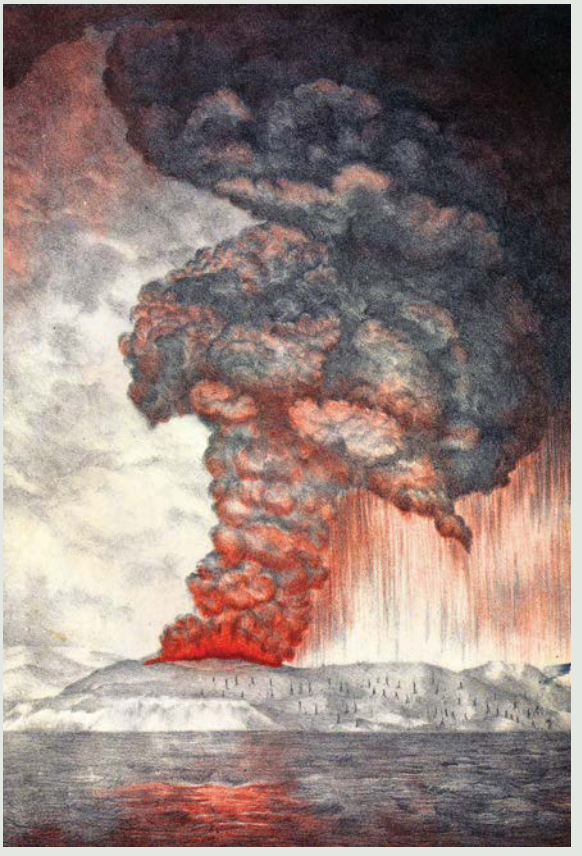

FIGURE 8 A volcanic eruption

12 A crocodile was struck by a landslide that quickly buried the animal. Apply your knowledge of fossil formation to determine whether the crocodile's skeleton would form a fossil.

Design and discuss

13 Use the timescale of life on Earth (Figure 7) to help you answer the following: Why did land plants appear before land animals? You may need to do further research to answer this question.

12.2 Evidence of speciation

KEY IDEAS

In this topic, you will learn that:

+ genetic divergence describes divergence from a common ancestor due to different selective pressures. Adaptive radiation is a type of genetic divergence.

Genetic divergence

genetic divergence evolution that leads to descendants becoming different in form from their common ancestor due to different selection pressures

niche the role of an organism in an environment

Genetic divergence, or divergent evolution, occurs when a population of interbreeding organisms diverges (i.e. separates) into two or more species. This may occur when there is competition for a particular resource, or when a new **niche** becomes available in an environment.

Most populations will have some variation in their physical characteristics, and these occur because of mutations in their DNA. These differences may be large (such as a new colour) or small (such as a slightly louder mating call). These variations allow the organisms to exploit a different resource and therefore have an advantage over other members of the same species. Although these variations may have been present in the population for some time, it is not until environmental pressure acts upon the population that the variations become important for survival. Eventually, two groups of organisms will become so different that they will no longer be able to breed together in natural conditions to produce viable offspring. This means they have become reproductively isolated and are considered different species.

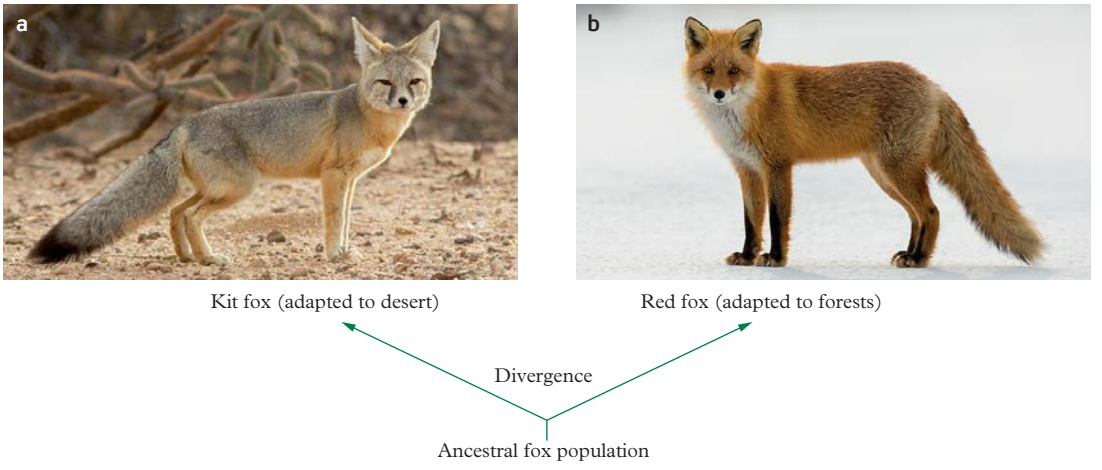

FIGURE 1 Divergence of **a** the kit fox (*Vulpes macrotis*) and **b** the red fox (*Vulpes vulpes*) from a common ancestor due to different selection pressures in their environments

homologous structures similar structures indicating shared ancestry, but may have different functions

geographic isolation when a population is separated due to a geographical barrier

adaptive radiation an evolutionary process in which organisms diversify rapidly from an ancestral species into several divergent forms

Because of their recent common ancestry, different species can have some common structures that have developed slightly different purposes. These are called **homologous structures**. For example, some plants have evolved different functions for their leaves. There are plants that evolved coloured leaves to attract insects, while others (such as pitcher plants) evolved leaves shaped like a container to trap insects.

Two forces can drive divergent speciation: a change in the environment, and **geographic isolation**, which is when a population is separated because of a geographical barrier. **Adaptive radiation** of species is an example of genetic divergence.

FIGURE 2 A common ancestor leaf has diverged as a result of different selection pressures.

Video
Genetic divergence

Adaptive radiation

Adaptive radiation is driven by a single lineage's adaptation to the environment, and can occur rapidly. Groups of organisms descended from a common ancestor that are reproductively isolated from others can accumulate mutations over time and this can result in new species. For this to occur, the common ancestor must have a **key adaptation** or novel phenotypic trait that allows the organism to evolve and take advantage of a new niche or resource. Although the variation(s) may have been present for some time, it is not until a selection pressure acts upon the population that these variations are selected for.

key adaptation
a novel phenotypic trait that allows an organism to evolve and exploit a new niche or resource, resulting in the subsequent radiation and success of a taxonomic group

One of the most spectacular evolutionary radiations in the animal kingdom, in terms of both species richness and diversity of body form, is seen in the Crustacea. Key adaptations in size and shape allowed these species to exploit new regions or niches that had less competition. Continued evolution emphasised the adaptations until they were reproductively isolated. Modern-day versions of these animals include:

- giant crabs
- immobile barnacles
- amorphous forms (i.e. no distinct body shape)
- microscopic plankton.

The plankton of the open ocean are the most abundant multicellular animals (with differentiated tissues) on Earth. Crustacea also occupy most habitats on Earth and are found in such diverse places as deep open trenches, mountain tops and deserts.

A further example of adaptive radiation is that seen in species co-evolving with Australian marsupials. Like many other mammals, Australian marsupials often have parasites such as the platyhelminth parasites. Since marsupials evolved from their original carnivorous diets to omnivorous diets, there were changes in their intestinal tracts that opened up niches for the parasitic worms.

FIGURE 3 Zooplankton seen through a microscope

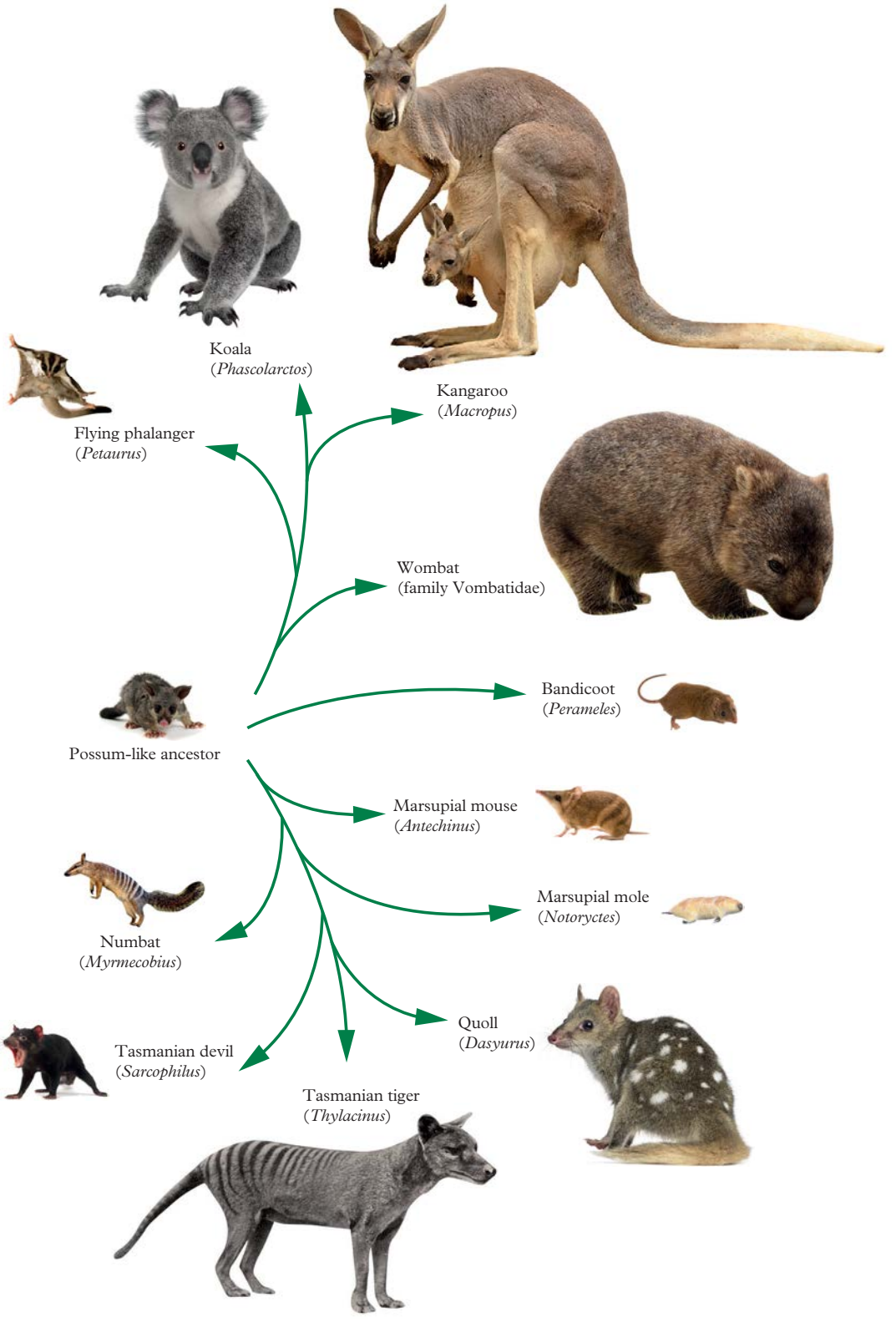

FIGURE 4 Adaptive radiation shown in the Australian marsupials

CASE STUDY 12.2

Cichlid fish in East Africa

Cichlid fish (family Cichlidae) are considered one of the most diverse, species-rich families of invertebrates. They serve as an excellent model of evolutionary change over time by observing the morphology of their jaws and teeth. There are approximately 1500 species of cichlid fish found in three East African lakes (Victoria, Malawi and Tanganyika). Lake Victoria is the largest lake in Africa and it is thought that about 12 000 years ago the lake almost completely dried up, leaving only small isolated pools with few fish. The now 500 diverse cichlid species in Lake Victoria are believed to have come from a single common ancestor, radiating explosively during the last 12 000 years.

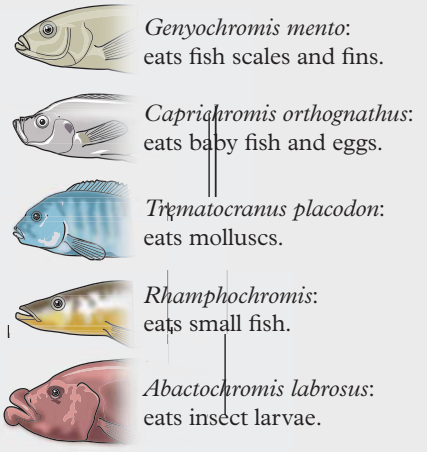

FIGURE 5 The cichlid fish of East African lakes have over 1500 species due to adaptive radiation.

The different species of cichlids are closely related and have developed phenotypic changes to the jaw and teeth to exploit a range of feeding niches. Some cichlids consume algae that grow on rock surfaces; they have flat teeth allowing them to nibble the algae from the rock. Other cichlids are insect eaters and have pointy teeth to allow them access to rock crevices where insects reside. Some cichlids (*Abactochromis* in Figure 5) also eat small fish, which can hide in tight spots, and these cichlids have developed large lips to suck the prey from their hiding spots.

For a long time, it was not fully understood how one common ancestor could radiate into so many different cichlid species in a relatively short period of time. Another notable observation was the fact that cichlid species in different lakes developed similar phenotypes even though they were from separate lineages. The answer to these questions was found when scientists sequenced the genomes of the fish. They found a particular master gene that controlled genes involved in jaw and teeth development in the embryo. This master gene generated physical variations that enabled cichlid fish to exploit a range of feeding niches and radiate into over a thousand species in a short period of time.

CHECK YOUR LEARNING 12.2

Describe and explain

1 Explain how variation can appear in a species.
2 Use an example to describe how adaptive radiation can occur.
3 Explain why the cichlid fish are an example of geographic isolation.
4 What are the two forces that drive genetic divergence?

Apply, analyse and compare

5 Why are Australian marsupials an example of adaptive radiation? Can you think of another group of organisms, not discussed previously in this chapter, that shows adaptive radiation?

12.3 Types of speciation

KEY IDEAS

In this topic, you will learn that:

- allopatric speciation occurs when two populations of the same species become geographically isolated and experience new mutations and selection pressures.
- sympatric speciation is the evolution of a new species from a single population while both inhabit the same geographical region.

speciation
the formation of a new reproductively isolated species as a result of evolution

Speciation is an evolutionary process where a single species evolves over time into two or more species that are unable to produce fertile offspring in natural conditions. Organisms can typically be considered two different species once there is no gene flow between their populations. There are several factors that can act as barriers to isolate a species' gene pool. These factors can act to prevent the species recognising a potential mate, by driving unsuccessful mating, or by blocking fertilisation occurring when mating is possible.

While this definition of speciation is useful, in practice it is limited to particular situations. For example, identifying the exact time an ancestor diverged from a modern living organism enough to be a different species is particularly difficult when using the definition. This means scientists need to modify their definitions to also define different species by body shape or occupation of different niches. In both of these situations, scientists are able to predict that gene flow would be limited or completely prevented between the two species.

Allopatric speciation

When a single population of organisms is physically separated by a permanent barrier, it can block gene flow between the two groups. The type of barrier that separates the two organisms is dependent on the ability of the organisms to move. Birds, wind-blown pollen, and dingoes are able to cross roads or rivers, while small bush mice might have more difficulty. In contrast, floods are not considered permanent, and therefore will not provide a permanent barrier.

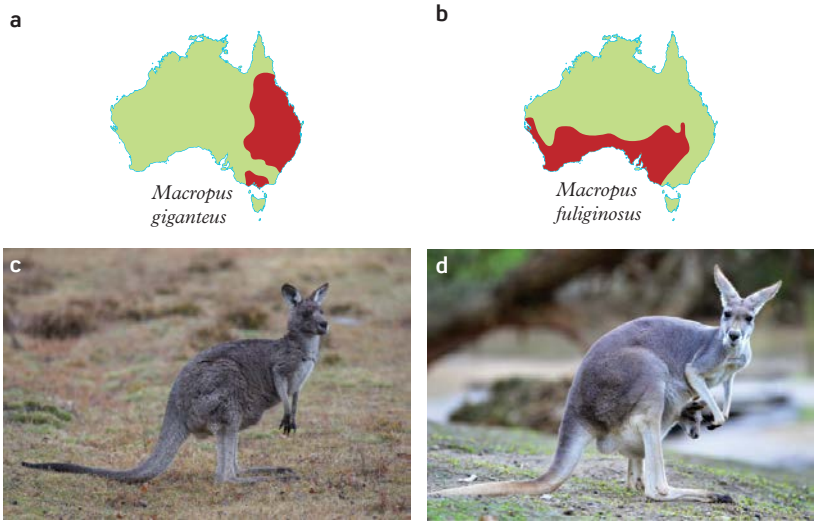

FIGURE 1 The distributions of two kangaroos species: **a** the eastern grey kangaroo (*Macropus giganteus*) and **b** the western grey kangaroo (*Macropus fuliginosus*) due to allopatric speciation

Once two populations are geographically isolated, different genetic mutations will accumulate over time, resulting in new alleles. Each population may experience new selection pressures that will select for or against the new phenotypes (natural selection), causing a change in allelic frequencies. Over time, the differences in the two populations will accumulate until they become reproductively isolated, and become different species. This process of speciation resulting from geographical isolation is called **allopatric speciation**.

allopatric speciation
the process of speciation as a result of a permanent barrier separating the ancestral species

Galapagos finches

One of the best-known examples of allopatric speciation is the evolution of finches on the Galapagos Islands. These finches are known as Darwin's finches, named after Charles Darwin, who first examined the variety of birds. The islands developed as a result of volcanic activity and have never been joined to the South American mainland. Genetic studies have indicated that all the finches shared common ancestors. Over millions of years, the finches radiated out from a single island to other islands (adaptive radiation). Each island presented its own set of selection pressures such as the type of shelter or type of food available (including seeds, fruits, cacti and invertebrates). The finches that survived on each island underwent a process of natural selection. For example, the large ground finch (*Geospiza magnirostris*) has a large blunt beak that can crack the hard shells of nuts and seeds, while the vampire ground finch (*Geospiza septentrionalis*) has a smaller sharper beak that allows it to drink the blood of large sea birds. Because these birds live on different islands, the water offers a natural barrier that restricts gene flow and prevents them from interbreeding.

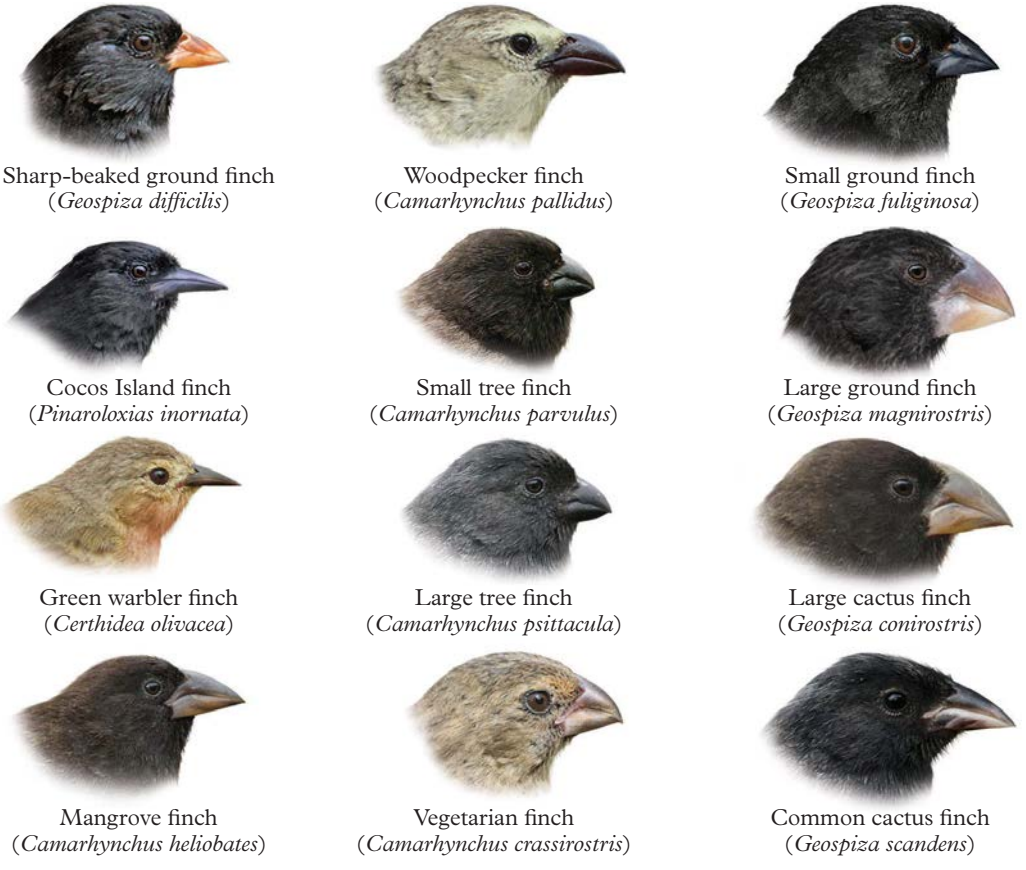

FIGURE 2 The different beak sizes and shapes of various Galapagos finches

CASE STUDY 12.3

Speciation in Galapagos finches

Most character changes in a lineage take place over a time period too long to be observed. But sometimes the evolution of a new species can occur in a matter of years. Such an example is a new finch species that arose on the island of Daphne Major in the Galapagos Islands in recent years.

When biologists Peter and Rosemary Grant first arrived on Daphne Major in 1973, there were only two species of finch present: the medium ground finch (*Geospiza fortis*) and the common cactus finch (*Geospiza scandens*). In 1981, a new male finch was blown to the island, and because of the distance was unable to return to its original island. Although similar to the medium ground finch, it had a much larger beak, an unusual hybrid genome and a new kind of song. After locating a mate that had hybrid chromosomes of her own, they produced offspring different from other birds on the island.

The male then mated with two females from one of the local species, the medium ground finch. After four finch generations, a drought killed off many of the birds on Daphne Major. Only a brother and sister pair of the hybrid line remained. The two family members mated with each other, producing offspring that were unique from their parent line. From that point on, as far as we know, this population of finches mated only with each other. They were never seen to breed with the cactus finches or the medium ground finches on the island. A new species, *Geospiza conirostris*, had evolved.

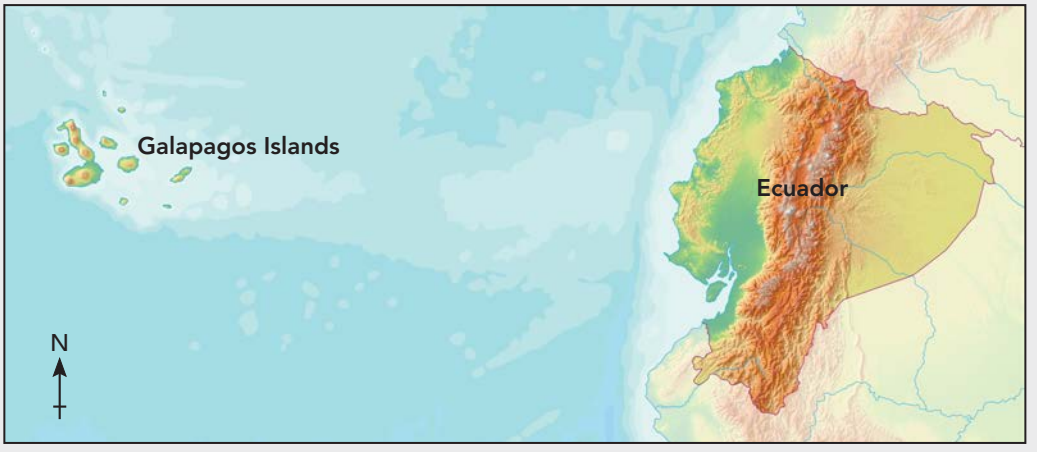

FIGURE 3 The Galapagos Islands are off the north-western coast of Ecuador.

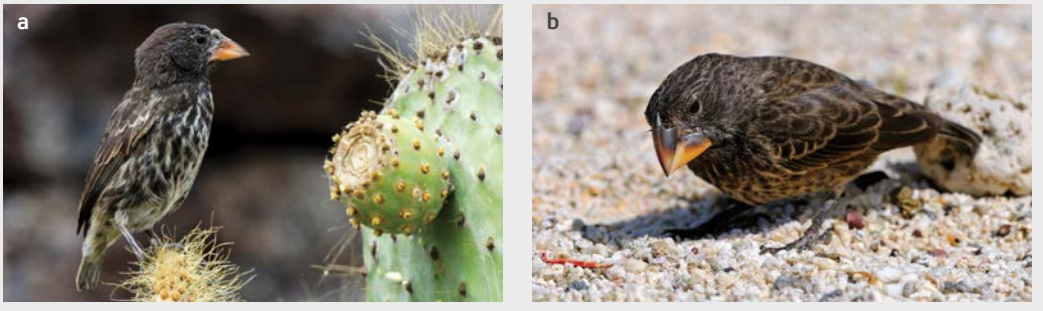

FIGURE 4 An example of rapid evolution on Daphne Major in the Galapagos Islands. **a** The medium ground finch: the new species, *G. conirostris* and **b** the indigenous species, *G. fortis*.

Sympatric speciation

Speciation does not always need a permanent barrier to occur. Sometimes two or more descendent species can evolve from one ancestral species, while occupying a single geographical location – in what is termed **sympatric speciation**. Sympatric speciation can occur when gene flow is restricted by polyploidy (more than two sets of chromosomes), when a species occupies different environmental niches, or when a species' sexual selection preferences change.

sympatric speciation when two or more descendent species evolve from a single ancestral species within a single geographical location

Polyploidy

When an error occurs during cell division, it can result in an extra set of chromosomes. This is most common in plants and can result in a reproductive barrier between the original parent species and the new polyploid species. This is due to the inability of homologous pairs to form during meiosis. Both sets of plants can survive alongside each other as the reproductive barrier is molecular rather than physical.

Video
Allopatric and sympatric speciation

Environmental niches

When an environment changes, it can result in some species being unable to survive. When that occurs, a new environmental niche (e.g. shelter location or resources) becomes available. An example of this is two species of palm trees – *Howea forsteriana* and *Howea belmoreana* – that are found on Lord Howe Island in Western Australia. Genetic studies indicate they share a common ancestor but had become reproductively isolated from each other despite producing vast amounts of wind-spread pollen in overlapping ranges. *H. forsteriana* prefers to grow in soils containing chalky lime found at low altitudes, while *H. belmoreana* grows in neutral and acidic soils found 90 metres above sea level. Each type of soil affects the timing of the palms' flowering, suggesting that this was the mechanism of initial reproductive isolation.

FIGURE 5 The two different Australian palms: **a** *H. forsteriana* and **b** *H. belmoreana*

Study tip

Meiosis is the process by which gametes (egg/ova and sperm/pollen) are formed. An important part of this process is when the matching homologous chromosomes form pairs before the cell can divide. This can be difficult in hybrid organisms.

Sexual selection

Some species are particularly selective when choosing their mate. When mating preferences change, this can result in sympatric speciation. An example of this is the speciation of cichlid fish, *Pundamilia pundamilia* and *Pundamilia nyererei*, found in East Africa's Lake Victoria. Although there are many factors influencing the mechanism of their evolution, including exploiting environmental niches, it is also thought that female mate preference was a factor. When researchers placed males and females of *P. pundamilia* and *P. nyererei* in a tank together, their choice of mate varied according to the kind of lighting present. In natural light, the females only mated with males from the same species; however, in an orange light, the females mated with males from either species. The resulting hybrid offspring were viable and fertile. This suggests that the gene flow barrier is a result of sexual selection due to colouration.

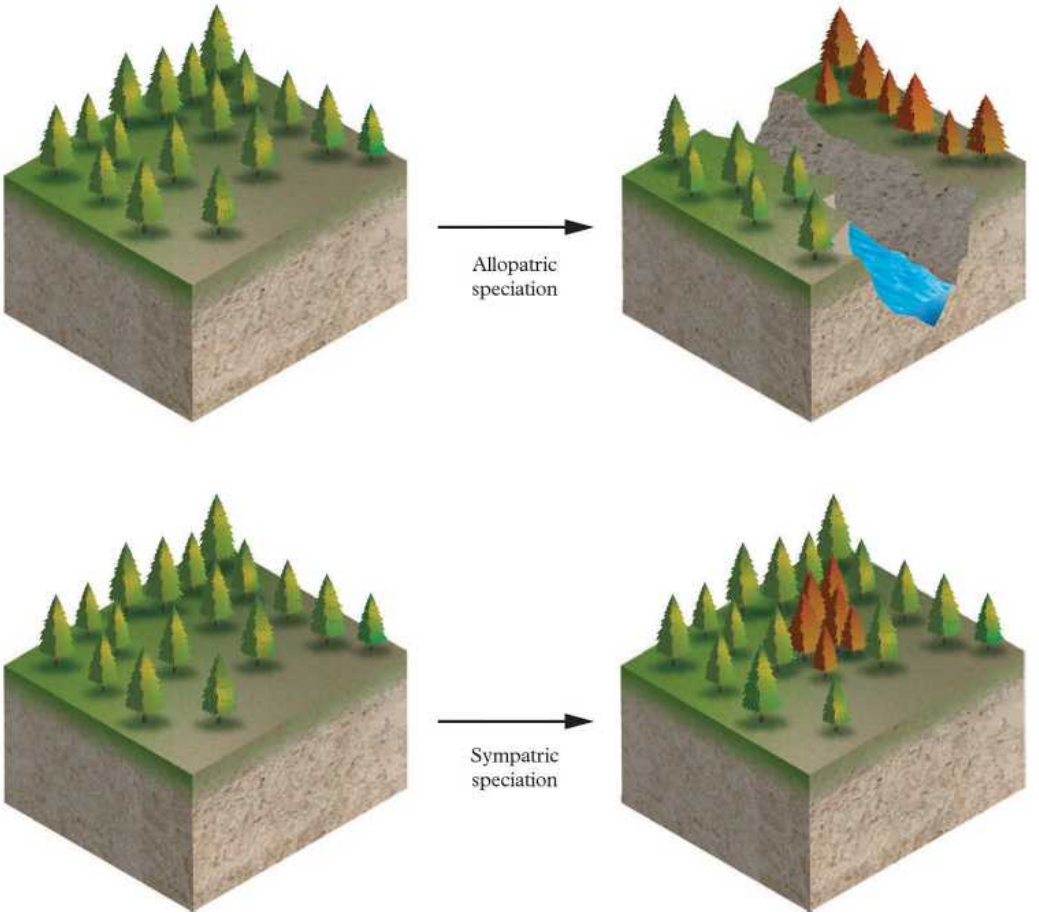

FIGURE 6 A comparison of allopatric speciation and sympatric speciation

CHALLENGE 12.3

African indigobirds

African indigobirds (*Vidua* spp) provide an example of how new species can emerge without first being separated by a geographical barrier. These birds do not directly care for their chicks; instead, they lay their eggs in the nest of other birds. When the young chicks hatch, they are exposed to the songs of their adoptive parents. When the indigobird chicks grow up, they are attracted to mates that sing the song of the adoptive parents and seek out nests similar to their adopted home. This means populations of indigobirds will divide into different groups according to their preferred mating song and nest site.

Robert Payne and Michael Sorenson of Boston University undertook genetic analysis that showed that the different groups of indigobirds accumulated genetic differences due to their distinct song preferences. These genetic differences resulted in physical changes in the pattern of mouth spots of the indigobird chicks, making them more likely to be accepted by the adoptive mother. The combination of physical changes, song and nest preferences contribute to the reproductive isolation between the different indigobird groups.

1 What type of speciation is described above? Provide both a definition and evidence to support your answer.
2 Why would learning different songs result in reproductive isolation?
3 Would this type of speciation occur quickly (within two generations) or take many generations to occur?

FIGURE 7 An African indigobird (*Vidua chalybeata*) at Kruger National Park in South Africa

CHECK YOUR LEARNING 12.3

Describe and explain

1 What is speciation?
2 What is an environmental niche?
3 Provide two examples of permanent barriers that could lead to allopatric speciation.
4 Identify the different types of sympatric speciation.

Apply, analyse and compare

5 Compare allopatric speciation and sympatric speciation.
6 By referring to Case study 12.3, explain how a drought resulted in the new species of finch, *Geospiza conirostris*.
7 What defines the two species of Australian palms on Lord Howe Island, and how did this speciation occur?

Design and discuss

8 On Daphne Major, the *Geospiza conirostris* offspring of the immigrant male finch and female *Geospiza fortis* learnt their mating song from their immigrated parent. Explain how this could have caused reproductive isolation.
9 A scientist found that small populations of birds that lived in urban areas were evolving a higher-pitched birdsong than similar populations that lived in suburban areas. Although the birds often learnt the songs of their parents, the ability to hear higher-pitched sounds is a genetic trait. Discuss whether this is an example of allopatric speciation or sympatric speciation.
10 Research other known examples of allopatric and sympatric speciation and describe your findings.

CHAPTER 12

Review

Chapter summary

12.1
- Fossils are evidence of species change over geological time.
- The geological timescale provides the sequence of events in Earth's history.
- Relative dating and absolute dating are two methods of dating fossils.

12.2
- Genetic divergence describes divergence from a common ancestor due to different selective pressures. Adaptive radiation is a type of genetic divergence.

12.3
- Allopatric speciation occurs when two populations of the same species become geographically isolated and experience new mutations and selection pressures.
- Sympatric speciation is the evolution of a new species from a single population while both inhabit the same geographical region.

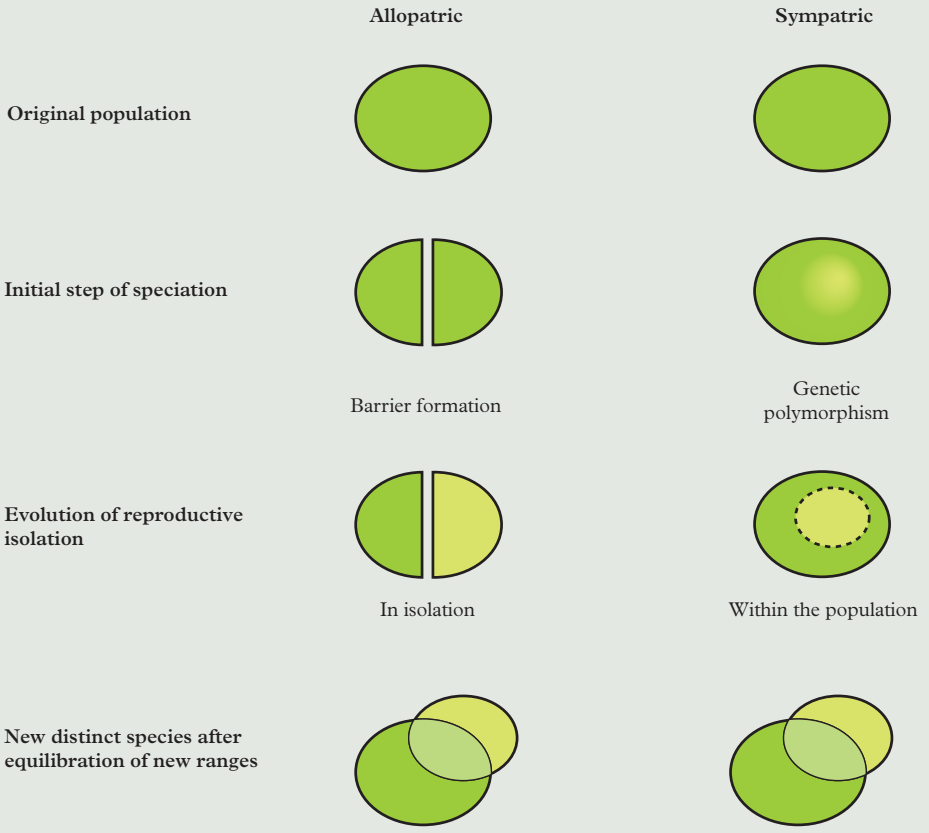

FIGURE 1 A summary of the differences between allopatric speciation and sympatric speciation

Key formulas

Age of fossil	Number of half-lives passed × half-life

Revision questions

Multiple choice

1 Which is important for an index fossil?
- **A** Short period of time in a wide geographical area
- **B** Short period of time in a narrow geographical area
- **C** Long period of time in a wide geographical area
- **D** Long period of time in a narrow geographical area

2 A fossil is more likely to form if the organism:
- **A** remains uncovered by sediment on the surface after it dies.
- **B** does not contain hard body parts.
- **C** is quickly buried by sediment before it decomposes.
- **D** dies in a moist, warm environment.

3 Which scenario is an example of where allopatric speciation could occur?
- **A** Certain members of a population have more offspring than others.
- **B** Finches with thin, sharp beaks eat fish and small mammals, while finches with larger beaks eat nuts and seeds.
- **C** A facial tumour disease kills all members of the Tasmanian devil population.
- **D** A river separates members of a possum population that used to occupy the same geographical area.

4 Scientists observed a rock stratum and hypothesised it was from the Devonian period. Without dating the fossils or the rock in the stratum, on what did the scientists base this claim?
- **A** The absence of insects and presence of fish
- **B** A range of diverse mammals
- **C** Large numbers of different aquatic plant species
- **D** The presence of the transitional fossil *Archaeopteryx*

5 The adaptive radiation seen in the Galapagos finches is the result of:
- **A** migration from the islands to the mainland.
- **B** polyploidy.
- **C** different food sources on the various islands acting as selective agents.
- **D** the loss of niches on different islands.

6 Samples of sedimentary rock from four different sites is shown below.

Which is the oldest fossil?

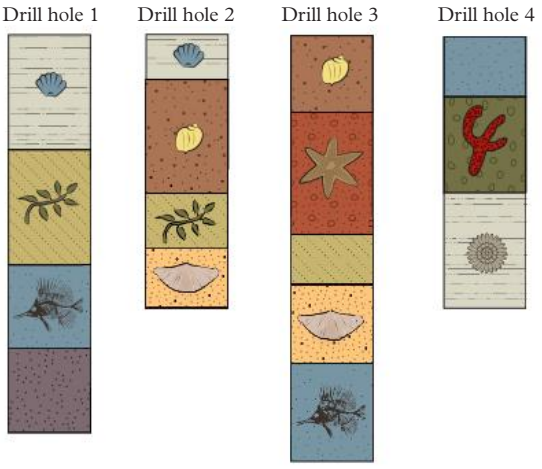

FIGURE 2 Drill cores of sedimentary samples with fossils

- **A** 
- **B** 
- **C** 
- **D** 

7 Absolute dating is a method of dating fossils that uses the radioactive decay of particular isotopes. Carbon-14 is a commonly used isotope, which decays to nitrogen-14.

A fossil is found to contain 20% carbon-14. Using Figure 3, what is the approximate age of the fossil?

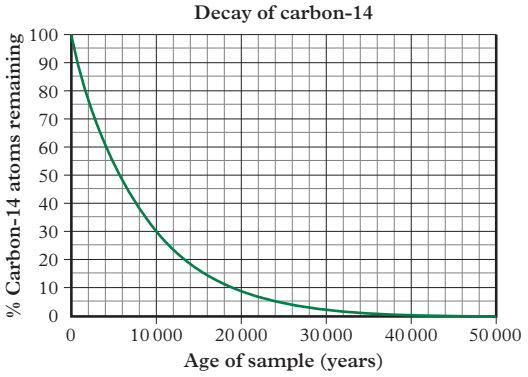

FIGURE 3 Carbon-14 decay with time (years)

A 12 500 years
B 15 000 years
C 17 500 years
D 20 000 years

Short answer

Describe and explain

8 Use examples to explain two different reproductive barriers that could occur in sympatric speciation.

9 What is a transitional fossil?

10 Members of a species living in the same valley begin to diverge from each other over time so that they no longer mate with members of the other group. Use definitions to explain why this is likely to be sympatric speciation rather than allopatric speciation.

11 Explain what is meant by the law of faunal (fossil) succession. How is it used to determine the relative age of a fossil?

12 What is meant by half-life? Explain how it can be used to work out the age of a fossil.

13 What is the difference between genetic divergence and adaptive radiation?

Apply, analyse and compare

14 Why is the fossil record incomplete?

15 Consider why sympatric speciation is more common in plants than animals.

16 Potassium-40 has a half-life of 1.3 billion years. After three half-lives have passed:

a What percentage of the original radioactive element would be present in the fossil?

b If a fossil sample originally included 10 g of potassium-40, how much would be left?

17 If 12.5% carbon-14 was found in a fossil where the remaining 87.5% had decayed into nitrogen-14:

a How many half-lives have passed?

b How old is the fossil if the half-life of carbon-14 is 5730 years?

c How many years would it take for the amount of carbon-14 to be 0.0625 g, if the original amount was 1.0 g?

18 Compare relative dating and absolute dating. Outline the limitations and applications of each type.

19 Why is the speciation of the *Howea forsteriana* and *Howea belmoreana* palm trees on Lord Howe Island a result of sympatric speciation rather than allopatric speciation?

20 Suggest three possible factors that could produce different ecological niches and reduce gene flow.

21 Which parts of an organism are more likely to be preserved as fossils? Explain why.

22 Trilobites were a class of animal that populated Earth's oceans and evolved for almost the entire length of the Palaeozoic era.

a What can you observe in Figure 4 that would suggest trilobites are a good index fossil?

b Explain how palaeontologists would use trilobites to date Palaeozoic rock.

FIGURE 4 A Trilobite fossils

23 Uranium-235 has a half-life of 700 million years. Rock surrounding a fossil was found to have ¼ the original amount of uranium-235. Using this information, calculate the approximate age of the fossil.

24 Carbon-14 has a half-life of 5370 years. Calculate how much carbon-14 would be left in a 3.0 g sample after 11 460 years.

25 Around 10 000 years ago, a population of squirrels were separated from each other when the Grand Canyon was formed. Since then, the squirrels have become two separate populations: the Kaibab squirrels and the Abert squirrels.

FIGURE 5 **a** Kaibab squirrel (*Sciurus aberti kaibabensis*) and **b** Abert squirrel (*Sciurus aberti*)

a Describe the process that could have resulted in the ancestor species of squirrel becoming two separate species.

b Examine Figure 5 to identify one physical difference between the two species. Suggest why these physical features have evolved differently.

26 *Howea forsteriana* is a type of palm that grows in soils containing chalky lime and at low altitudes. *Howea balmoreana* grows in neutral or acidic soils 90 metres above sea level. Both types of palm share a genetic ancestor. Discuss how this example demonstrates sympatric speciation.

Design and discuss

27 'The process of a living organism becoming fossilised is very rare'. Discuss this statement.

28 For each of the following fossils, discuss whether using carbon-14 to date the fossil would be suitable. If carbon-14 is not suitable, explain why and suggest another dating method.

a A footprint of a woolly mammoth

b An insect found in sedimentary rock dating to the Carboniferous period

c A bone of the sabre-toothed tiger (*Smilodon fatalis*) that existed up to 11 000 years ago

29 Discuss why adaptive radiation is an important process in biology, and provide examples.

30 'All living organisms share a common ancestor.' Discuss this statement.

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Chapter quiz
Check your understanding of this chapter.

Check your Teacher obook pro for these resources and more:

QuizletLive
Launch a quiz for your students on key concepts in this chapter.

Exam essentials

Responding to questions

In your exam, you may be expected to identify key information about a process and link it to the important information in the stem of the question.

Describe, Explain, Relate to the question (DER)

The DER method is a useful approach for responding to questions that ask you to explain:

- **D**escribe or identify the key information
- **E**xplain why it is important
- **R**elate the information to the stem of the question

Consider how the DER method has been used in the responses to the real exam question below.

QUESTION 8b (2020 Biology Written Examination)

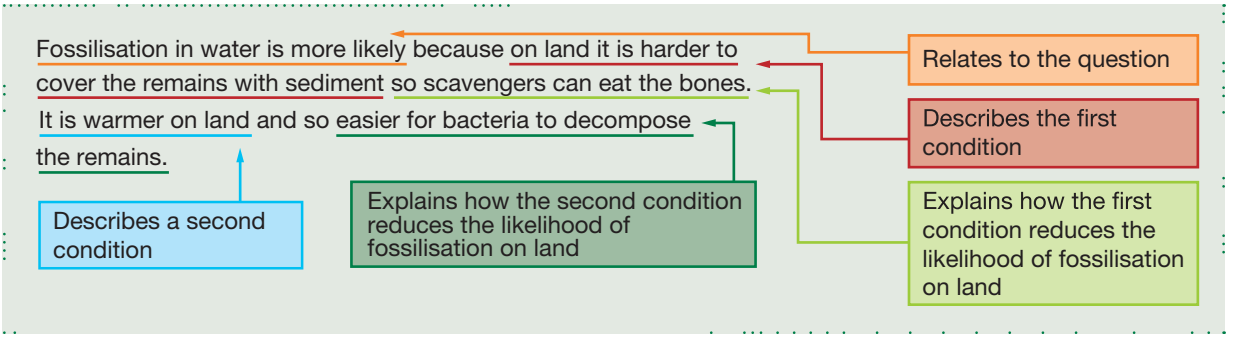
b Fossils of species of fish are more likely to be found than fossils of land-dwelling animals.

Explain why this is the case with reference to two conditions required for the fossilisation of an organism.

2 marks

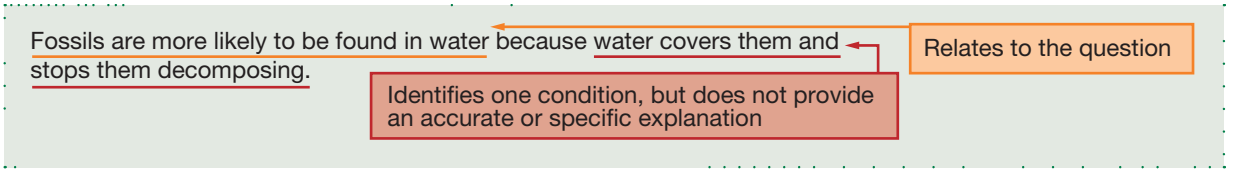
Source: 2020 Biology Written Examination Question 8b, Short answer, reproduced by permission © VCAA

Response 1



Response 2 would receive full marks as it describes the conditions for fossilisation and explains the reasons why this is less likely on land and therefore more likely in water (relating to the question).

Response 2



Response 1 does not provide the two conditions needed for fossilisation or explain why these conditions help fossilisation. The key information that needs to be included for each mark is the condition of fossilisation followed by explaining why this helps fossilisation.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the ir response.

QUESTION 7 b–c (2019 Biology Written Examination)

In 2014 palaeontologists discovered a frozen, well-preserved, complete specimen of an extinct species, the steppe bison, in Siberia. The intact specimen was dated at 9300 years old.

b Scientists are debating the cause of the steppe bison's extinction. Give **one** possible cause of its extinction.

1 mark

The steppe bison became extinct due to unprecedented climate change.

Though steppe bison are extinct, palaeontologists know a lot about them as they have found several frozen, intact steppe bison bodies.

c Describe what would have occurred to lead to the preservation of the animal from when the steppe bison died to when the frozen, well-preserved remains were discovered.

4 marks

After death, the steppe bison would have been covered by sediment and water, which became sedimentary rock. In this process, minerals preserve the fossil. The fossil would then have been discovered when erosion of the rock revealed the preserved fossil.

Source: 2029 Biology Written Examination Question 7b-c, Short answer, reproduced by permission © VCAA

Marking guide

Question 7b	- 1 mark for correctly identifying one possible cause of extinction (e.g. hunting by humans, ice age or cold environment, habitat destruction) Note: climate change is not specific enough. Students must specify an increase or decrease in temperature.
Question 7c	- 3 marks for identifying three of the following: organism not eaten by scavengers; body frozen and does not decay; lack of oxygen; uplift, erosion or melting of glacier exposes remans - 1 mark for correctly recognising that the steppe bison was frozen or rapidly buried (not covered by rock) Students need to relate their response specifically to the question. Prepared answers about the fossilisation process are unlikely to earn full marks.

Fix the response

Consider where you did and did not award marks in the above responses. How could the responses be improved?

Write your own response to the same question to receive full marks from an examiner.

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The DER method

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CHAPTER 13

Species relatedness

There are many forms of evidence to support evolution. Comparative anatomy and molecular homology help to determine recent common ancestors and predict the evolutionary history of living organisms.

Comparative anatomy examines morphological structures in species to determine their relatedness to one another. There are structures (homologous and vestigial) on organisms that can be analysed to indicate genetic divergence from a common ancestor. Molecular homology compares different organic molecules (DNA, RNA or amino acids) and is the most recent form of evidence of evolution.

Phylogenetic trees are a visual representation of the evolutionary history of different organisms. They are constructed from the analysis of characteristics or molecular homology and are typically based on the simplest explanation (a concept known as parsimony).

KEY KNOWLEDGE

→ evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences

→ the use and interpretation of phylogenetic trees as evidence for the relatedness between species

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

13A What is the difference between divergent evolution and adaptive radiation?

13A Groundwork resource
Divergent evolution

13B How do selective pressures affect phenotypes?

13B Groundwork resource
Selection pressures

13C What effects do DNA mutations have on polypeptides?

13C Groundwork resource
DNA mutations

FIGURE 1 The emu (*Dromaius novaehollandiae*) has wings but is unable to fly. It was believed that the emu and other flightless birds evolved from a single flightless ancestor species, but molecular evidence suggests the emu lost its power of flight separately to other flightless birds.

PRACTICALS

NO-TECH PRACTICAL	**13.2** Molecular differences between species

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.

13.1 Structural morphology

KEY IDEAS

In this topic, you will learn that:

- comparative anatomy gives an indication of species relatedness
- homologous structures are the result of divergent evolution
- vestigial structures provide evidence of common ancestry.

Studying the structural morphology of different species provides evidence of their common ancestors and therefore gives an indication of species relatedness. Structural morphology is the study of the size and shape of animals. The biological field of comparing structures of organisms is known as comparative morphology or **comparative anatomy**. Anatomical structures of fossils are compared with those of living organisms, to determine any relationship between them.

comparative anatomy
comparing structures between organisms to determine relatedness

Homologous structures

Features of organisms that share a fundamentally similar structure, based on common ancestry, are termed **homologous structures**. Homologous structures suggest that the organisms in question all shared a recent common ancestor from which they diverged. The greater the number of homologous structures between two species, the more recently they shared a common ancestor. Often homologous structures develop different functions due to different selection pressures. New and distinguishing characteristics of different species that come from a common ancestor are therefore the result of genetic divergence (as covered in Chapter 12).

homologous structures
structures that are structurally similar between species due to a recent common ancestor

Study tip

The term '*Homos*' is Greek for 'the same'. '*Heteros*' is Greek for 'the other (of two), another, different'.

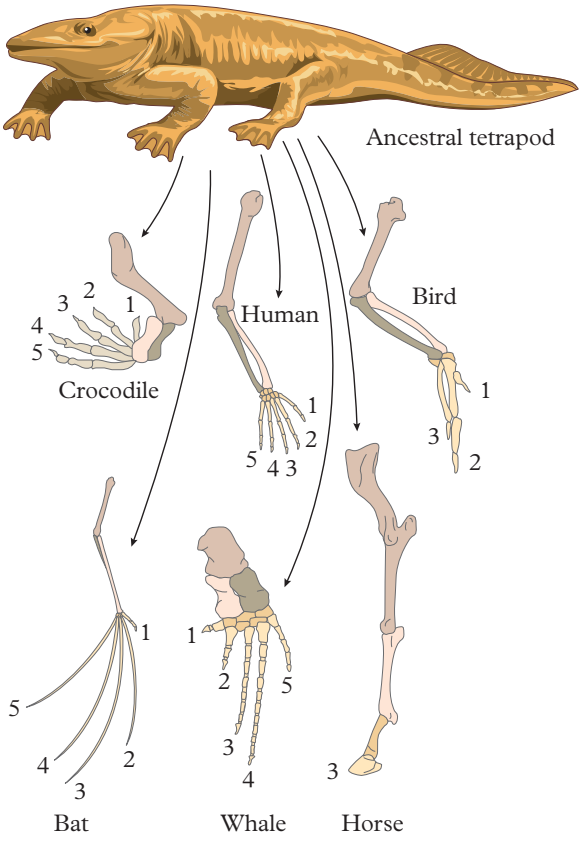


FIGURE 1 Homologous structures: the forelimb of terrestrial vertebrates. Colour shows matching bones; numbers show corresponding digits. All originate from a common ancestor, the tetrapod.

The following structures are homologous:

- the wing of a bat
- a whale's flipper
- a horse's forelimb
- a human arm.

These all look different and serve different functions, but studying the bones reveals that they all have the same internal structure.

Figure 1 shows the same arrangement of the bones from the shoulder to the digits.

All organisms have clear homologous structures. For example, all flowering plants have roots, stems, branches, leaves, chlorophyll and flowers – these features are considered homologous characteristics. These similar organisms diverged from a common ancestor due to different selection pressures.

If one group of plants is exposed to strong winds and cold temperatures, those that are smaller and lower to the ground will survive and produce seeds. In contrast, plants exposed to hot dry environments are more likely to survive if their leaves are narrow in order to prevent water loss. Over millions of years, the two groups of plants will become more diverse from each other due to the different environmental pressures. Their new defining features of being either low to the ground or having narrow leaves is an example of divergent evolution.

Vestigial structures

The possession of **vestigial structures** is also taken as evidence of a common ancestral origin. These structures are smaller and simpler than those in related species and typically have lost, or almost lost, their original function.

vestigial structures structures that had functional importance in ancestral species but have reduced in size over time and have limited or no use in their present form

Examples of vestigial structures include:

- reduced bones in horse limbs
- reduced stomata on the petals of flowering plants
- the coccyx in humans
- hind limb bones in snakes and whales
- reduced eyes of salamanders.

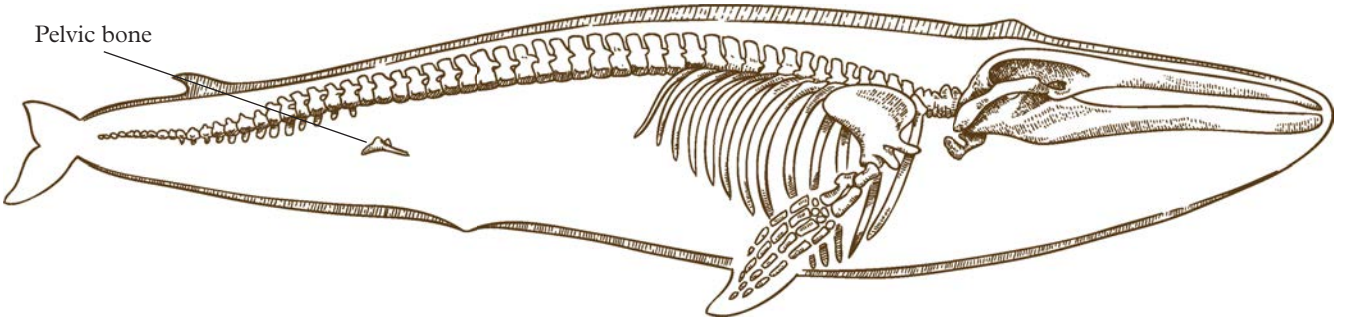


FIGURE 2 The skeleton of a baleen whale (*Mysticeti*) contains pelvic bones. These bones resemble those of other mammals, but are only very small in the whale and do not share the same apparent function of leg development.

CHECK YOUR LEARNING 13.1

Describe and explain

1 What are homologous structures?

2 Why is the coccyx considered a vestigial structure in humans?

Apply, analyse and compare

3 Identify a vestigial structure in humans that is not mentioned in this topic.

4 Use Figure 1 to explain how each of the terrestrial vertebrates came to have different shaped forelimbs.

5 What is the significance of homologous structures in terms of evidence of species relatedness? Use examples of animals and plants in your answer.

6 Determine whether the following are vestigial or homologous structures.

a The wings of bats

b The flippers of whales

c The forearms of humans

d The coccyx in humans

e The hind limb bones of a snakes

Design and discuss

7 Humans are still evolving. Can you predict a part of the human body that will likely become a vestigial structure, and why this might be the case?

13.2 Molecular homology

KEY IDEAS

In this topic, you will learn that:

- differences in DNA and amino acid sequences are used to determine relatedness between species
- mitochondrial DNA can be used to determine relationships between closely related species.

Structural morphology (comparative anatomy) was the main evidence used to determine relatedness between species before molecular techniques were available. But structural comparisons can be ambiguous because some features, such as **analogous structures** that have the same function with a different structure, are caused by **convergent evolution** and not due to a recent common ancestor. For this reason, differences in DNA and amino acid sequences are a much more robust technique for investigating relatedness between species.

analogous structures structures with a similar function but which have not arisen due to common ancestry

convergent evolution the independent development of similarities between species as a result of them having similar ecological roles and selection pressures

Comparisons of genetic material, amino acid sequences and proteins are used as evidence of relatedness between species. If species have very similar molecules, it provides evidence that they shared a recent common ancestor. The more differences in the sequences, the further back in time they shared a common ancestor.

DNA and amino acid sequence comparisons

Mutations can occur in the DNA sequence of any organism. Mutations can occur during DNA replication and for the most part these errors are repaired before the cell starts to divide. However, sometimes these errors are not repaired and become a permanent part of the genome. If these mutations occur in a germline cell then they can be passed onto the next generation.

As species diverge from one another, they can accumulate mutations in their DNA sequences that can ultimately affect the amino acid sequence of their proteins. The further back two species shared a common ancestor, the more differences there are between their DNA sequences.

Not every mutation in the DNA sequence results in a change in an amino acid sequence. Two species could have exactly the same amino acid sequence for a protein, but may have different DNA sequences. This is because the genetic code is degenerate, meaning more than one codon codes for an amino acid. You can refresh your memory on amino acids and degeneracy in Chapter 2. Even if the point mutation causes a change to the amino acid, it may not change the phenotype. This is because the amino acid change may be similar biochemically. Comparison of DNA sequences in determining relatedness between species is therefore more powerful than comparison of amino acid sequences or proteins.

conservative substitution an amino acid substitution that doesn't cause a change in the protein

semi-conservative substitution an amino acid substitution that may change the resulting protein

non-conservative substitution an amino acid substitution that results in a completely different protein

There are different causes of change due to amino acid substitutions, which include the following.

- **Conservative substitution**: where there is no change to the protein because the amino acid change is biochemically similar.
- **Semi-conservative substitution**: where the amino acids change but the substituted amino acid has a similar shape (not biochemically similar) and may cause a change to the protein structure and function.
- **Non-conservative substitution**: the result of an amino acid change that is different biochemically, leading to a protein with major functional and structural changes.

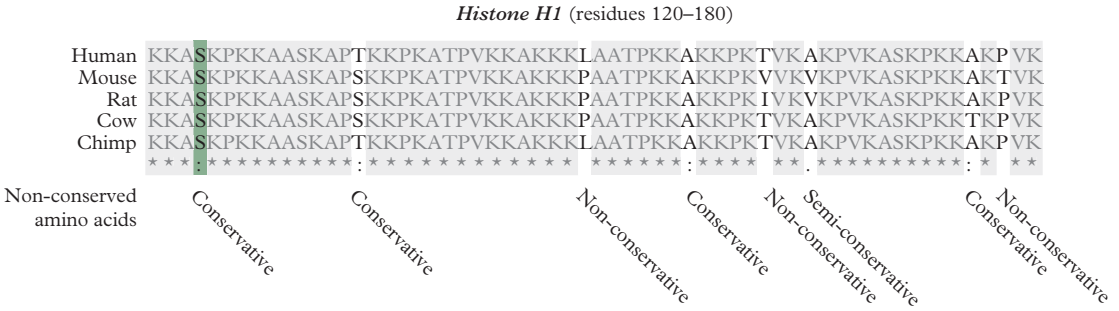


FIGURE 1 A section of the *histone H1* gene for the human, mouse, rat, cow and chimp. The type of amino acid substitution is given. The asterisk underneath each of the amino acids represents those that have not changed.

FIGURE 2 A mouse has conserved and non-conserved regions of the *histone H1* gene with a human, rat, cow and chimp.

Each protein is made up of a chain of amino acids (a polypeptide chain). A change in one amino acid along this chain results in changes to the shape of the final protein. New technologies can rapidly analyse the amino acid sequence in a protein. When the sequences of amino acids of the same protein are compared, the number of differences in the amino acids can be determined. The greater the number of differences in amino acids between two organisms, the more distantly related they are.

WORKED EXAMPLE 13.2

DETERMINING RELATEDNESS BETWEEN SPECIES

Use Table 1 below to determine which species of bear are most closely related and which are more distantly related.

TABLE 1 Amino acid differences in the lyst protein among four different bear species

	Panda bear	Black bear	Brown bear	Polar bear
Panda bear	0			
Black bear	33	0		
Brown bear	34	1	0	
Polar bear	40	7	8	0

Video
Worked example 13.2: Determining relatedness between species

SOLUTION

1 When reading the table, it is important to read across and then downwards. Reading across, the panda has zero differences in the lyst protein compared to itself. Then reading downwards, the panda bear has 33, 34 and 40 differences compared to the black, brown and polar bear, respectively.

2 The species that are most closely related will have the least number of differences. This is the brown and black bears, with one difference between them for the lyst protein.

3 The species that are most distantly related will have the most differences. This is the polar and panda bears, with 40 differences.

Molecular clock

Comparisons between the molecules of particular organisms can be used to provide more information about possible evolutionary pathways. Some molecules, such as DNA, mutate at a constant rate, and the rate differs from one part of the DNA to another. This means sections of DNA are acting like a clock that starts when the two groups of organisms begin to diverge. This is referred to as a **molecular clock**.

molecular clock the rate of accumulation of mutations, used to determine species relatedness

As time passes, mutations accumulate in the DNA. Many of these sections of DNA contain the code for a protein, so increasing changes in the DNA results in changes in the proteins as well. The greater the number of changes in the DNA or proteins, the more time has passed for these mutations to accumulate. By calibrating the DNA and protein clock with fossil dating, an estimate of 'real' time differences can be determined.

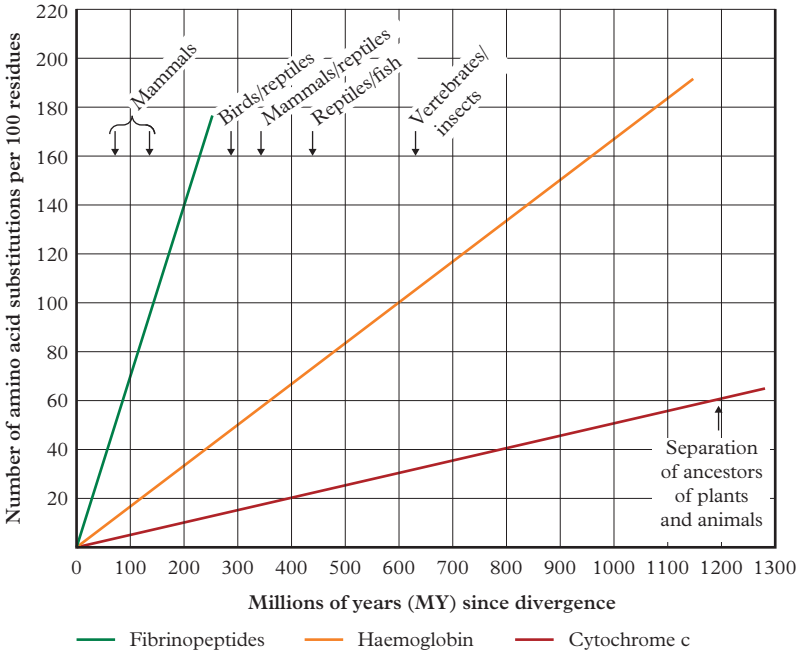


FIGURE 3 The number of amino acid substitutions over time (millions of years) for three different proteins

A limitation of the molecular clock hypothesis is the assumption that the rate of evolutionary change is constant and the same across species. There is a positive correlation between number of mutations and time since divergence; however, the actual rate of genetic change is not constant. Occasionally, there can be a rapid accumulation of changes, while at other times the rate can slow. Therefore, using just differences in genetic material is not an accurate measure of time.

Mutations are not always neutral and can be affected by natural selection. A mutation may give rise to a phenotype that will be selected against due to selection pressures. Therefore, the mutation rate of genes and proteins will not be constant.

As seen in Figure 3, some sections of DNA mutate at different rates compared to others. Cytochrome c is a protein coded by the *CYCS* gene and displays a slower mutation rate compared to haemoglobin and fibrinopeptides. A slower mutation rate indicates a gene sequence that produces a protein essential for survival. **Conserved genes** are those that are essential for survival and rarely accumulate mutations. Highly conserved sequences are often used for comparative DNA analysis because they are found in many species.

conserved gene a gene that has remained relatively unchanged throughout evolution

Mitochondrial DNA

In sexually reproducing species, all mitochondria are inherited from the mother. The mitochondria (like the chloroplasts in plants and algae) are able to self-replicate. Like the bacteria from which they are thought to have originated, mitochondria contain a circular molecule of **mitochondrial DNA (mtDNA)** that contains the code for the enzymes essential for cellular respiration.

mitochondrial DNA (mtDNA)
DNA located in the mitochondria

In humans, the mitochondrial genome consists of about 16 500 base pairs that are known to code for 13 proteins, 22 tRNAs and 2 rRNAs. During fertilisation, only the nucleus of the sperm (containing no mitochondria) enters the ovum. Even when sperm cells are injected directly into the cytoplasm of the ovum (during IVF), no paternal mtDNA will be found in the zygote.

nuclear DNA
DNA found in the nucleus of the cell

Using the mtDNA to determine the maternal relationship between two groups of organisms has many advantages over **nuclear DNA**, as follows.

- Direct genetic lines can be traced, since only maternal mtDNA is passed on to offspring.
- The mtDNA has a higher mutation rate because there are no repair mechanisms like there are in nuclear DNA. This makes it easier to compare differences between closely related individuals. By determining the rate at which this mutation occurs, a molecular clock can be calculated and can give the point in time at which different groups diverged.
- Mitochondria are present in large numbers in each cell, so fewer cell samples are required to obtain large amounts of mtDNA.

Although the human mitochondrial genome was one of the first human chromosomes to be analysed and sequenced in its entirety, only recently has technology been able to compare the whole genome sequence.

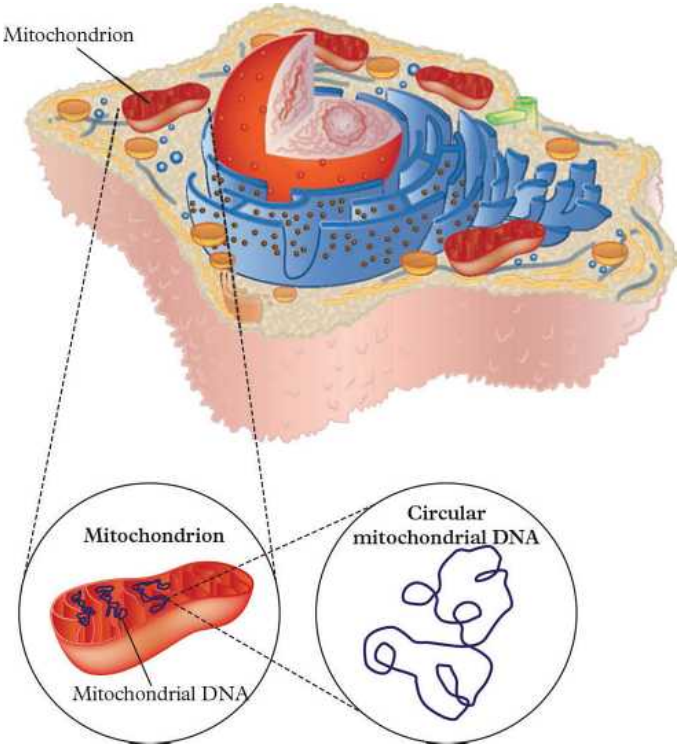


FIGURE 4 Circular strands of DNA (mtDNA) are found in mitochondria. Mitochondria are passed on from the maternal line only.

CHECK YOUR LEARNING 13.2

Describe and explain

1 How does mtDNA differ from nuclear DNA?
2 What are the limitations of the molecular clock hypothesis?
3 For conserved genes such as *CYCS*, which produces cytochrome c, explain why they remain relatively unchanged over evolutionary time.
4 What is the difference between conservative, semi-conservative and non-conservative substitution?

Apply, analyse and compare

5 Compare homologous and analogous structures.
6 Why is mtDNA useful in determining relationships between species that share a recent common ancestor?

Design and discuss

7 Discuss the advantage of DNA sequence comparison over comparative anatomy in order to determine species relatedness.

13.3 Phylogenetic trees

KEY IDEAS

In this topic, you will learn that:

+ phylogenetic trees are based on derived characteristics or differences in molecules
+ phylogenetic trees can be rooted or unrooted and may be scaled or unscaled.

phylogeny
the evolutionary history of taxonomic groups

derived characteristic
a feature that sets members of that clade apart from other individuals

cladistics
a method of grouping organisms that uses evolutionary lines of descent instead of structural similarities

phylogenetic tree
a visual representation of the likely hypothesis of evolutionary relationships between different organisms, showing the path through evolutionary time from a common ancestor to the different taxa

The evolutionary history of a species over time is called **phylogeny**. A phylogenetic classification system shows the evolutionary history of a species or group. It shows the timeline where different groups separated from a recent common ancestor.

In this classification system, it is assumed that as groups of organisms diverge and evolve from a common ancestral group, they retain some unique common characteristics, known as **derived characteristics**. For example, most mammals have hair, so this is considered a derived characteristic since it is unique to all mammals. The basic idea behind **cladistics** is that members of a group share a common evolutionary history and are more closely related than members of another group.

FIGURE 1 A leopard can purr just like a domestic cat can. This is a derived characteristic.

In this system, organisms are classified according to the order in time that groups arise along a **phylogenetic tree**. The phylogenetic tree is composed of a series of branches where each separation is defined by a new feature. For example, the reptile, horse, seal, dog and cat are all vertebrates that have a mixture of characteristics that existed in the common ancestor of all vertebrates (pentadactyl limbs). At one point in the evolutionary history, the ancestor of mammals (horse, seal, dog and cat) evolved hair and mammary glands, while the ancestor of reptiles did not. In more recent evolutionary history, the ancestor of the seal, dog and cat evolved skeletal changes that the horse ancestor did not. This continued until the early ancestors of each organism diverged from each other.

Phylogeny and classification

The Linnaean system of classification, developed by Carl Linnaeus around 1760, organises organisms into a hierarchy of groups (taxa) to reflect their evolutionary relationships. Those groups are:

- Domain
- Kingdom
- Phyla
- Subphyla
- Class
- Order
- Family
- *Genus*
- *Species*.

Taxonomy is the study of classifying organisms based on their **shared characteristics**, which also implies their evolutionary relationship.

shared characteristic
a feature that all members of a group have in common

Relying on comparative anatomy for phylogenetic classification has limitations. For example, species who share a recent common ancestor may have lost morphological features or diverged so much that comparative anatomy is not always possible. The other limitation is that distantly related species, due to convergent evolution, develop similar features that are not necessarily evidence of evolutionary relatedness. There is also the incidence of traits being gained and lost multiple times during the evolutionary history of one single species.

Phylogenetic trees

The evolutionary relationship between two or more organisms over time can be shown using a phylogenetic tree. These diagrams use the similarities between organisms (homologous structures or molecular sequences) to represent the length of time that has passed since the two species shared a recent common ancestor.

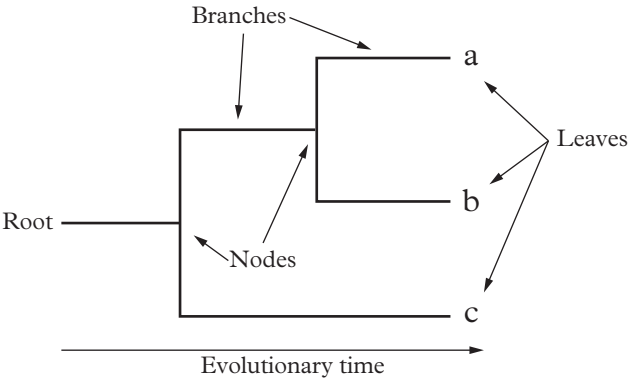


FIGURE 2 The ancestral line of each taxon is represented by a branch, with the taxon name on the leaves. The nodes represent a common ancestor.

Figure 2 shows the following features of phylogenetic trees:

- The individual taxonomic group (known as a *taxon*, plural *taxa*) is placed on a **leaf**.
- The ancestral line is represented by a **branch**.
- Each common ancestor is represented by a **node** (the point at which two branches combine).
- Each group of taxa that evolve from a common ancestor is called a **clade**. A clade includes a common ancestor and all its descendants.
- The beginning of the tree is the '**root**' and represents the last common ancestor.
- The first species to diverge (species c) from the original common ancestor is known as the **outgroup**.
- Species that share a recent common ancestor (species a and b) are considered sister taxa.

leaf
part of the phylogenetic tree that represents a taxonomic group (taxon)

branch
part of the phylogenetic tree that represents a lineage from a divergent event

node
part of the phylogenetic tree that represents the common ancestor

clade
a group of taxa and their common ancestor

root
the start of a phylogenetic tree, representing the ancestral lineage

outgroup
the first taxon to diverge from the original common ancestor in a phylogenetic tree

The last common ancestor is typically unidentified on a phylogenetic tree. For all life on Earth, the last common ancestor is still unknown, but is suggested to be an archaea.

Today, most phylogenetic trees are built based on DNA or RNA sequence data instead of being derived from anatomical characteristics. In Figure 3, organisms are grouped based on the number of differences in their nucleotide sequences. This molecular data, alongside comparative anatomy and ecological data, helps to strengthen our understanding of evolution and aids in the refinement of the classification.

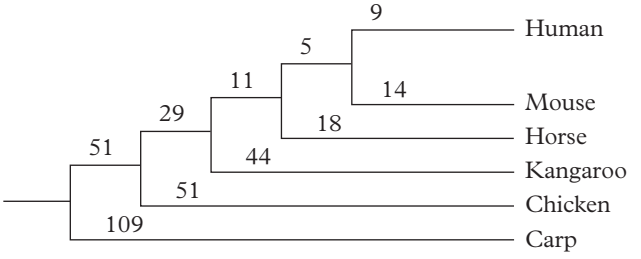


FIGURE 3 A phylogenetic tree constructed from amino acid substitutions in the alpha-haemoglobin chain. The numbers on the nodes and branches indicate the number of substitutions.

When constructing phylogenetic trees, it is assumed that the tree with the simplest explanation, the least number of evolutionary events, is most likely to show the correct evolutionary relationship. This concept is known as parsimony. Often scientists construct phylogenetic trees using large numbers of characteristics and can come into conflict. It is vitally important for them to consider parsimony when constructing evolutionary trees.

Types of phylogenetic trees

There are two main types of phylogenetic trees – rooted and unrooted trees – and both of these trees may be either scaled or unscaled. Scaled phylogenetic trees are called **phylograms** and unscaled trees are called **cladograms** (Figure 4). The length of the branch in a phylogram indicates the number of nucleotide changes since divergence, but this is not the case for cladograms.

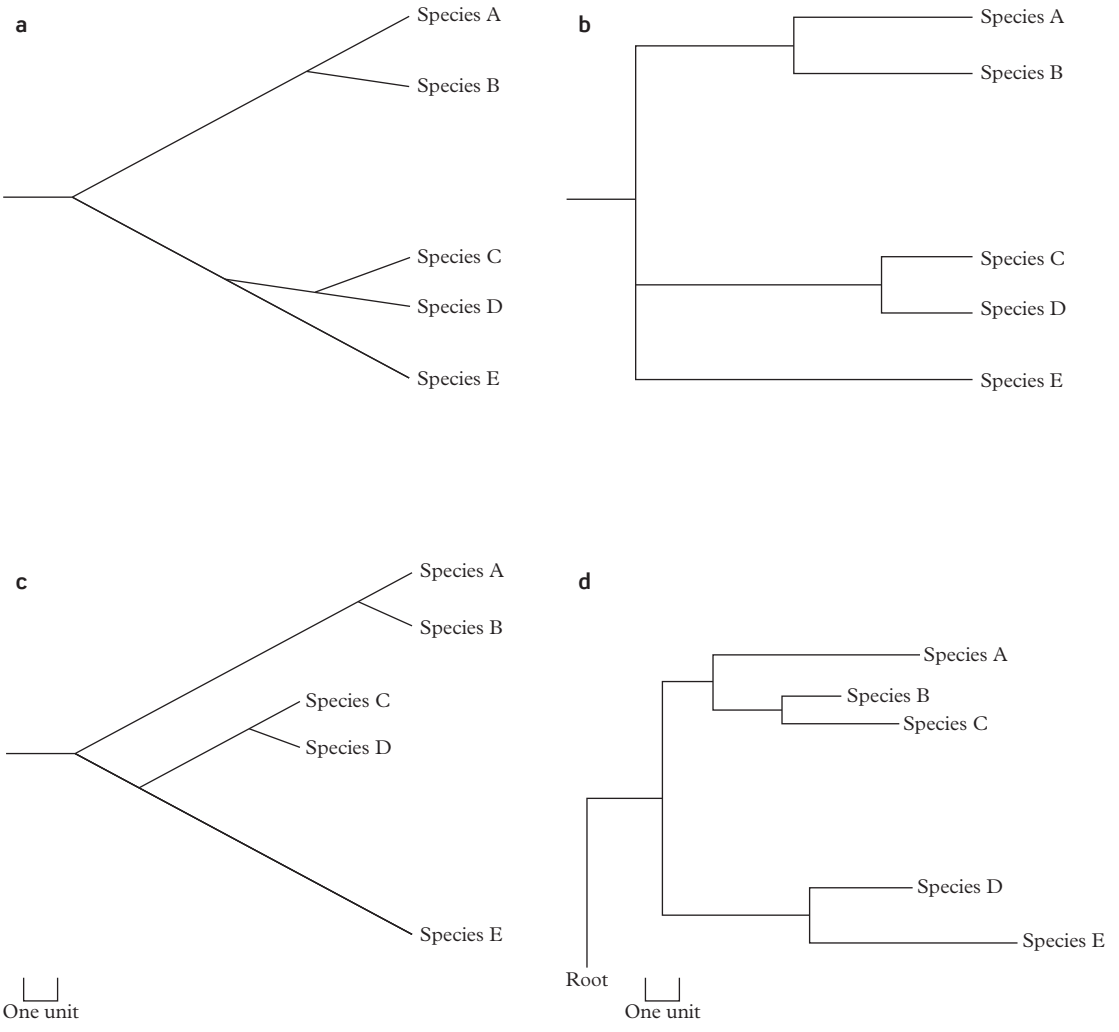
phylogram
a scaled phylogenetic tree

cladogram
an unscaled phylogenetic tree

Phylogenetic trees may be horizontal, vertical or the branches may be diagonal. Although the format of the tree may be different, the branch points and their positions relative to one another should represent the same information.

Study tip

The order of the branching from the root (common ancestor) to the tip (taxa) represents the order of evolutionary divergence.



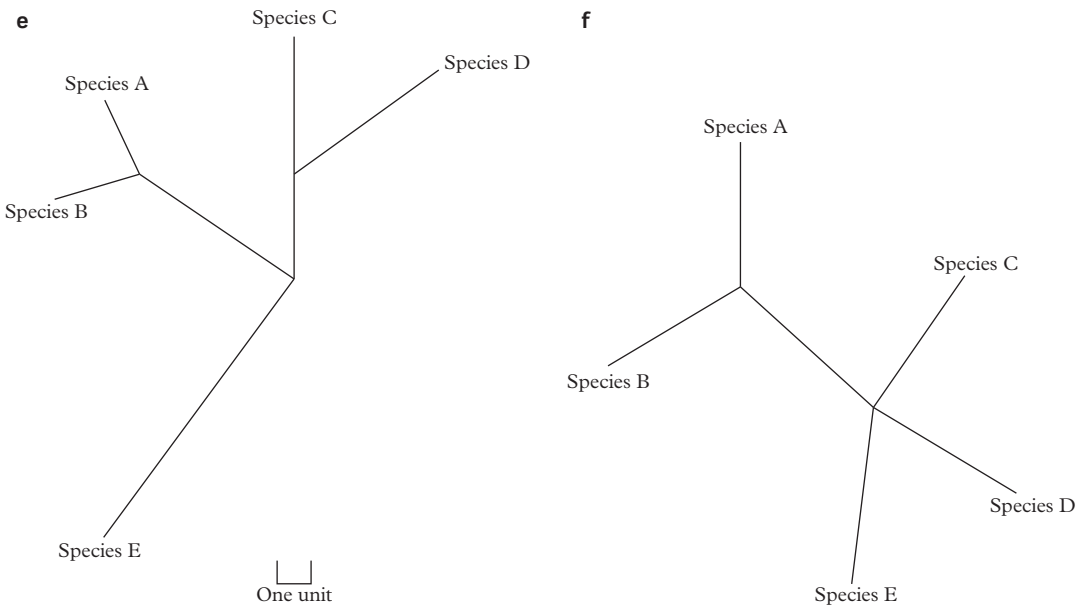


FIGURE 4 Phylogenetic trees are represented by different formats. **a** and **b** Unscaled branches that do not indicate the number of differences or time are known as cladograms. **c** and **d** Scaled branches are known as phylograms. **e** and **f** There are also unrooted trees that are both scaled (phylogram) and unscaled (cladogram).

WORKED EXAMPLE 13.3A

CONSTRUCTING A PHYLOGENETIC TREE USING HOMOLGOUS STRUCTURES

A table of organisms with a list of their different features has been provided. Construct a phylogenetic tree to show the relatedness of these species, based on the data provided in Table 1.

TABLE 1 Derived characteristics of the cat, horse, reptile, seal and dog

	Hair	Carnassial teeth	Retractable claws	Involuted cheek teeth	Flippers
Cat	Yes	Yes	Yes	Yes	No
Horse	Yes	No	No	No	No
Reptile	No	No	No	No	No
Seal	Yes	No	No	Yes	Yes
Dog	Yes	Yes	No	Yes	No

SOLUTION

1 There are several things to consider from the data you have been given.
First, identify the outgroup, the species with no or little relationship to the other species. This becomes the first branch of the tree. From Table 1, the reptile is the outgroup since it has no similar characteristics to the other organisms. Then try to identify the derived trait that is shared between the largest number of organisms. In this case, the characteristic is hair, being shared by four out of five of the organisms.

Video
Worked example 13.3A: Constructing a phylogenetic tree using homologous structures

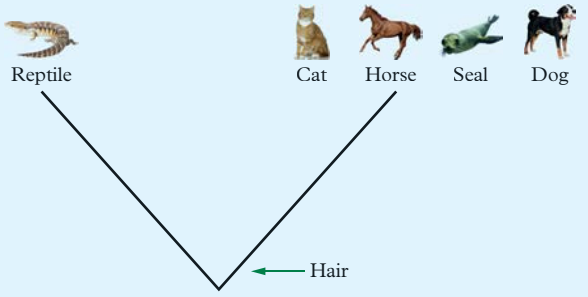


FIGURE 5 Identifying the outgroup and most commonly shared characteristic

2 Next, identify the derived trait shared by the next largest group of organisms, and so on.

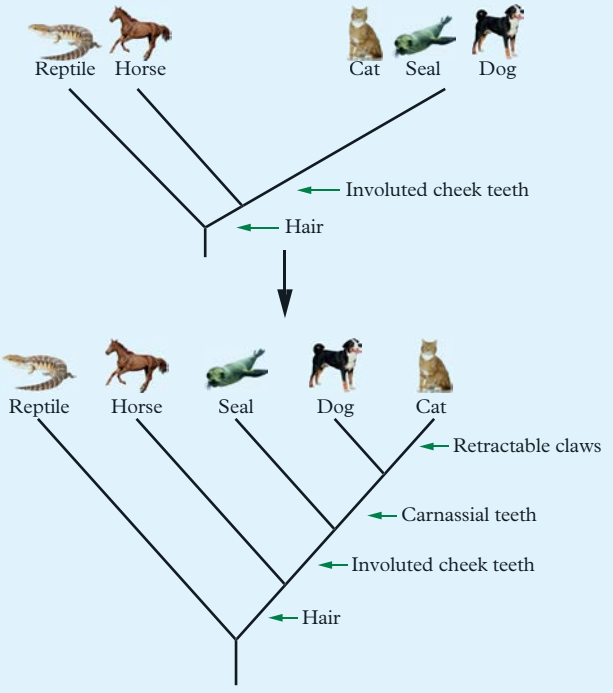


FIGURE 6 Building the phylogenetic tree based on commonly shared characteristics

3 For traits that are derived, but not shared (only found in a single species), you can still put them on the tree, after the node. This is shown with the flippers on the branch for the seal in Figure 7.

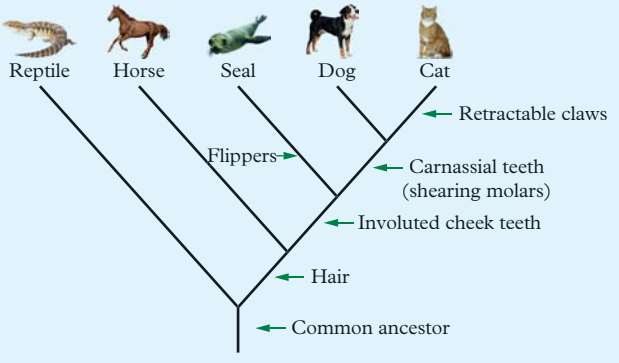


FIGURE 7 The final phylogenetic tree

WORKED EXAMPLE 13.3B

CONSTRUCTING A PHYLOGENETIC TREE USING MOLECULAR SEQUENCES

Use the table from Worked example 13.2 to construct a phylogenetic tree that shows the relatedness between the four species of bears.

TABLE 2 Amino acid differences in the lyst protein among four bear species

	Panda bear	Black bear	Brown bear	Polar bear
Panda bear	0			
Black bear	33	0		
Brown bear	34	1	0	
Polar bear	40	7	8	0

SOLUTION

1 The difference in the amino acids of the lyst protein can be used to create a phylogenetic tree. Use Table 2 to identify the outgroup. This is the bear with the largest number of different amino acids to each of the other bears – the panda bear. It has 40 different amino acids to the polar bear, 34 to the brown bear and 33 to the panda bear.

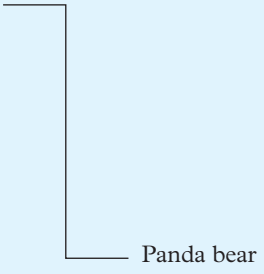


FIGURE 8 Panda bear as the outgroup

2 Now find the bears that are the closest to each other. The brown and black bear have only one amino acid difference between them. These are a group of their own, separate to the panda bear branch. They could look like this:

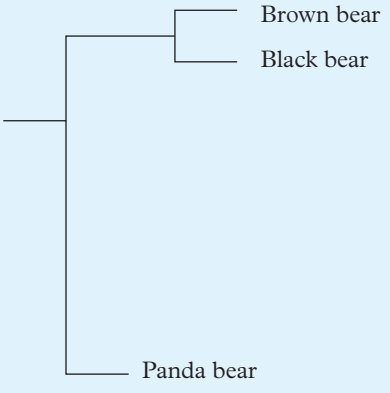


FIGURE 9 Brown bear and black bear forming a separate group

Video
Worked example 13.3B: Constructing a phylogenetic tree using molecular sequences

3 Now you need to place the polar bear on the tree. Is it closer to the panda bear or brown and black bear group? It is 40 amino acids different from the panda bear and only 7 different from the black bear and 8 different from the brown bear. So it will be furthest from the panda and close to the brown and black bear. It should look something like this:

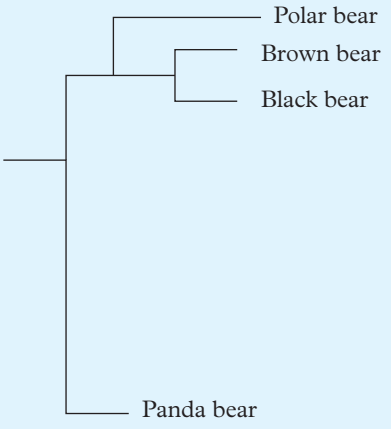


FIGURE 10 Polar bear branch furthest from panda bear and closest to brown and black bear

CASE STUDY 13.3

Phylogenetics and falsification

Angiosperms, also known as flowering plants, are one of the most diverse plant groups with at least 260 000 species classified into 453 families. The classification of angiosperms into their various families began in 1789 by a French botanist named Antoine Laurent de Jussieu. Classifications were based on structural morphology and continuously revised through the 1900s.

Until recently, figworts (Scrophylariaceae family) were the eighth-largest family of angiosperms and one of the original families proposed by de Jussieu. Following de Jussieu, the family grew from 16 genera to 275 genera and more than 5000 species.

Three chloroplast genes were sequenced and examined. It was found that of the 5000 figwort species, less than half showed enough similarity to be considered part of the figwort family clade. Taxonomists moved various figwort genera to different families, created new families and merged existing families based on this new data. After this molecular analysis, the figwort family became only the 36th-largest family of angiosperms. This suggests that the morphological classification previously used included analogous structures.

Reclassification of the figwort family was made possible with molecular analysis. Updated phylogenetic trees of the figwort family are now based on this molecular data rather than structural morphology.

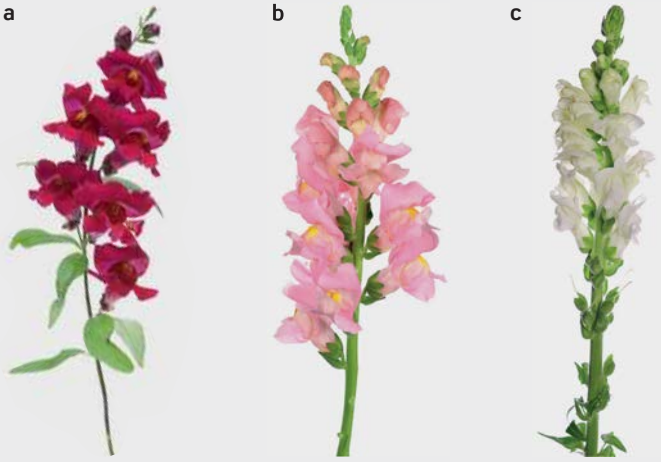


FIGURE 11 **a** Red snapdragon; **b** pink snapdragon; **c** white snapdragon. Common snapdragon (*Antirrhinum majus*) has been removed from the figwort family and is now in the plantain family.

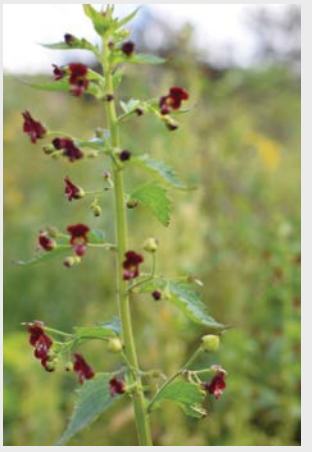


FIGURE 12 Nettle-leaved figwort (*Scrophulara peregrine*) has remained in the figwort family.

CHECK YOUR LEARNING 13.3

Describe and explain

1 What does a phylogenetic tree represent?

2 How is a clade different from sister taxa?

3 Explain the difference between a branch and a node.

Apply, analyse and compare

4 Compare scaled and unscaled phylogenetic trees. What are the main differences?

5 How is phylogeny related to the Linnaean classification system?

Design and discuss

6 **a** Construct a phylogenetic tree using the data in Table 3.

b What feature separates primates from amphibians?

c Which species was first to diverge from the common ancestor?

d Which species will share the most similarities in their DNA with the crocodile?

7 All organisms share a common ancestor if you go back far enough in evolutionary time. The last universal common ancestor (LUCA) of all life on Earth has been hypothesised. Research one current hypothesis for LUCA and describe your findings.

TABLE 3 The derived characteristics of different organisms

	Vertebrae	Bony skeleton	Four limbs	Hair	Amniotic egg	Eggs with shells
Ray-finned fish	Yes	Yes	No	No	No	No
Amphibians	Yes	Yes	Yes	No	No	No
Rodents and rabbits	Yes	Yes	Yes	Yes	Yes	No
Primates	Yes	Yes	Yes	Yes	Yes	No
Sharks	Yes	No	No	No	No	No
Birds	Yes	Yes	Yes	No	Yes	Yes
Crocodiles	Yes	Yes	Yes	No	Yes	Yes

CHAPTER 13

Review

Chapter summary

13.1
- Homologous structures are physical structures in organisms that are the same, but may have different functions. They are used as evidence of evolution.
- Vestigial structures are structures that remain in an organism, but are no longer used. They are indications of a last common ancestor.

13.2
- Differences between nuclear DNA, mtDNA and amino acid sequences can be used to determine relationships between different species.
- The molecular clock hypothesis is used to indicate time since divergence, but it has its limitations.

13.3
- Phylogenetic trees can be constructed from derived traits of organisms or molecular sequences.
- Phylograms are scaled phylogenetic trees and cladograms are unscaled phylogenetic trees.
- Construction of phylogenetic trees is based on the concept of parsimony, the tree with the simplest explanation.

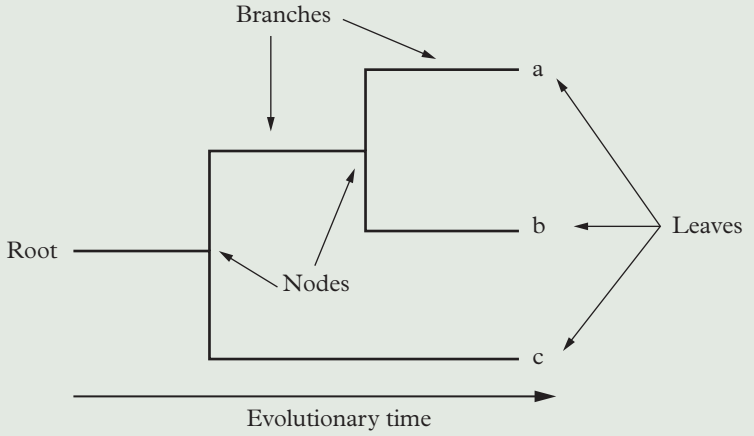


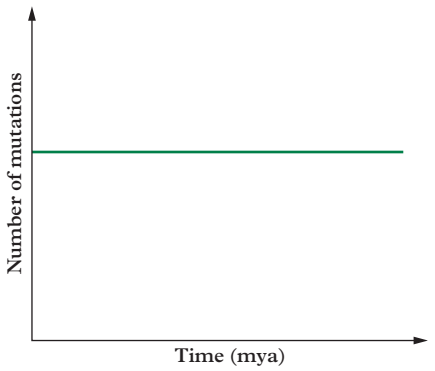
FIGURE 1 The basic structure of a phylogenetic tree

Revision questions

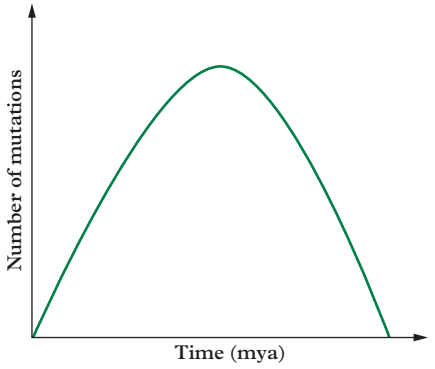
Multiple choice

1 Which graph best represents the rate of accumulated mutations over time – that is, the molecular clock hypothesis?

A



B



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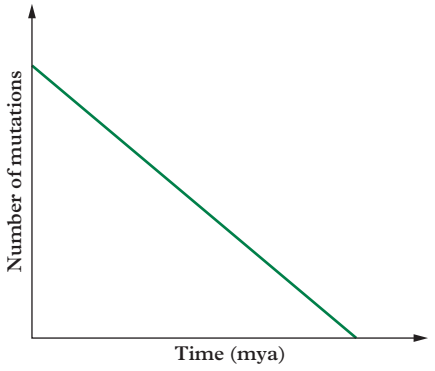

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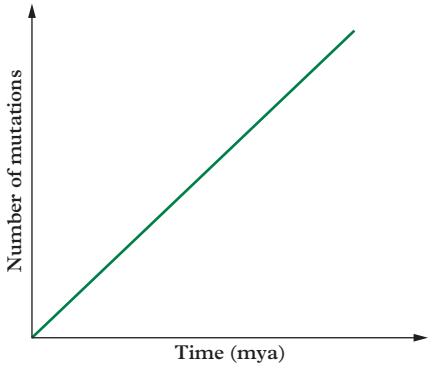

2 Cladograms are different from phylograms because of which of the following?

A Cladograms include an evolutionary timescale.

B Cladograms are only based on molecular evidence.

C Cladograms are unscaled.

D Cladograms are unrooted and unscaled.

3 Based on Figure 2, which of the following statements is true?

A Species E is more closely related to Species C than Species D.

B Species C and D are more closely related than Species A and B.

C Species A and B share the most recent common ancestor.

D Species D is an outgroup.

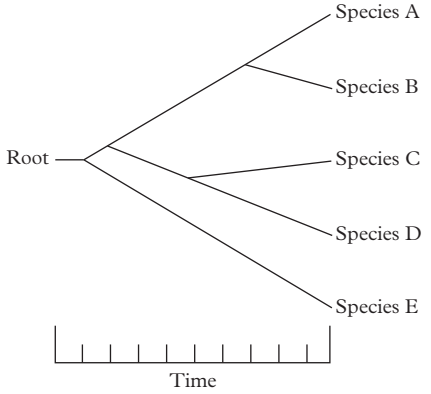

FIGURE 2 A phylogenetic tree

4 Based on Figure 3, which species is most closely related to Species D?

A Species E

B Species C

C Species B

D Species A

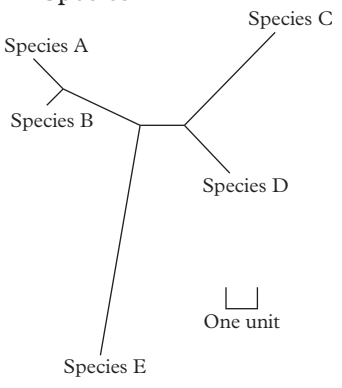

FIGURE 3 A phylogenetic tree

5 What is the name given to the study of the similarities and differences between the anatomies of animals?

A Molecular homology

B Comparative anatomy

C Homologous structures

D Heterogeneous structures

6 An amino acid substitution occurred due to a DNA mutation. The amino acid changed but the substituted amino acid had a similar shape to the original amino acid. There was a small change to the protein but it was able to carry out its original function. This amino acid substitution would be considered:

A silent.

B non-conservative.

C semi-conservative.

D conservative.

7 Mitochondrial DNA is different to nuclear DNA because mitochondrial DNA has:

A more genes.

B a faster mutation rate.

C repair mechanisms.

D a paternal lineage.

Short answer

Describe and explain

8 Explain how genetic divergence gives rise to homologous structures.

9 Describe the four different types of phylogenetic trees.

10 Explain how amino acid differences in a protein can indicate evolutionary relationships.

11 Describe how vestigial structures arise.

Apply, analyse and compare

12 Compare mtDNA and nuclear DNA for molecular homology analysis.

13 Construct a phylogenetic tree using the data provided in Table 1.

TABLE 1 Data for phylogenetic tree

	Cells	Legs	Antenna	Wings
Worm	Yes	0	0	0
Spider	Yes	8	0	0
Carpenter ant	Yes	6	2	4
House fly	Yes	6	2	2
Dragonfly	Yes	6	2	4

14 Analyse the difference between structural morphology data and molecular evidence when constructing phylogenetic trees. Are there any limitations?

Design and discuss

15 Use the following information and Table 2 on the next page to answer the questions below.

Evolutionary biology and the relatedness of species is based upon the accumulation of evidence. Similarities and differences can be examined between species by using fossil evidence, patterns of embryological development and patterns of biogeographic distribution of various groups of organisms.

Comparisons of the base sequences of homologous genes in a range of different kinds of organisms and of the amino acid sequences of their homologous proteins allow inferences to be made about degrees of relationship of various groups by evolutionary descent.

a Why is cytochrome c considered conserved?

b Which species is most closely related to the snapping turtle? Explain.

c Which two species shared the most recent common ancestor? Explain using data.

d Yeast has many differences in the cytochrome c molecule compared to all of the other species. What does this suggest about yeast?

e What are the limitations of this data?

f Construct a phylogenetic tree for a snapping turtle, moth, duck, rabbit and chicken based on the information provided in Table 2.

TABLE 2 A comparison of the number of different amino acids in the cytochrome c molecule from a variety of species

	Human	Monkey	Pig	Horse	Dog	Rabbit	Kangaroo	Chicken	Duck	Rattlesnake	Snapping turtle	Tuna	Moth	*Neurospora*	*Candida*	Yeast
Human	0															
Monkey	1	0														
Pig	10	9	0													
Horse	12	11	3	0												
Dog	11	10	3	6	0											
Rabbit	9	8	4	6	5	0										
Kangaroo	10	11	6	7	7	6	0									
Chicken	13	12	9	11	10	8	12	0								
Duck	11	10	8	10	8	6	10	3	0							
Rattlesnake	14	15	20	22	21	18	21	19	17	0						
Snapping turtle	15	14	9	11	9	9	11	8	7	22	0					
Tuna	21	21	17	19	18	17	18	17	17	26	18	0				
Moth	31	30	27	29	25	26	28	28	27	31	28	32	0			
Neurospora	48	47	46	46	45	46	49	47	46	47	49	48	47	0		
Candida	21	21	20	21	49	20	21	21	21	21	23	48	47	42	0	
Yeast	45	45	45	46	45	45	46	46	46	47	49	47	47	41	27	0

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Exam essentials

Responding to questions

In your exam, you may be expected to justify an answer.

Justifying your answer

To justify your answer, you need to provide evidence to support your decision. This can be done through a series of short steps. The number of steps is usually directly related to the number of marks in the question. A well-justified response would include:

- data from the stem of the question
- a statement that shows how the data and the question are linked
- a link back to the question.

The following question is taken from the 2020 VCE Biology Examination. Read the question carefully, then consider whether evidence has been provided to justify the responses.

QUESTION 8a (2020 VCE Biology Written examination)

a Molecular homology can be used to construct a phylogenetic tree.

Based on the information above, state which **two** species of cichlid fish would be expected to have the most similar amino acid sequences in their proteins. Justify your answer. 3 marks

Source: 2020 Biology Written Examination Question 8a, reproduced by permission © VCAA

Response 1

- *Amatitlania siquia* and *Hypsophrys nematopus* share the most recent common ancestor at 25 mya.
- Therefore, they will have less time to accumulate differences in the DNA.
- Therefore, they will have more amino acids in common than other species.

Uses names and data from the question stem.

Relates the data to the question and a logical sequence of dot points.

This response will receive 3 marks since it correctly identifies both species that more recently shared a common ancestor in the diagram and linked this to having less time to accumulate differences in the DNA.

Response 2

The two species that have the most recent common ancestor at 25 mya and they will have the most in common.

Needs to relate back to the question and provide linking information that justifies the decision made.

This response would not receive any marks since it does not name the two species or explain why they would have the most similar amino acid differences.

Think like an examiner

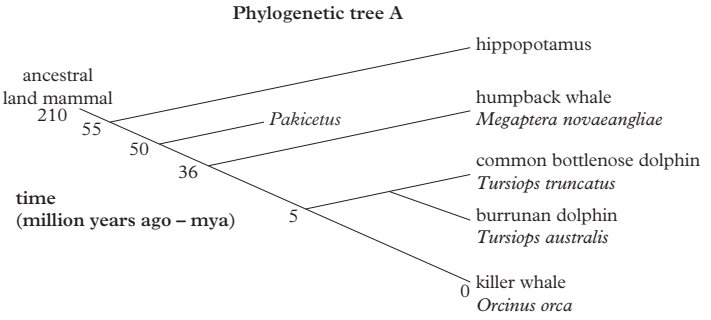
To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 9a&b (2018 Biology Written Examination)

Cetaceans (whales, porpoises and dolphins) are marine mammals belonging to the order Artiodactyla (even-toed hoofed mammals). The closest living relatives of cetaceans are hippopotamuses. Phylogenetic tree A summarises the evolutionary relationships of four present-day cetacean species and the hippopotamus.



a What does the length of the line that represents the evolution of *Pakicetus* suggest? 1 mark

The line is short and this means they are not alive anymore.

b A fossil named *Ambulocetus* was found in 1992 and dated at 49 million years old. Some palaeontologists believe that it is a transitional fossil between the ancestral land mammal shown in Phylogenetic tree A and present-day cetaceans.

Predict **two** structural features of the *Ambulocetus* fossil that would provide evidence to support the hypothesis that it is a transitional fossil and suggest a survival advantage of each feature. 3 marks

The transitional fossil will have gills to help it breathe in water and strong legs for walking.

Source: 2018 Biology Written Examination Question 9a&b, reproduced by permission © VCAA

Marking guide

Question 9 a	- 1 mark for stating that the short line suggests that *Pakicetus* is extinct.
Question 9 b	- 1 mark for a feasible feature of a transition fossil in an aquatic environment. - 1 mark for a feasible feature that is an advantage in a terrestrial environment. - 1 mark for stating how both features were an advantage to the organism's survival.

Fix the response

Consider where you did and did not award marks in the above responses. How could the response be improved?

Write your own responses to the same questions to receive full marks from an examiner.

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CHAPTER 14

Evolution of humans

Before Darwin published his book *On the Origin of Species by Means of Natural Selection*, questions were raised about the origin of humans. While it is no longer claimed that we evolved from apes (we share a common ancestor), the story of our ancestors continues to evolve as new evidence is found. Many species of humans often coexisted with each other. More recently, DNA evidence has suggested that gene flow occurred between the different species of human outside of Africa. It is only in the last 30 000 years that a single species of humans survived. Out of all the different species of humans and their ancestors that have existed in the last 7 million years, why were *Homo sapiens* the only one that survived?

KEY KNOWLEDGE

- → the shared characteristics that define mammals, primates, hominoids and hominins
- → evidence for major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure
- → 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
- → 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.

Source: *VCE Biology Study Design (2022–2026) reproduced by permission © VCAA*

FIGURE 1 Human evolution can be seen through the changes in skull shapes over time.

GROUNDWORK QUESTIONS

Before you start this chapter, try the following groundwork questions. If you need help with any of the questions, have a go at the corresponding groundwork resource on your obook pro.

14A What features do humans have that classify us as mammals?

14A Groundwork resource
Mammals

14B What is the binomial system of classification?

14B Groundwork resource
Binomial classiþcation

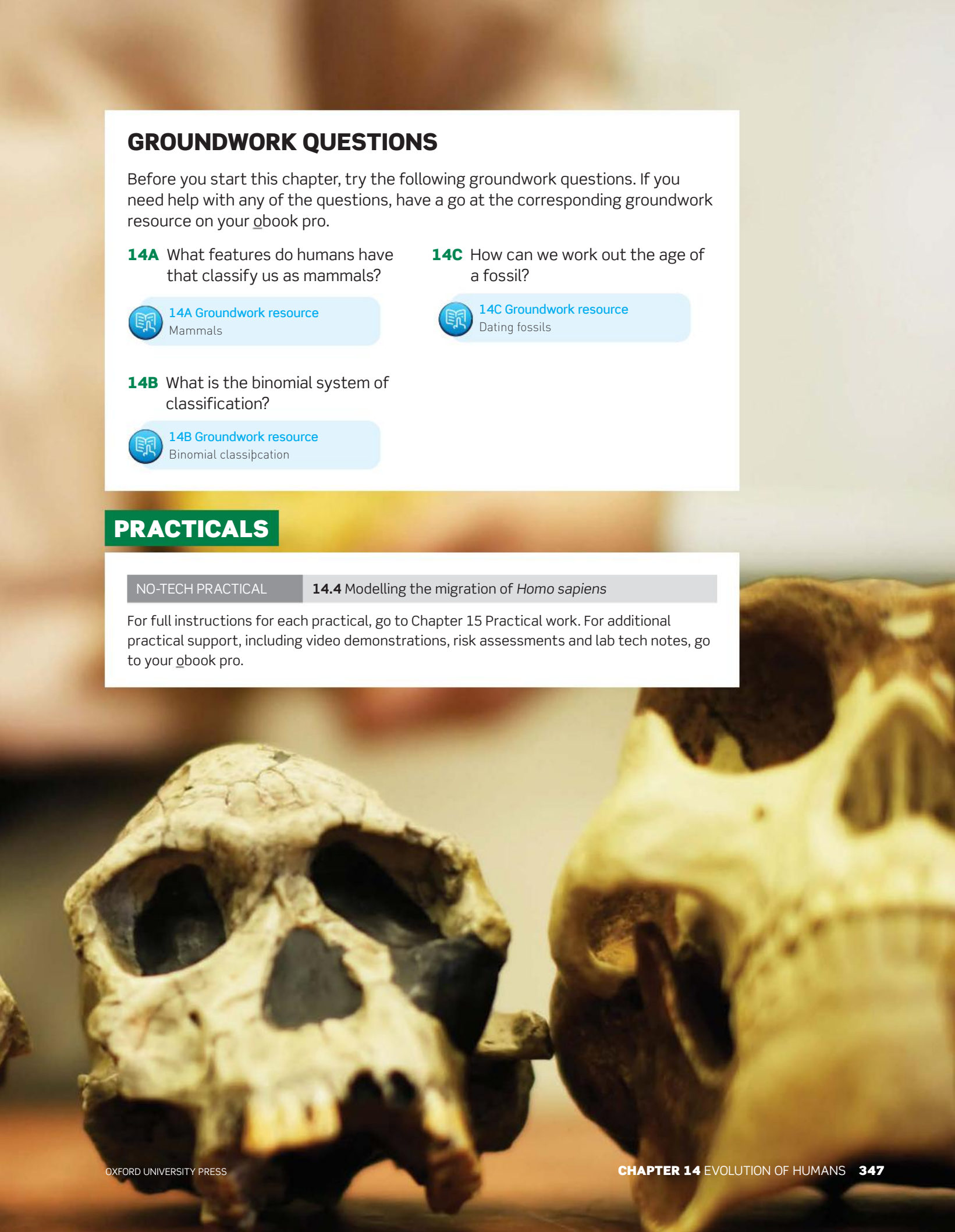
14C How can we work out the age of a fossil?

14C Groundwork resource
Dating fossils

PRACTICALS

NO-TECH PRACTICAL	**14.4** Modelling the migration of *Homo sapiens*

For full instructions for each practical, go to Chapter 15 Practical work. For additional practical support, including video demonstrations, risk assessments and lab tech notes, go to your obook pro.



14.1 Shared characteristics from mammals to hominins

KEY IDEAS

In this topic, you will learn that:

- all mammals share common characteristics, including the production of milk from mammary glands
- primates (including humans) share common characteristics, including opposable thumbs and flattened nails rather than hollow claws
- hominoids are a subdivision of primates that includes the great apes and humans
- hominins are a tribe of modern *Homo sapiens* and all their extinct ancestors.

Modern humans (*Homo sapiens*) share common ancestors with other mammals, from whales and elephants to mice and bats. As a result, all mammals inherited common characteristics from their ancestors.

Mammals

The dividing of organisms into groups according to their characteristics allows a shared understanding of evolution. Although not the largest group of organisms on Earth, mammals are one of the biggest and most diverse group of vertebrates with a set of unique defining features.

Three mammalian groups

placentals
a subgroup of mammals that provide nutrients and remove waste from a foetus through a placenta

marsupials
a subgroup of mammals that nurse their young in a pouch

monotremes
a subgroup of mammals that lay eggs; includes the platypus and echidna

Mammals are broken into three groups: **placentals**, **marsupials** and **monotremes**.

One of the oldest groups of mammals is the monotremes. Although their ancestors appeared approximately 220 million years ago in the fossil record, there are only two groups of monotremes still in existence today – four species of echidna and the platypus. These mammals do not give birth to live young; instead, they lay soft-shelled eggs through a single opening called a cloaca. Once the young hatch, they are able to crawl onto their mother's abdomen to lick milk off the mother's fur.

FIGURE 1 The echidna is a monotreme that lays eggs and feeds its young by secreting milk onto its stomach fur.

Marsupials are known for the front pouch found on the adult females. They give birth to immature young that must crawl along their mother's fur-covered skin and into the pouch or fold of skin. Once attached to the mammary nipple, the young remain there until they are large enough to survive on their own.

FIGURE 2 All female mammals are able to feed their young with milk from mammary glands.

The young of placental mammals are able to obtain nutrients via the placenta before they are born. The placenta is an organ attached to the uterus that provides oxygen and nutrients, and removes carbon dioxide and waste from the developing young. This enables the embryo to remain protected inside the mother's uterus for longer, allowing them to become relatively mature before they are born.

Common traits among mammals

Mammary glands

One of the key distinguishing characteristics of mammals is their ability to produce milk from mammary glands. These glands are modified and enlarged sweat glands that secrete milk containing proteins, sugars, fats and vitamins.

Hair

At some stage in their life, all mammals have hair growing from their body. This can appear in several different forms, including thick hair, long whiskers, defensive quills and even horns. The hair protects the mammals from the cold, protects the skin, and acts as camouflage or even as an extra sensor.

Middle ear bones

Inside the middle ear of mammals are three small bones: the malleus, incus and stapes (commonly called the hammer, anvil and stirrup). These small bones evolved from the lower jawbones of their ancestors and transmit sound vibrations from the outer membrane to the inner ear.

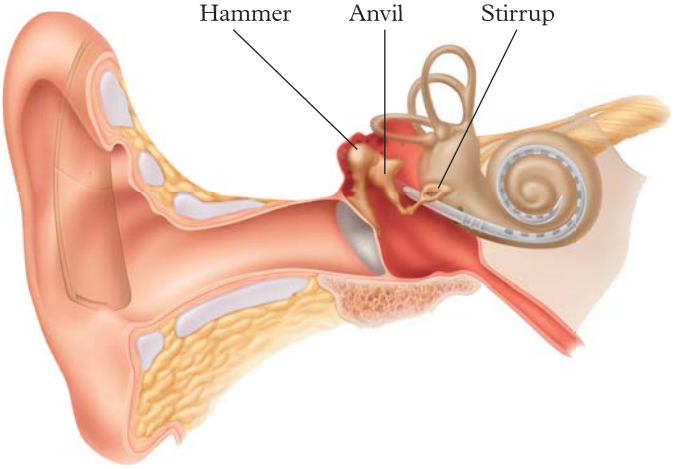


FIGURE 3 The three small bones found in the middle ear are common to all mammals.

Single jawbone

Most mammals have a powerful bite due to a single lower jawbone that connects directly to the skull. The four different types of teeth (incisors, canines, premolars and molars) are connected directly to the jawbone and can only be replaced once during a lifetime.

Other characteristics

There are many other characteristics possessed by mammals, but there are always exceptions between some species. For example, most mammals are able to maintain a constant body temperature; however, bats and mole rats use different methods to regulate their internal temperature. Constant body temperature cannot be described as a distinguishing characteristic of mammals since other groups of animals (bees, birds and the hawk moth) are also able to regulate their body temperature.

Sweat glands are unique to mammals, but not all mammals have them. Whales do not need them because they are able to cool themselves in the water. Pigs also do not have sweat glands.

Similarly, having a four-chambered heart or giving birth to live young are not unique features to all mammals. Birds and crocodiles have four-chambered hearts, sharks are able to give birth to live young, while monotreme mammals lay eggs.

CASE STUDY 14.1

Why does Australia have so many marsupials?

Australia has a marsupial population that is unique. This does not mean that marsupials evolved on this continent. The fossil record suggests that the first marsupials originally evolved in the northern supercontinent Laurasia during the Cretaceous period at least 125 million years ago. Approximately 66 million years ago, the marsupials made their way along a land bridge to South America and filled many of the niches not filled by placental mammals.

As recently as 40–35 million years ago, South America and Australia were connected to Antarctica. This allowed the marsupials to move through the temperate rainforest that covered Antarctica at the time, to reach Australia. This is supported by the 55-million-year-old fossil remains of both placental mammals and marsupial mammals found at Tingamarra (Queensland). These fossils are similar to those found in Antarctica and South America.

It is thought that the sometimes harsh environment of the Australian continent gave marsupial mammals an advantage since they were able to jettison their young from their pouch when times were tough. Placental mammals were unable to do this because the mothers had to wait until their long gestations were over before they could divert their resources to their own survival.

FIGURE 4 Early Australian marsupials such as *Djarthia* may have migrated from South America through Antarctica.

Primates

One order of placental mammals that include humans and the great apes are the **primates**. This group shares all the common characteristics of mammals along with some that are unique to primates. Many of these characteristics are adaptations for climbing trees.

Grasping fingers

Most primates have **pentadactyl limbs** with five individual digits that enable them to grasp tree branches. Some primates are able to touch the pad of the other four fingers with their first digit (the thumb). This structure is described as an **opposable thumb**. Not all primates have a fully opposable thumb. The chimpanzee, for example, has four elongated fingers with a relatively short thumb, preventing their thumb from being fully opposable. The ability to use individual fingers to grasp objects is a key characteristic of primates.

Flattened nails

The flattened fingernails that primates have at the end of their digits (especially the largest digit) are different to the rounded, hollow claws of other mammals. It is thought that nails and broad fingertips evolved to aid in grasping of food sources and small branches.

Stereoscopic vision

All primates have forward-facing eye sockets that allow their fields of vision to overlap. The slightly different angles of the eyes allow the primate (including modern humans) to judge how far away an object is located. This depth perception makes it easier for a primate to jump between tree branches.

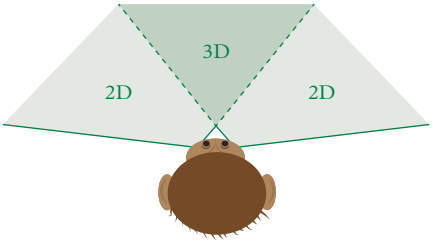


FIGURE 5 Primates have their eyes facing forward. The slightly different angles allow them to judge the distance to objects.

primates
a classification group of unique placental mammals that have common characteristics, including using individual fingers to grasp objects

pentadactyl limb
a limb with a single upper limb bone, two long bones on the lower limb and five individual digits (fingers or toes) at the end; these digits can be fused in some animals

opposable thumb
a thumb that can be placed opposite other fingers, allowing the organism to grasp with two digits

Study tip

Modern humans are classified as:

- Kingdom: Animalia
- Phylum: Chordata
- Class: Mammalia
- Order: Primates
- Family: Hominidae
- Genus: *Homo*
- Species: *sapiens*

FIGURE 6 Mountain gorillas have opposable thumbs and flattened fingernails that are common in primates.

Relatively large brain

All primates have relatively large brains compared to their body size. They are also heavily reliant on their vision rather than smell (like many other mammals). This correlates to a natural cleft (narrow opening) between the visual areas on each side of the brain that is unique to primates.

Collarbones

The clavicle (or collarbone) of primates is unique since it helps keep their arms out to the side. This allows primates to move their arms in more directions than other mammals that do not have collarbones.

Hominoids and hominins

While all primates have some characteristics in common, there are also many differences.

Hominoids

The order of primates is divided into many smaller groups. The group that includes the great apes (gorilla, chimpanzee, bonobo and the orangutan), gibbons and humans is called the **hominoids**.

hominoids
a classification group that includes modern humans and the great apes

FIGURE 7 Hominoids are able to walk upright and some can even walk on their knuckles, like this baby chimpanzee.

Locomotion

Compared to other primates, hominoids have a shortened spine relative to their body length, a wide chest and a broad pelvis. This makes them more stable when walking in an upright posture, walking on their knuckles or swinging along branches (brachiation). This partially upright posture allows them to keep their hands free when manipulating food or caring for their young.

No tail

One unique characteristic of hominoids is their lack of a tail. Instead, bony segments at the base of the spine fuse together to form a triangular **sacrum**.

sacrum
a small triangular bone at the end of the spine formed by the fusion of vertebrae

Hominins

A further subdivision of the hominoid grouping is the tribe of **hominins**. This group contains all members of genus *Homo* and all their extinct erect-walking ancestors. All hominins have structural differences, including fully erect posture, bipedal motion (two-legged walking) and larger brains. There are also some behavioural characteristics that are unique in hominins, including specialised tool use and the ability to communicate through language.

hominins
a classification group that includes modern humans and all their extinct ancestors

CHECK YOUR LEARNING 14.1

Describe and explain

1 Describe the characteristics that both the gorilla and kangaroo have in common.
2 Explain the differences between the kangaroo (marsupial) and the gorilla (placental mammal).
3 Describe the characteristics that are unique to the gorilla (primate).
4 Explain what an opposable thumb is.
5 Describe the characteristics that make humans a primate and not part of the canine family with a domesticated dog.

a

b

FIGURE 8 **a** Gorilla; **b** kangaroo

Apply, analyse and compare

6 Draw a table that compares the characteristics of hominoids and hominins.
7 Analyse the accuracy of the statement: 'Humans are hominoids and hominins.'
8 Compare the advantages and disadvantages of the reproductive method of marsupials, monotremes and placentals.
9 Who am I?
- I can feed my live young milk from mammary glands.
- I have three middle ear bones.
- I have flattened nails.
- I have collarbones.
- I have a tail.

Design and discuss

10 Use your knowledge of natural selection to discuss the advantage of mammary glands.
11 Discuss the advantage of stereoscopic vision for both herbivores and carnivores.
12 A student claimed that Australia has no placental mammals. Use examples to justify your agreement or disagreement with the statement.
13 The first mammals were very small creatures that lived in the same time period as dinosaurs. It is thought that a large meteor collided with Earth, causing small particles to fill the atmosphere and causing an ice age. Discuss what characteristics provided mammals with an evolutionary advantage.

14.2 The human fossil record

KEY IDEAS

In this topic, you will learn that:

- the human fossil record is as example of a classification scheme that is open to different interpretations
- interpretations of the human fossil record can be challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis*, and evidence of new putative *Homo* species.

direct evidence evidence-based or direct observation without any assumptions

putative evidence a hypothesis or logical inference made from observations

Study tip

Direct evidence is usually something that is actually seen or can be touched. Putative evidence refers to the suggestions made as a result of direct evidence.

All the information that you have learnt in the Biology course this year represents the current theories that are supported by a large body of evidence. This evidence may be **direct evidence** such as fossilised bones and teeth containing DNA, or **putative evidence** such as footprints, shell engravings, teeth marks and the discarded shells found in ancestral rubbish tips, called middens. It is important to understand the difference between direct and putative evidence.

As new evidence is discovered, scientists use it to challenge current models or frameworks. The similarities between different fossilised skulls allow scientists to hypothesise possible relationships between different species, changing the direct evidence of the fossil's existence to putative evidence of a relationship. The same is true for the theory of human evolution. The human family tree is constantly rearranged as new fossil evidence is discovered and new relationships are hypothesised. Part of being a good scientist is being able to adapt your viewpoint on a subject when new evidence is presented. You may even prove yourself and your work wrong. Scientists need to evolve with the science they produce.

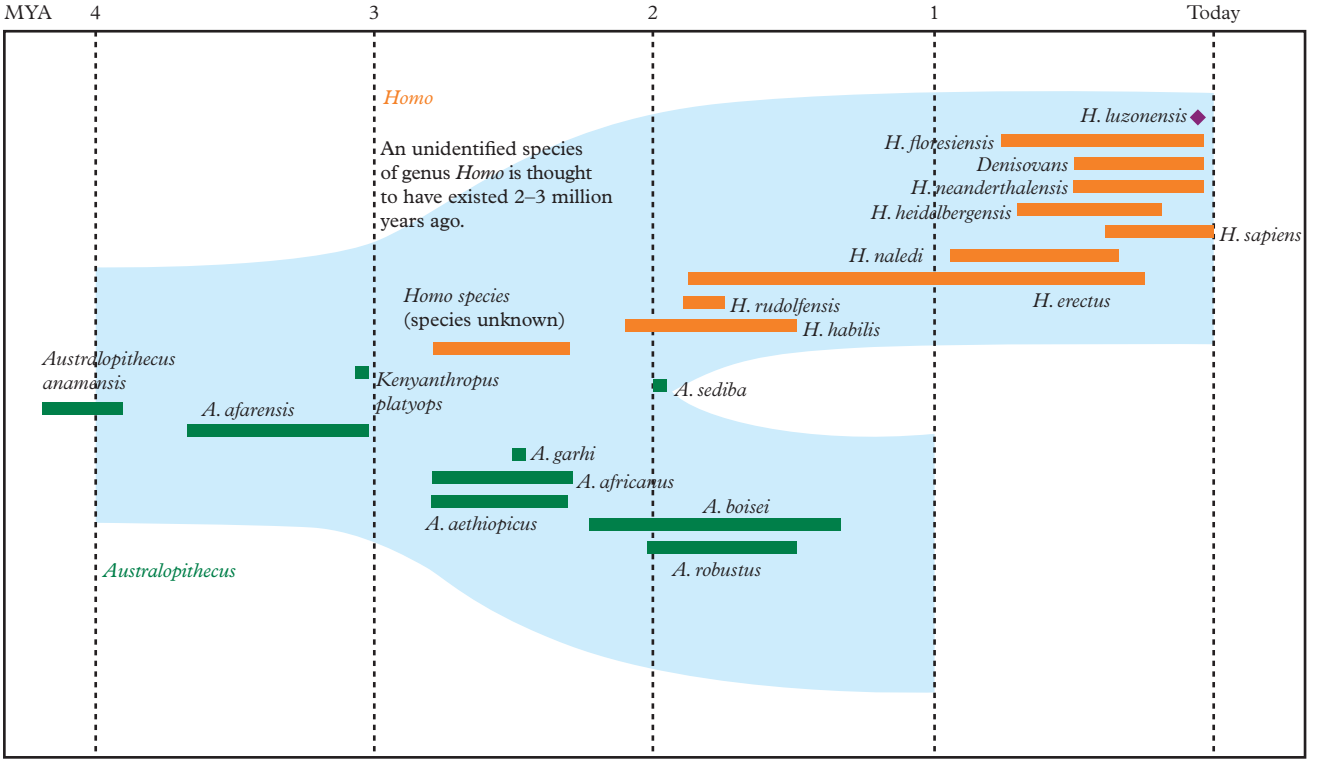


FIGURE 1 The fossilised remains of many species of humans have been discovered. Dating techniques suggest that some species coexisted.

The earliest humans

The earliest fossils that have been identified as unique to modern humans and different to the ancestors of chimpanzees were found in Africa. The earliest of these species (6 million years old) were found in the desert of Ethiopia in 1994. The skeleton was of a muscular female who was approximately 1 metre tall and weighed approximately 50 kg. 'Ardi', as the skeleton became known, had a small brain capacity (400 cc) and did not have the stiff wrist of a chimpanzee. Her upper pelvis was shorter and broader than an ape's, suggesting that she did not walk using her knuckles. Despite this, the nature of her hip and thigh muscles (as shown by the linkage marks on the pelvis) indicates that she would not have been able to run or jump as well as a modern human. Her grasping big toe suggested that she largely moved from tree to tree. Putative evidence suggests that this was necessary in the savannah environment in which she lived.

Study tip

Cubic centimetres (cc) is a measure of volume. One cc is equivalent to one mL.

Australopithecus

'Lucy' was the name given to an *Australopithecus afarensis* skeleton found in 1974. The fossil remains were of a young female who lived 3.9 million to 2.9 million years ago. The skeleton suggested that she was able to walk upright (1.2–1.4 metres tall) before hominins were able to use stone tools and before their brain became larger than 400 cc. Since Lucy, many other *Australopithecus afarensis* skeletons have been found. There has been some variation in the size of these fossils, suggesting there may have been sexual dimorphism (males being larger than females).

FIGURE 2 The skull of *Australopithecus afarensis* indicates a cranial capacity of 400 cc.

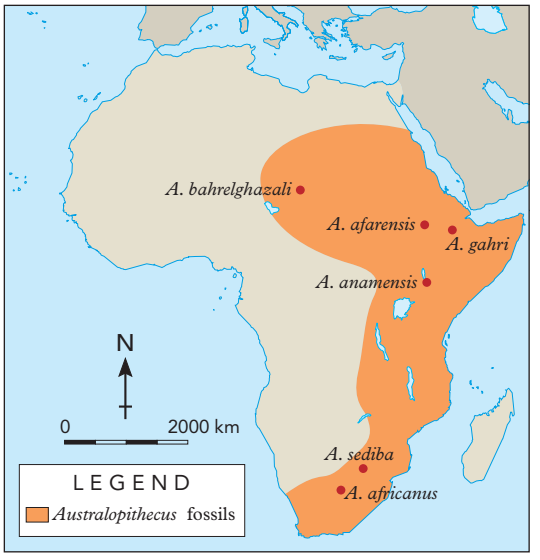


FIGURE 3 There are a number of different species of *Australopithecus* that have been identified, including *A. afarensis*, *A. africanus* and *A. sediba*.

Genus *Homo*

The use of tools was once considered one of the distinguishing characteristics of genus *Homo*. The first tools found near fossilised ancestral remains were very primitive and most likely used for scraping skins. The fossilised skeletal remains of the toolmakers (2.33 million to 1.4 million years ago) suggest that they were 110–130 cm tall with an average cranial capacity of 610 cc. Their skulls showed the beginnings of a forehead and a smaller arched brow than their earlier ancestors. The **foramen magnum** was located in the centre of the base of the skull, suggesting that they were bipedal.

foramen magnum a large oval opening at the base of the human skull through which the spinal cord connects with the brain

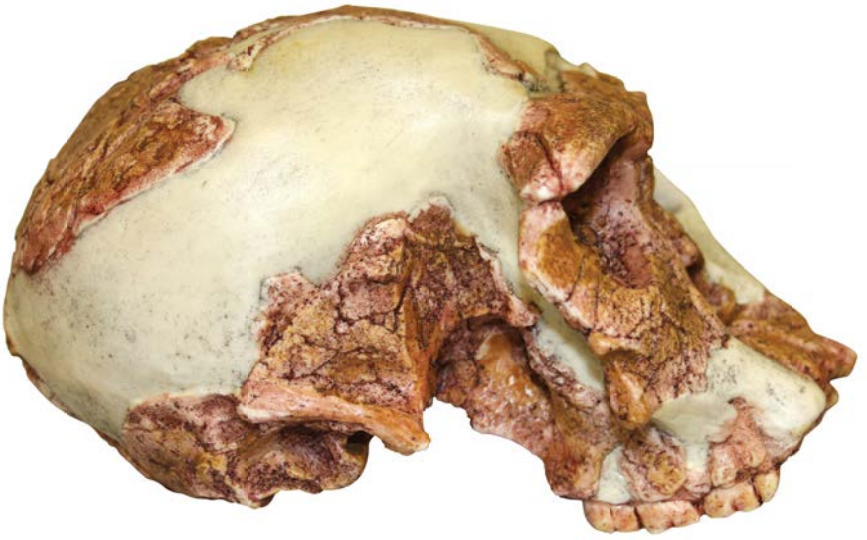


FIGURE 4 *Homo habilis* was once considered the first toolmaker.

The first traveller

It was not until 1.8 million years ago that the first human ancestors migrated out of Africa, with their fossilised remains being discovered in Europe and Asia. The fossilised bones have been dated to between 1.8 million and 300 000 years and suggest they had a long, low skull with small forehead and a cranial capacity of 750–1225 cc. The species had a protruding jaw, no chin and were the first hominin to have a protruding nose. Their remains have been found from Kenya (Turkana boy) to China (Peking man). Some palaeontologists split this species into two based on the location of the remains, *Homo erectus* (found outside Africa) and *Homo ergaster* (found inside Africa).

FIGURE 5 *Homo erectus* was the first of our ancestors to migrate out of Africa.

Study tip

Homo neanderthalensis and *Homo sapiens* are the only species that you need to know for the exam.

Homo neanderthalensis

Fossilised remains of *Homo neanderthalensis* are only found in Europe and the Middle East. Direct evidence based on the age and location of the remains suggests that they coexisted with *Homo sapiens* between 230 000 and 30 000 years ago. This caused scientists of reconsider the hypothesis that *Homo neanderthalensis* was a direct ancestor of *Homo sapiens*. Species that coexist cannot be considered ancestors. Instead they would have shared a common ancestor and then continued to evolve.

Homo neanderthalensis get their name from the Neander Valley in Germany where the first fossils were found. The fossils indicate that they were heavily built with thick bones, therefore suggesting that they may have had large muscles. Their cranial capacity is the largest of any of the genus *Homo* (1450 cc) and many of them had an **occipital bulge** (a projection at the rear of the skull). Putative evidence suggests that the bulge is an adaptation that allowed their larger neck and jaw muscles to attach to the skull. Their noses were prominent, and they had sloped foreheads and almost no chin. These adaptations are thought to be the result of living in cold climates. Neanderthals have been found buried with a range of complex tools and necklaces.

occipital bulge
a prominent lump or projection found at the back (occipital area) of the skull

Although evidence suggests that *Homo neanderthalensis* and *Homo sapiens* were able to interbreed to produce fertile offspring, this occurred rarely. For this reason, it is thought that there was a partial reproductive barrier between the two groups, leading to them being defined as two different species.

Many humans living outside Africa today have Neanderthal DNA in their genome, providing direct evidence of interbreeding between the two species. A 2011 study suggested that some of these genes may include ancient variants of immune system genes that protect against some pathogens. These genes do not exist in modern humans found on the African continent.

FIGURE 6 *Homo neanderthalensis* had the largest cranial capacity of all of our human ancestors.

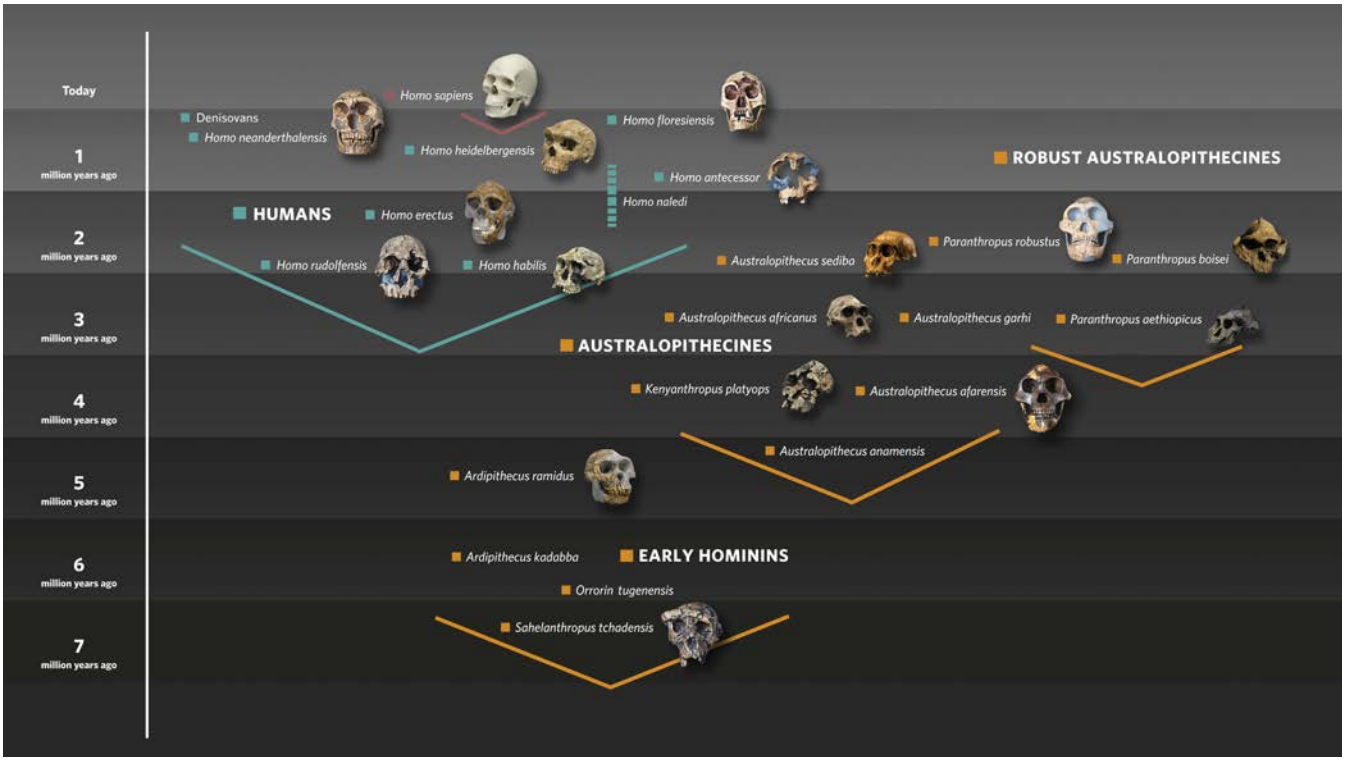


FIGURE 7 An updated evolutionary tree of hominins

Coexisting with *Homo sapiens*

A number of relatively small 'hobbit-like' skeletons found on the Indonesian island of Flores caused a sensation when they were first discovered in 2004. Initially, scientists were not sure if the skeletons belonged to a group of children or individuals with dwarfism on the island; however, the number of complete skeletons found suggests (putative evidence) that it was a different species. These remains were unique since they were 1 metre tall and had a cranial capacity of 380 cc. This is much smaller than any other species in the *Homo* genus. Yet their relatively recent existence (until only 18 000 years ago) and the existence of a number of stone tools suggest they are a dwarfed species that evolved from our earliest travelling ancestors. It is not unusual for dwarfed forms of animals to be found on smaller isolated islands. This may be due to a decrease in predators and/or a requirement for less food.

FIGURE 8 *Homo floresiensis* (left) is nicknamed 'the hobbit' because it was much smaller than modern *Homo sapiens* (right).

Until recently, everything that was known about a different extinct species (or subspecies of *Homo sapiens*) came from direct evidence of teeth and bone fragments found in the Denisova Cave in Siberia. More recently in 2019, a jawbone was discovered high on the Tibetan Plateau. DNA evidence suggests that the nicknamed 'Denisovans' shared a common ancestor with the Neanderthals before diverging 550 000–765 000 years ago. The discovery of a bone fragment that shared the DNA of a Denisovan father and a Neanderthal mother at the Denisova Cave provides direct evidence that interbreeding occurred after that time.

Although a newly discovered jawbone in China did not contain any useable DNA, a comparison of ancient proteins confirmed it was related to the bone fragments in Siberia. Genetic overlap (3–5%) has been found between Denisovan DNA and modern-day people of Asian descent, particularly Melanesians (a group of Pacific Islanders living in Papua New Guinea). This suggests that early *Homo sapiens* interbred with the Denisovans.

CHALLENGE 14.2

Homo naledi: A new species

In 2013, fossilised remains of a new species, *Homo naledi,* were found in the Rising Star cave systems, north-west of Johannesburg in South Africa. The curved finger bones, small cranial capacity and broad rib cage suggest similarities to 6 million-year-old fossilised remains found in Ethiopia, of early hominoids such as *Australopithecus*. On the other hand, the lower body represented more human-like features with evidence of locomotion and hand-use.

Intriguingly in 2015, the fossils of *Homo naledi* were dated to be between 236 000–335 000 years old. In 2017, these dates were published.

1 Why would the age of the fossils be the cause of much discussion by palaeontologists?

2 Is the date range of 236 000–335 000 years the date that *Homo naledi* first evolved? Why or why not?

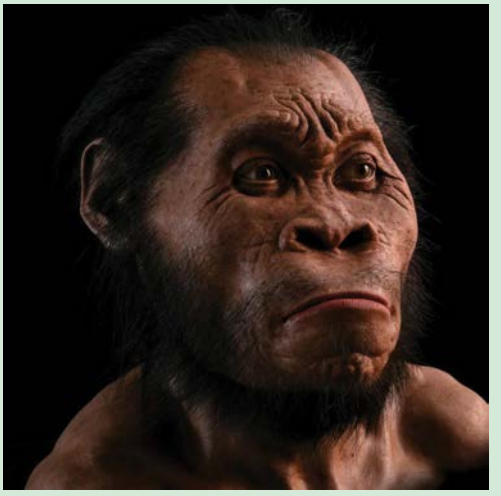


FIGURE 9 The fossilised remains of *Homo naledi* have caused much debate about the human evolutionary tree.

CHECK YOUR LEARNING 14.2

Describe and explain

1 Describe the key physical characteristics of *Homo sapiens* and *Homo neanderthalensis*.

2 Recent genetic analysis has suggested that *Homo sapiens* interbred with other species. Identify which species passed their DNA on to modern humans.

3 Why is toolmaking no longer considered a characteristic unique to the genus *Homo*?

4 Explain how scientists determine whether two animals are different species.

5 Explain how scientists determine whether two sets of fossilised remains are different species.

6 Explain why smaller organisms are usually found on islands.

7 Describe why *Homo sapiens and Homo neanderthalensis* are described as different species despite evidence of interbreeding.

8 Explain why it is important to know the difference between direct and putative evidence.

Apply, analyse and compare

9 For many years *Homo neanderthalensis* was considered a direct ancestor of *Homo sapiens*. What evidence was discovered that resulted in this theory being replaced?

10 Use examples to compare direct evidence and putative evidence.

Design and discuss

11 Genetic studies of body lice provide putative evidence that one of the two species that infect humans today evolved more than a million years ago, in association with another human species. How could this have occurred?

12 One of the characteristics that first defined genus *Homo* was a cranial capacity greater than 400 cc. Discuss why the 'hobbit' fossils were still classified as *Homo* despite their cranial capacity being smaller than 400 cc.

13 Discuss why finding the remains of *Homo neanderthalensis* buried with complex tools and necklaces suggests they were capable of complex thought.

14.3 Trends in hominin evolution

KEY IDEAS

In this topic, you will learn that:

- evolution from the genus *Australopithecus* to *Homo* can be seen in the change in limb structure and brain size
- the structure of hominin legs and arms evolved to allow for bipedal motion.

Video
Trends in hominin evolution

Modern humans (*Homo sapiens*) share a common ancestor with chimpanzees approximately 13 million years ago. The first of our uniquely human ancestors (genus *Australopithecus*) were more similar to those ancestors than to us. How our ancestors changed and evolved structurally, physiologically and culturally can be described with a number of overall trends.

Structural and physiological trends

Evidence of hominin evolution can be seen in structural and physical trends such as changes in limb structure and brain size.

Arms

The earliest ancestor of modern humans, *Australopithecus*, has upper limbs that are much longer and stronger for their size than those of bipedal *Homo sapiens*. This provides putative evidence that our earliest ancestors were largely tree-dwellers. It is suggested that *Australopithecus* spent a large part of their time walking along tree branches while using their arms to hold on to the upper branches. This means longer arms with hands able to grip branches would have been an advantage to their survival. As the number of trees decreased and a grassland savannah developed, the trees became fewer and further apart. This meant more time was spent on the ground, and the upper limbs could be utilised to hold babies or carry other items as they moved between the trees. The length of the arms became less important than the ability for bipedal motion.

FIGURE 1 The different relative limb lengths of **a** *Australopithecus* and **b** *Homo sapiens*

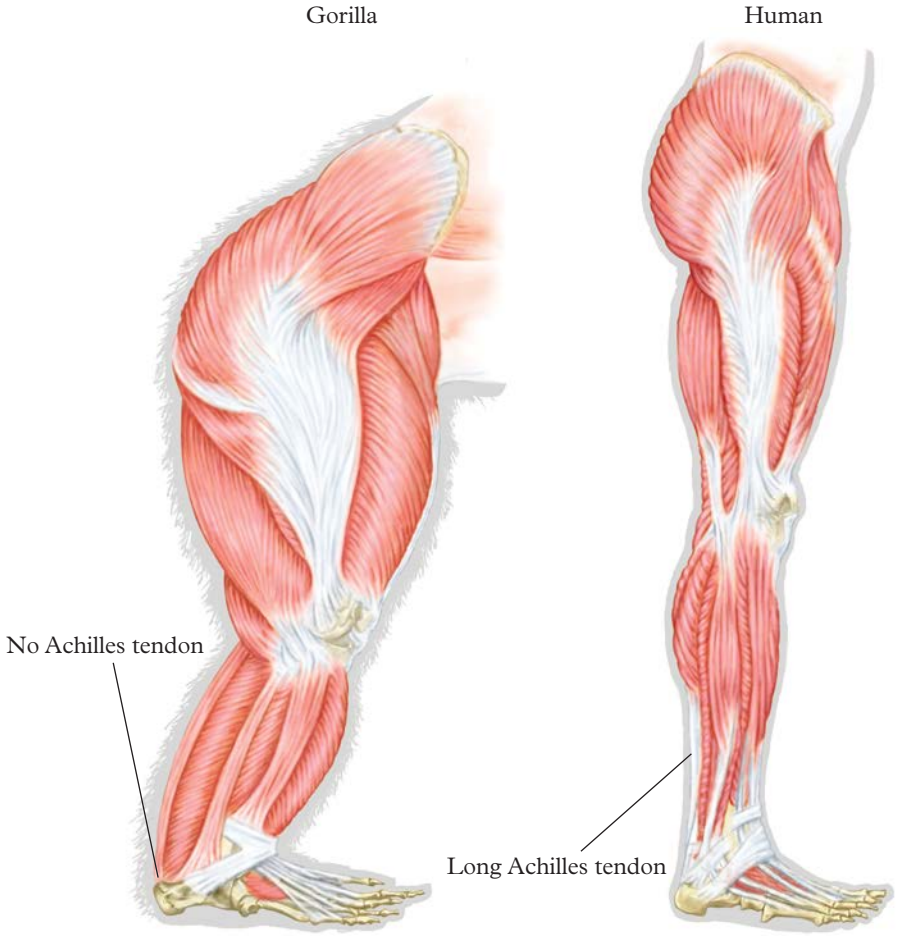


FIGURE 2 Humans have a long Achilles tendon that helps them run long distances. The lack of an Achilles tendon in the gorilla means the calf muscle attaches directly to the ankle bone. This makes gorillas poor bipedal runners.

bipedalism
the ability to walk upright on two legs

Study tip

The language you use when describing the evolution of *Homo sapiens* is important. *Homo sapiens* did not evolve from apes or chimpanzees. We do share a common ancestor with chimpanzees, and then both of these branches of the primate family continued to evolve.

Legs and feet

Bipedalism had significant effects on other parts of the body. The Achilles tendon, which links the calf muscle to the heel in the foot, is unique to our ancestors. This tendon acts like a spring that stretches and stores elastic energy as the foot moves up and down. This is more efficient than the chimpanzee in bipedal forward-striding motion. The longer the Achilles tendon, the more power can be generated in the leg, which increases performance in sports such as long-distance running.

Walking upright also places stress on the ball joints at the top of the pelvic bone. An angled joint allows some of the weight to be passed further down the femur bone, rather than on the thin connection to the ball. Having the knees angled under the hips allowed our human ancestors to stand on one foot. This structural change, along with increased development of the gluteus maximus muscle and the development of waist muscles, allowed human ancestors to run long distances. This provided advantages in hunting, and escaping from predators. It also allowed our early ancestors to move to new areas when food supplies were limited.

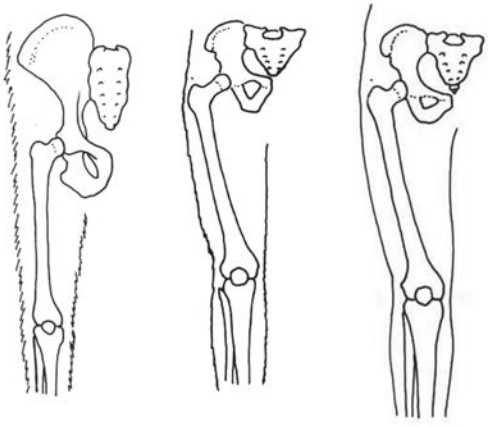


FIGURE 3 Evolving the knee to sit under the hip has allowed our ancestors to easily place their weight on one foot. This meant they were able to walk and run more easily (ancestral form – left; modern human – right).

CASE STUDY 14.3

Continual evolution of *Homo sapiens*

An extremely useful genetic mutation found in many Western Australia Indigenous populations allows for survival at high temperatures like those in the desert areas. Like many other animals, the metabolism of *Homo sapiens* is moderated by the hormone thyroxine. When the level of free thyroxine increases, there is a general increase in all the chemical reactions in the body. Most chemical reactions are exothermic, where heat is generated by the organism. Increasing the chemical reactions in the body increases the amount of heat generated inside the organism.

The level of thyroxine is moderated by a number of factors, including a carrier molecule called thyroxine-binding globulin (TBG). The TBG binds most of the free thyroxine in the blood so that only 0.03% of the total thyroxine in the blood is free to bind to receptors. At external temperatures above 37°C, the TBG changes its shape so that more thyroxine becomes free to bind to its receptors, increasing the overall metabolism and generating even more heat. This can be a disadvantage in the extreme temperatures in the desert environments of Western Australia.

Over 40% of Western Australia's Indigenous population have been found to have two substitution mutations in the *TBG* gene. These mutations result in the amino acids threonine and phenylalanine being substituted for alanine and leucine respectively. These two new amino acids change the shape of the TBG and allow it to remain bonded to the thyroxine at higher temperatures. Individuals with the mutations will only experience a small increase (10%) in free thyroxine at 39°C instead of the 23% increase experienced by other populations. This means that the individuals with the mutations will not generate as much heat at high desert temperatures. The advantage of being able to survive the high temperatures of a desert environment has allowed the frequency of this new allele to increase in Indigenous Peoples over time.

FIGURE 4 Western Australia gets so hot that Indigenous populations have evolved to withstand such heat.

Cranial capacity

The **cranial capacity** of a fossil refers to the size of the internal volume of a skull. The skulls of our early human ancestors in Africa (genus *Australopithecus*) were much smaller than ours with an average of 400 cc. Over time, the size of the skulls became larger with the first *Homo* skull beginning at 510 cc. The largest cranial capacity belongs to our cousins (not a direct ancestor), *Homo neanderthalensis*, who reached a massive 1450 cc. This is larger than the average *Homo sapiens* today (1350 cc).

cranial capacity the size of the internal volume of a skull

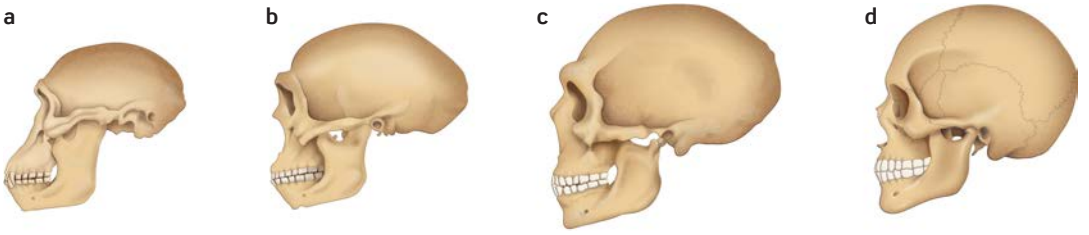


FIGURE 5 Cranial capacity: **a** *Australopithecus*; **b** *Homo erectus*; **c** *Homo neanderthalensis*; **d** *Homo sapiens*. The cranial capacity of *Homo sapiens* and *Homo neanderthalensis* is much larger than the earlier ancestors.

Study tip

Since the brain of our early ancestors is soft tissue, there is little evidence for how it changed over time. Therefore, only the cranial capacity can be used as putative evidence of changes in brain size.

CHECK YOUR LEARNING 14.3

Describe and explain

1 Describe how the structure of our ancestral leg bones changed over time.
2 What is cranial capacity and explain how the cranial capacity of *Australopithecus* skulls is different to that of *Homo sapiens*.
3 Describe what bipedalism is and the advantages of bipedal motion.
4 Explain why being able to run for long distances was an evolutionary advantage.

Apply, analyse and compare

5 The arms of *Homo sapiens* are relatively much shorter than those of their *Australopithecus* ancestors. Suggest how the change of locomotion from brachiation (swinging along tree branches) to bipedal motion might have supported this change.
6 Walking upright required the pelvic bones to become cup-like so that they were able to support the internal organs. Suggest how this may have affected the size of babies at birth.
7 Compare how the Achilles tendon changed over time and the selection pressures that might have driven that change.
8 Suggest why arm length was more important to the earliest ancestors of *Homo sapiens* than leg length.

Design and discuss

9 'The evolution that resulted in an increased cranial capacity that occurred over thousands of years is thought to contribute to the increased chances of survival of *Homo sapiens*.' What assumption is being made with this statement?
10 Read Case study 14.3. Not all changes to humans were structural. Describe how the allelic frequency of the mutated *TBG* gene would have increased in Western Australia.
11 The inside surface of the skull can often show patterns that correlate to the development of specific areas of the brain such as the frontal cortex. If the frontal cortex is involved in higher-order thinking, suggest how the size and complexity of patterns in this area of the skull may have changed over time.

14.4 Migration of modern humans

KEY IDEAS

In this topic, you will learn that:

- fossil and DNA evidence can be used to demonstrate migration of modern human populations around the world
- Aboriginal and Torres Strait Island Peoples are the oldest continuous culture outside Africa.

The only species of genus *Homo* remaining alive today is that of modern humans, *Homo sapiens*. Tracing back the lineage of our early ancestors is often difficult since there were many different species living throughout Africa at the same time, and one of these species (*Homo erectus*) was the first to migrate to Europe and Asia. This has led to two theories about how modern humans evolved.

Origin of *Homo sapiens*

Out-of-Africa hypothesis
a hypothesis that *Homo sapiens* evolved in Africa and then migrated to Europe and Asia approximately 70 000 years ago

multiregional hypothesis
a hypothesis that *Homo sapiens* evolved through the gene flow between different groups of ancestors in regions outside Africa

There are two main hypotheses about the origin of *Homo sapiens*: the **Out-of-Africa hypothesis** and the **multiregional hypothesis**. Both hypotheses agree that our earliest migrating ancestor, *Homo erectus,* evolved in Africa approximately 1 million years ago before migrating into Europe and Asia.

Out-of-Africa hypothesis

The Out-of-Africa hypothesis suggests that *Homo sapiens* evolved from their ancestors in Africa approximately 200 000 years ago. It is thought that a series of small groups migrated out of Africa, but that they had all died or retreated by 80 000 years ago. The most significant wave or migration is thought to have occurred approximately 60 000–80 000 years ago. Over time, this group of individuals grew and spread across the world, reaching Australia around 65 000 years ago. Evidence that will be discussed later supports this hypothesis.

Multiregional hypothesis

The multiregional hypothesis suggests that many different ancestral species of *Homo sapiens* ancestors left Africa and settled in different parts of the world. Each small group evolved over time and interbreeding generated gene flow between the settlements, resulting in the eventual evolution of modern humans. Although genetic studies agree that modern humans interbred with other species (Neanderthals and Denisovans), mitochondrial evidence suggests that the Out-of-Africa hypothesis is more likely.

Evidence supporting the Out-of-Africa hypothesis

For many years, scientists hunted for fossils that would be the 'missing links' between our earliest ancestors and modern humans. Modern technologies such as DNA testing have provided further evidence supporting the theory that modern humans first evolved in Africa before migrating throughout the rest of the world.

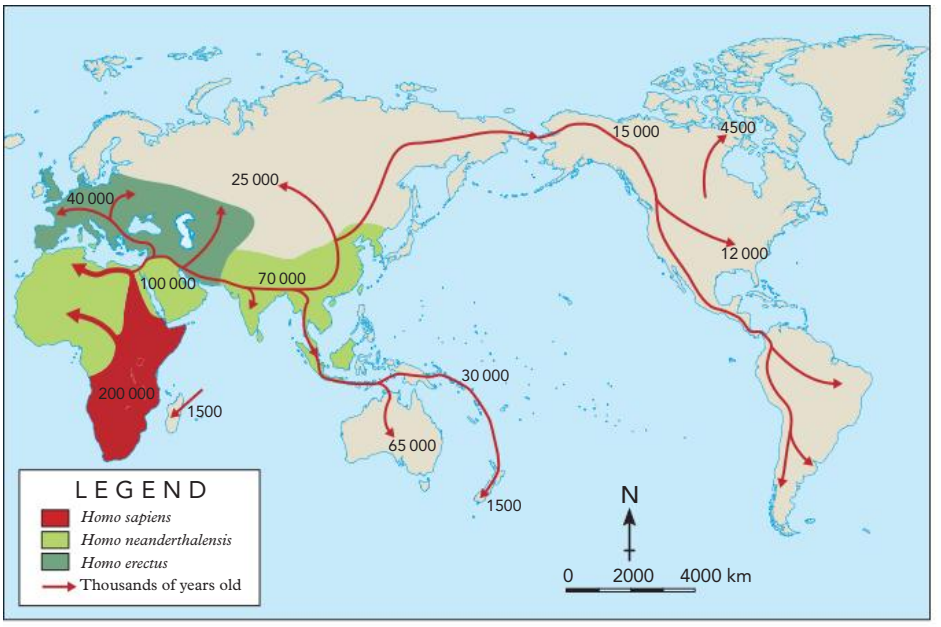


FIGURE 1 The Out-of-Africa hypothesis suggests that *Homo sapiens* evolved in Africa before migrating across Europe and Asia.

Fossil evidence

Modern humans are considered to have a more 'gracile' (slender) and lighter build than that of their 'robust' heavy-boned ancestors. *Homo sapiens* have skulls that are flatter at the back rather than the occipital buns found on earlier ancestors. Most of our early ancestors had prominent jaws with defined chins, vertical foreheads without the heavy brow ridges.

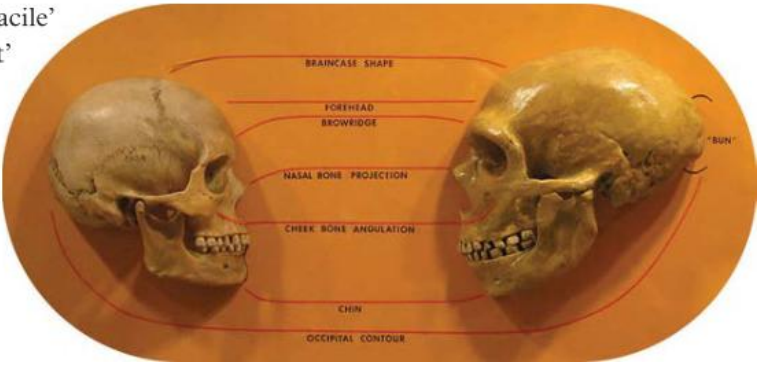


FIGURE 2 Fossils of the modern human (left) and Neanderthal (right), showing the different skull shapes

The types of tools used by *Homo sapiens* are also found to be different to that of their earliest ancestors. Modern human stone tools and weapons were usually finely crafted from stone, bones or antler tips, while the tools of earlier hominins were chunky flakes of stone.

These physical features and tools are first seen in 180 000-year-old fossils found in Africa, suggesting that this is the birthplace of modern humans. Fossils with these physical features found outside Africa have been dated at approximately 60 000–80 000 years, suggesting that this is the date of the first successful migration out of Africa.

DNA evidence

The ability to extract DNA from bone fossils (especially teeth) has allowed scientists to compare conclusions made from the anatomical structure of the fossils to the genomes of our ancestors.

Whole-genome evidence

Comparison of whole genomes of currently living *Homo sapiens* across the world indicates that people living in Africa have the highest level of diversity.

Video
DNA evidence to explain migration of modern humans

This suggests that modern humans first evolved on the African continent and split into many small groups. Similar to a founder's population, the group of early *Homo sapiens* that left Africa would have had a low genetic diversity compared to the populations left behind in Africa. Africa had the longest time to develop and maintain rich diversity. Satellite populations would suffer from the founder effect – and have reduced diversity; the longer ago the split from each other, the more time was available to accumulate novel mutations, and therefore the differences between these disparate populations would be greatest in those separated for the longest amount of time.

Mitochondrial DNA (mtDNA)

mitochondrial DNA (mtDNA)
DNA located in the mitochondria

Mitochondrial DNA (mtDNA) is often used for comparing evolutionary lines because a single cell contains many copies of the smaller mitochondrial DNA and is passed directly from mother to children. Mitochondrial DNA also mutates at a faster rate than nuclear DNA (which is often repaired by nuclear proteins). These mutations in the mitochondrial DNA are passed on to future generations, allowing scientists to use a molecular clock to follow the changes back through previous generations. This extended family tree can be used to show the pathways *Homo sapiens* used as they migrated throughout the world.

Using this method, scientists have calculated that a single woman who lived approximately 200 000 years ago was the source of the mitochondria in every person alive today. This 'mitochondrial Eve' would not have been the first modern human, or the only woman alive at that time; however, she would have been the only woman who produced the lineage of daughters that are our most recent mitochondrial ancestors. Recent comparisons of mitochondrial DNA between people found outside Africa and different groups of people still living in Africa suggest that the Makgadikgadi wetland in Botswana was the possible homeland of 'mitochondrial Eve'.

Testing of sediment core samples of this area suggests that the first significant migration of *Homo sapiens* from Africa occurred when changing rainfall patterns were opening up habitable land in the area. Corridors of greenery may have attracted migrating groups around the desert regions.

Pathway of migration

Although there is evidence of several waves of migration of modern humans from Africa, many of the fossilised remains appear to have limited ages, suggesting that the lineage may have died out. The most successful migration was thought to have occurred 60 000–80 000 years ago. Evidence suggests that a group of 1000 to 5000 people crossed a land bridge at the southern end of the Red Sea and followed the sea where the weather was favourable and there was plenty of food. Tools similar to those used in Africa 74 000 years ago have been found at locations in India. There is also evidence of groups moving into Europe approximately 40 000 years ago. The extreme cold, and existing groups of *Homo neanderthalensis*, may have slowed the migration of *Homo sapiens*. The last of the Neanderthals are thought to have died 15 000 years ago. Why this occurred is not known. Various hypotheses include dwindling resources and epidemics. There is little evidence of fighting occurring between the two species. Instead, there is evidence of interbreeding, resulting in 1–4% of modern human genomes outside Africa containing Neanderthal DNA today.

The most detailed maps of *Homo sapiens* migration are made through the comparison of mitochondrial DNA. Each group of mutations in this DNA signifies a time and place of female migration. These groupings of unique ancestral mitochondrial DNA located throughout the world are called haplogroups.

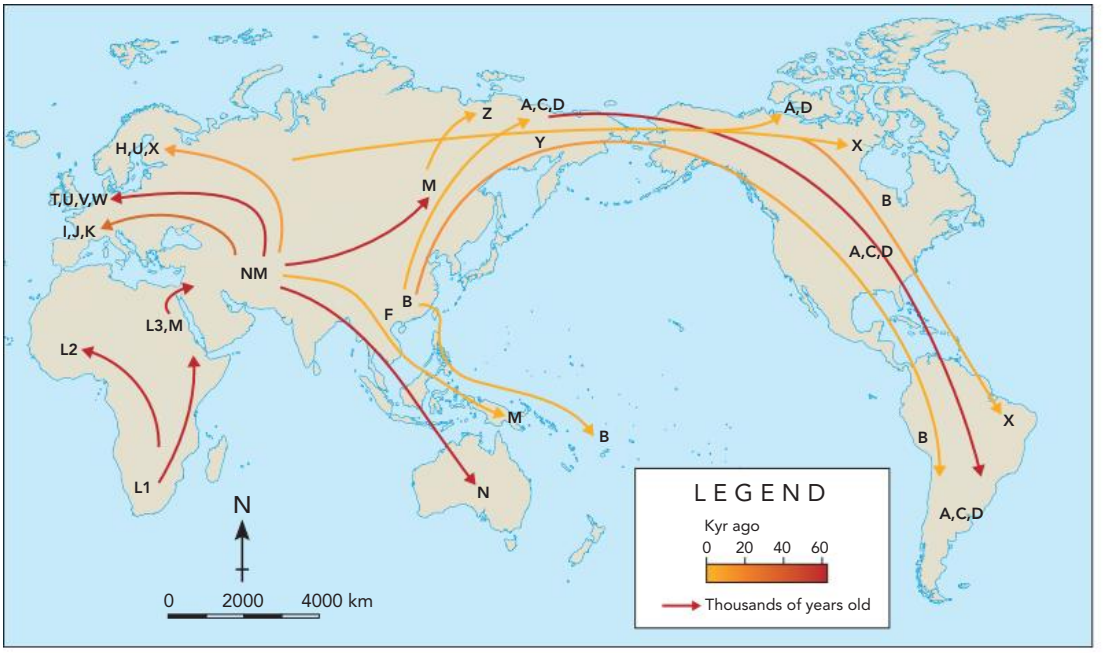


FIGURE 3 Global migration patterns of hominins with genome variations

Aboriginal and Torres Strait Islander Peoples populations

The first humans to inhabit Australia were the Aboriginal Peoples. As ancestors moved along the coastlines, genomic evidence suggests that populations (like other *Homo sapiens*) met and possibly mated with *Homo neanderthalensis*. There is also evidence that the early Aboriginal Peoples mated with other species of the genus *Homo*. Fossilised remains of finger bones and teeth found in the Denisova Cave in Russia identified DNA from an unknown genome (now called Denisovan). Up to 6% of the genome of New Guineans and 3–5% of the Aboriginal Peoples' genomes have been found to be identical to that of Denisovan DNA. This is mirrored in modern European populations also.

In modern times, these *Homo sapiens* are divided into two Peoples: Aboriginal Peoples, who are descended from the first modern humans to inhabit mainland Australia over 50 000 years ago, and Torres Strait Islander Peoples, who descended from the residents of a group of islands in the Torres Strait, north of Queensland. The First Peoples did not make the stone tools found in other migrating groups. Instead, the early fossilised tools were simple Neanderthal-style flaked stones and scrapers. This may have been due to the use of more sophisticated wooden tools that would have quickly decayed (so there would be no fossil evidence for these wooden tools).

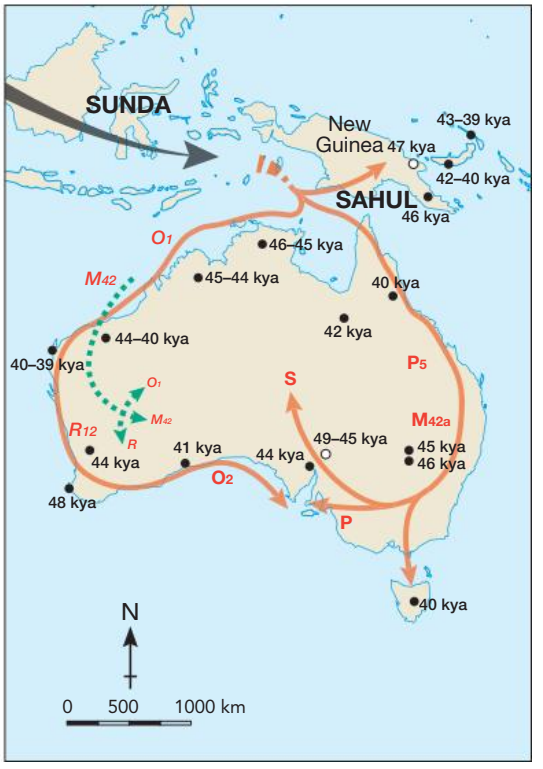


FIGURE 4 The various periods of rapid movement of the First Peoples in Australia. The route travelled by the First Peoples from the Sundra (mainland Asia) to Sahul (Australia-New Guinea) is still being debated.

The story of the Indigenous Peoples is unique since it would have required at least one 87 km sea voyage (possibly the earliest known) between Timor and the northern Kimberley Coast. Estimates of the number of people travelling based on mitochondrial DNA lineages

have ranged from 72 to 300 people. This journey would have taken 4–7 days on a raft and required extensive knowledge of sailing and navigation, and planning of food, water and resources. This knowledge is thought to have been beyond other groups of *Homo sapiens* living during the same time period. This makes First Nations Australians the oldest continuous culture in the world.

As well as being the first *Homo sapiens* to travel to Australia, Aboriginal and Torres Strait Island Peoples have undeniable respect for and understanding of the land. Connection to Country and Place is expressed through cultures, languages, spiritualties, identities, laws and family.

CASE STUDY 14.4A

Mungo Man

Lake Mungo is a dry lake located 90 kilometres north-east of Mildura. Between 1969 and 1974, three sets of remains of Indigenous Peoples were discovered in the area. One of the fossilised individuals, nicknamed Mungo Man, was dated at approximately 40 000 years old and constituted some of the oldest *Homo sapiens* remains found on the Australian continent. The body was laid out with bent legs and hands covering the pelvis in a ritualistic manner. Ochre from 200 km away was found near the body. Archaeologists removed the remains for study at the Australian National Museum. It was determined that he was 170 cm tall, 50 years of age and with severe arthritis at the time of death.

As the recovery and analysis of ancestral remains is of great sensitivity to Aboriginal and Torres Strait Islander Peoples, many Indigenous Peoples protested against the desecration of the Mungo Man remains. In 2017, Mungo Man was returned to the First Peoples of the area. After much discussion between scientists who wanted to continue their research and the First Peoples of the land, it was decided that the remains be reburied at the site where they were originally discovered.

Out of respect for Elders past and present, the Ngiyampaa, the Mutthi Mutthi, the Paakantji, and all Aboriginal Peoples, no images of the remains of Mungo Man have been included in this text.

CASE STUDY 14.4B

Evidence of First Peoples

New evidence suggests that the oldest Indigenous Peoples may have arrived in Australia as early as 65 000 years ago. This is much earlier than the 47 000 years ago that was previously considered. The 20 000 year overlap with Australian megafauna also suggests that the First Nations were not directly responsible for the animals' extinction.

FIGURE 5 An ground-edge stone axe found in Kakadu was one of the tools surrounded by sand dated to 65 000 years ago.

The new date was determined using a combination of carbon dating and optically stimulated luminescence. The optically stimulated luminescence technique measures the radioactive signature of a grain of sand. When grains of sand are exposed to sunlight, it removes any natural luminescence signal. When the sand is buried, it is exposed to low levels of natural radiation in the surrounding sediment, causing it to store the ionising radiation. If the sand is dug up and removed to a laboratory in total darkness, the radiation remains intact.

In the laboratory, the sand is exposed to a blue-green light, releasing the radiation as luminescence. The amount of light released is an indication of when the grains of sand were last exposed to sunlight. This technique was used to determine the age of the Indigenous artefacts, including ochre, reflective paint surfaces and unbroken ground-edge stone axes found in Kakadu National Park.

CHALLENGE 14.4

Ancestral DNA testing

The trend of wiping the inside of your cheek and sending it off to have your DNA analysed has increased rapidly in recent times. There are many companies offering to compare your DNA to their database to tell where your ancestors came from and their migratory pathway out of Africa. There are many difficulties inherent with this form of DNA testing.

Most of the people who are on the database of these companies have paid to have their DNA tested. This means the database is usually made up of largely Western countries who have enough income to pay for the test. Therefore, there are large areas – and many countries – that are not represented on the database. When a person receives their results stating that they are 20% Italian, it could mean that their ancestors are from Italy, or that the ancestors are actually from Morocco and shared common ancestry with people from Italy. There is no limit to how far back these comparisons can be made.

Another concern is the proportion of DNA obtained from your parents. Ultimately, your mother's DNA is 50% from her mother (your maternal grandmother) and 50% from her father (your maternal grandfather). However, this does not mean that you will have 25% from each of these grandparents. Instead, you might have inherited 40% from your maternal grandfather and 10% from your maternal grandmother. This will change the percentages of ancestry from each location for every sibling in the family.

1 How accurate are ancestral DNA tests in determining the location of your ancestors?

CHECK YOUR LEARNING 14.4

Describe and explain

1 Explain the anatomical differences between a *Homo sapiens* skull and a skull from *Homo neanderthalensis*.

2 Describe the DNA evidence supporting the Out-of-Africa hypothesis.

3 Explain who 'mitochondrial Eve' is.

4 When did the first human ancestors leave Africa?

5 When did the most significant wave of *Homo sapiens* migrate out of Africa?

6 Where is the oldest continually living culture outside of Africa?

Apply, analyse and compare

7 Compare the Out-of-Africa hypothesis and the multiregional hypothesis.

8 Suggest why the people of New Guinea have similar amounts of Denisovan DNA to that of the Aboriginal Peoples.

Design and discuss

9 Refer to Case study 14.4A. Discuss what the discovery of Mungo Man can tell us about the migration patterns of the First Peoples.

10 Discuss the cognitive skills that were needed for the first *Homo sapiens* to reach Australia.

11 Discuss the cultural and ethical challenges that arose with the excavation of Mungo Man.

CHAPTER 14

Review

Chapter summary

14.1
- All mammals share common characteristics, including the production of milk from mammary glands in females, hair at some stage of their lives and the three middle ear bones.
- Primates (including humans) share common characteristics, including opposable thumbs and flattened nails, collarbones, steroscopic vision and large brains.
- Hominoids are a subdivision of primates that includes the great apes and humans.
- Hominins are a tribe consisting of modern and living *Homo sapiens* and all their extinct ancestors.

14.2
- The interpretation of the human fossil record is constantly being contested, refined and replaced as new evidence (either direct or putative) arises.
- *Homo sapiens* is the only surviving member of genus *Homo*.

14.3
- Major trends in hominin evolution include a change in cranial capacity and the size of arms and leg structures, which allowed for bipedal motion.

14.4
- Fossil and DNA evidence can be used to demonstrate migration of modern human populations around the world.
- The Aboriginal and Torres Strait Island Peoples are the oldest continuous culture outside Africa.

Revision questions

Multiple choice

1 Which of the following characteristics is unique to mammals?
- **A** Opposable thumbs
- **B** No tail
- **C** Mammary glands
- **D** Constant body temperature

2 Which of the following characteristics is unique to primates?
- **A** Opposable thumbs
- **B** Mammary glands
- **C** Constant body temperature
- **D** Grasping fingers

3 Which of the following characteristics is unique to the hominoid group of primates?
- **A** Mammary glands
- **B** Grasping fingers
- **C** No tail
- **D** The ability to communicate

4 Which of the following characteristics is unique to the genus *Homo*?
- **A** The ability to communicate
- **B** Opposable thumbs
- **C** The ability to use tools
- **D** Fully erect posture with bipedal motion

5 Over the last 7 million years of human evolution, which of the following trends has occurred?
- **A** The development of a defined chin
- **B** An increase in brain size
- **C** Increase in the size of teeth
- **D** An ability to walk upright

6 Which of the following is evidence that *Homo neanderthalensis* was not a direct ancestor of *Homo sapiens*?
- **A** *Homo neanderthalensis* had a larger cranial capacity than *Homo sapiens*.
- **B** No *Homo neanderthalensis* remains have been found in Africa.
- **C** The two species coexisted in Europe and Asia.
- **D** *Homo sapiens* were better adapted for the environment.

7 What does the Achilles tendon help humans do most?
- **A** Cross their legs when sitting
- **B** Run
- **C** Walk upright (bipedal)
- **D** Lunge

8 Who am I?
- I am a mammal.
- I have a single jawbone.
- I have stereoscopic vision.
- I have no tail.
- I am bipedal.
- I have a cranial capacity bigger than *Homo sapiens*.

- **A** An orangutan
- **B** *Australopithecus*
- **C** *Homo floresiensis* (the hobbit)
- **D** *Homo neanderthalensis*

9 Bipedalism is a characteristic of the ancestors of *Homo sapiens*. Which of the following characteristics provides evidence of bipedalism in a fossil?
- **A** The size of the skull
- **B** An enlarged shoulder blade
- **C** The flaring nature of the rib cage
- **D** The position of the knee joints

FIGURE 1 Bipedalism is the ability to walk on two legs.

10 Which of the following explains the presence of Denisovan DNA in Aboriginal Peoples?
- **A** Early migration of Denisovans to Australia
- **B** Interbreeding with *Homo neanderthalensis*
- **C** Interbreeding with Denisovans
- **D** European immigration to Australia

11 Evidence for the multiregional hypothesis includes:

A *Homo sapiens* migrating out of Africa 70 000 years ago.

B the presence of Neanderthal and Denisovan DNA in modern humans.

C the similarity in mitochondrial DNA in modern humans outside Africa.

D the discovery of fossils belonging to *Homo erectus* in Europe.

12 Evidence for the Out-of-Africa hypothesis includes:

A the wider variety of mitochondrial DNA of modern humans in Africa compared with that found outside Africa.

B the presence of *Homo erectus* DNA in all modern humans living outside Africa.

C the interbreeding of *Homo sapiens* with *Homo neanderthalensis* and the Denisovans.

D after migrating out of Africa, *Homo erectus* populations becoming reproductively isolated.

Short answer

Describe and explain

13 Explain the assumption that is made when measuring the cranial capacity of fossilised skulls.

14 Refer to Case Study 14.4A on page 368. Explain the significance of Mungo Man being buried with ochre and his hands placed over his pelvis.

15 Describe the path of migration of early *Homo sapiens* from Africa to Australia.

16 Describe the features of fossilised leg bones that suggest that the human ancestor was bipedal.

17 Describe the shared characteristics that define mammals, primates, hominoids and hominins.

18 A 3.2 million-year-old foot bone suggested that the human ancestor had rigid arches similar to that of modern humans. Describe how this may have affected the ancestor's ability to climb trees.

19 Explain how mitochondrial DNA and whole genomes can be used as evidence for the migration of modern human populations around the world.

Apply, analyse and compare

20 Describe one form of direct evidence and one form of putative evidence of the migration pathway of the ancestors of Aboriginal and Torres Strait Islander Peoples.

21 Apply your knowledge of natural selection to determine the advantage of bipedalism.

22 Why is it significant that early *Homo sapiens* travelled a considerable distance over water to reach Australia?

23 A study of the Aboriginal Peoples' genomes suggest that there is genetic diversity between the populations on the east and west coast of Australia. What does this suggest about gene flow between the populations?

24 Compare the physical characteristics of *Homo neanderthalensis* and *Homo sapiens*.

25 Name the locations of the world that were inhabited by *Homo neanderthalensis*.

26 Which species of human ancestors are thought to have interbred? Provide evidence to justify your answer.

27 The first ancestors of modern humans who were thought to cook food were *Homo erectus* approximately 1.9 million years ago.

a Describe the advantage cooking food would have given this population.

b How would the cooking of food affect the structure of the muscles connected to the jaw?

28 The earliest ancestors of modern humans have traits that are not shared with modern-day African apes. Explain the reason for these observed differences.

29 In 1997, a team of researchers used Polymerase Chain Reaction (PCR) to amplify and extract many short strands of mtDNA from a *Homo neanderthalensis* fossil found in Germany.

The researchers generated a sequence of 379 bases from the Neanderthal individual. Each base was extracted in at least two separate amplifications.

They then compared this sequence against a database of 994 different mtDNA sequences from modern humans. Their findings are presented as a graph in Figure 2.

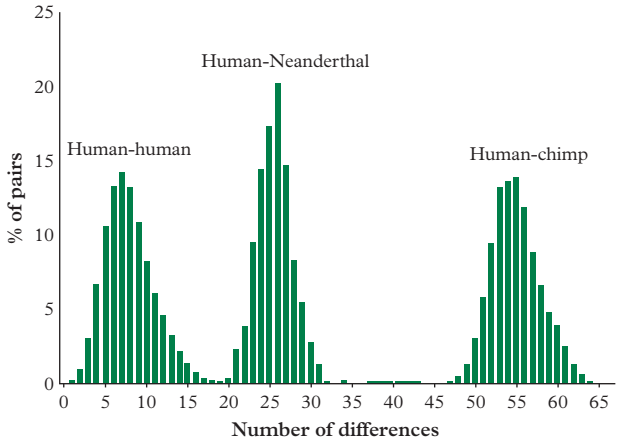


FIGURE 2 mtDNA sequences from modern humans

a Explain the number of differences between the human-neanderthal sample.

b Explain why there are differences in the human-human sample.

c Explain why it is important that the researchers extracted each base in at least two amplifications.

Design and discuss

30 Design your own timeline that shows the significant events that occurred with all the ancestors of *Homo sapiens*.

31 Discuss which characteristics you could use to distinguish between the skull of *Australopithecus* and the skull of genus *Homo*.

32 Recent testing has found *Homo neanderthalensis* DNA in the genome of modern northern African Peoples despite no Neanderthal fossils being found in Africa. Discuss a possible explanation for this finding.

33 Discuss the evidence that supports the hypothesis that *Homo neanderthalensis* did not evolve in Africa.

34 Radiocarbon dating of fossils of *Homo neanderthalensis* and *Homo sapiens* found in Europe suggests that the Neanderthals in different areas became extinct shortly after the arrival of modern humans. Discuss the possible implications of this evidence.

35 Consider Case study 14.3 on page 362. Suggest how the identification of the *TBG* gene mutation in the Indigenous Peoples of Western Australia indicates a long-lived connection to Country.

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Chapter quiz
Check your understanding of this chapter.

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QuizletLive
Launch a quiz for your students on key concepts in this chapter.

Exam essentials

Responding to questions

In your exam, you may need to identify different parts of a question in order to provide an answer that gives you full marks.

Identifying different parts of a question

Often questions worth multiple marks will have different parts to the question. These different parts may refer to past events, present events or even future events. It is important to answer all parts of the question, including referring to any different time periods.

The following question is taken from the 2015 VCE Biology Examination. Read the question carefully, then consider whether the responses identify the different parts of the question and provide a definition to help answer the question.

QUESTION 11a (2015 VCE Biology Written examination)

Recent DNA evidence has shown that:

- the genome of living humans of African descent does not contain Neanderthal DNA
- the genomes of living humans of European, East Asian and Australian Aboriginal descent all contain small amounts of Neanderthal DNA (1–4%).

a **i** Suggest how DNA from *H. neanderthalensis* entered the genome of present-day European, East Asian and Australian Aboriginal *H. sapiens*, and continues to be found in modern populations. 2 marks

ii What implication does this DNA evidence have for the classification of the two hominin species, *H. sapiens* and *H. neanderthalensis*, according to the common definition of a species? 1 mark

Source: 2015 Biology Written Examination Question 11a, Short answer, reproduced by permission © VCAA

Response 1

i *H. neanderthalensis* interbred with ancestor of European, East Asian and Australian Aboriginal *H. sapiens*, and produced children with both genomes. When *H. neanderthalensis* became extinct, the genes/DNA in the *H. sapiens* ancestors were passed on to present day European, East Asian and Australian Aboriginal *H. sapiens*.

Identifies 'the how' (the past).

Suggests how the DNA continues to be found (the present).

This response would receive 2 marks since it identifies that:

- there was interbreeding between *H. neanderthalensis* and the ancestors of (present-day) European, East Asian and Australian Aboriginal *H. sapiens*
- DNA was passed from one generation to the next.

Response 2

i *H. neanderthalensis* and a *H. sapiens* had sex and produced children with both genomes.

Only discusses ancestor and does not mention current generations.

This response would receive 1 mark because it identifies that the two groups interbred and produced children. It would not receive the second mark since it does not refer to how this would affect modern populations.

Think like an examiner

To maximise your marks on an exam, it can help to think like an examiner. Consider how many marks each question is worth and what information the examiner is looking for.

Mark the response

A student has given the following response in a practice exam. Imagine you are an examiner and use the marking guidance below to mark the response.

QUESTION 11a (2013 Biology Written Examination)

The skeletal structures of two extinct members of the hominin family tree, *Australopithecus africanus* and *Homo neanderthalensis*, are shown below.

a Examine the skeletal structures. For each of the features listed in the table below, describe the difference between the two species and state the significance of the difference. 4 marks

Source: 2013 Biology Written Examination Question 11a, reproduced by permission © VCAA

Feature	Description of difference	Significance of difference
pelvic structure	One is bigger than the other.	Makes it easier to walk on two legs.
arm to leg length ratio	A. africanus has longer arm to leg ratio than H. neanderthalensis.	H. neanderthalensis mostly walked upright and not on knuckles like A. africanus.

Marking guide

Question 11 a	- 1 mark for making a comparison of the pelvis structure of both skeletons. - 1 mark for relating the difference in pelvis to the survival advantage of the individual. - 1 mark for making a comparison of the arm to leg ratio of both skeletons. - 1 mark for relating the difference in arm to leg ratio to the survival advantage of the individual.

Fix the response

Consider where you did and did not award marks in the above response. How could the response be improved?

Write your own responses to the same questions to receive full marks from an examiner.

Check your Student obook pro for these digital resources and more: pro

Video tutorial
Identifying different parts of a question

Weblink
Past examinations and examiners' reports

UNIT 4

Area of Study 1 – Practice SAC

Analysis and evaluation of a selected biological case study

This practice School-assessed Coursework (SAC) should take 50 minutes and covers Key Knowledge from Unit 4, Area of Study 1. If you have access to the *Biology for VCE Units 3 & 4 Student workbook*, you can practise your key science skills by completing the Case Cracker activities before attempting this practice assessment.

The epidemiology of SARS-CoV-2

SARS-CoV-2 is a virus that causes the COVID-19 disease. This virus belongs to a family of viruses that usually occur in animals such as bats and pangolins (armadillo-like mammals found in Asia and Africa).

FIGURE 1 **a** Bats and **b** pangolins have each been hypothesised as the source vector of the SARS-CoV-2 virus that caused the COVID-19 pandemic.

This virus was first identified in the lower respiratory tract of patients with pneumonia in Wuhan, China in December 2019. The symptoms included a runny nose, fever, breathing difficulties, cough and lung infection. These early cases came from people linked to a local seafood market. A short time later, human-to-human transmission via close contact was observed. The method of transmission was found to be via droplets (sneezing and coughing), direct contact on contaminated surfaces or through aerosol transmission when respiratory droplets mix into the air.

One of the symptoms of severe COVID-19 infection was fluid (oedema) and lymphocytes accumulating in the lower lungs of a patient. This could lead to bacterial pneumonia.

To prevent the spread of the SARS-CoV-2 virus in January 2020, Wuhan authorities took a number of measures. They set up special clinics to test anyone with symptoms. These clinics could test hundreds of people each day. If the person tested positive, they were immediately placed in a special isolation ward. Roadblocks sealed off cities and public transport was shut down. Most residents were barred from leaving their homes (social distancing), instead ordering food and supplies online. These measures resulted in an initial decrease in the number of people infected with the disease.

FIGURE 2 A number of measures were taken to prevent the spread of COVID-19.

1 Explain how the SARS-CoV-2 virus is able to replicate in a patient.
2 It was recommended during the outbreak that people wash their hands often and not touch their face. Explain why these measures would minimise the risk of someone becoming infected with SARS-CoV-2.
3 One of the most severe symptoms of the disease is oedema and lymphocytes accumulating in the lungs. Explain how the presence of the virus in the lungs could lead to this inflammatory response.
4 Older patients (70–90 years of age) are more vulnerable to severe COVID-19. Suggest why this age group is less able to recover from the disease.
5 Explain why a person who has recovered from COVID-19 is unlikely to 'catch' the virus again.
6 What type of immunity would the person in the previous question have against SARS-CoV-2?
7 A mother with a 10-month-old baby was thinking of stopping breastfeeding during the COVID-19 outbreak. What would be your recommendation to the mother? Use your knowledge of immunity to justify your answer.
8 A new test for the presence of an antibody against the SARS-CoV-2 virus was developed in a laboratory. Explain how this test could be used to track the spread of the infection.
9 A number of vaccines have been developed in response to the SARS-CoV-2 virus. Explain how a vaccine can prevent a person from contracting the disease.
10 Governments and medical authorities around the world are vaccinating their citizens in an attempt to achieve herd immunity, which will prevent the spread of COVID-19. Define 'herd immunity' and explain how it could prevent the spread of infection. Comment on whether this has occurred in Australia or not. Discuss why.

Area of Study 2 – Practice SAC

Analysis and evaluation of generated primary and/or collated secondary data

This practice School-assessed Coursework (SAC) should take 50 minutes and covers Key Knowledge from Unit 4, Area of Study 2. If you have access to the *Biology for VCE Units 3 & 4 Student workbook*, you can practise your key science skills by completing the Data Drill activities before attempting this practice assessment.

Endangered koalas

The koala (*Phascolarctos cinereus*) is a marsupial that is unique to Australia. It is a specialist feeder whose diet is almost exclusively 40–50 species of eucalyptus leaves (out of 900 possible eucalypt species). These leaves contain toxic compounds that require the koalas to select the leaves carefully so that they are able to detoxify through a series of biochemical pathways. The microbes in their digestive system aid the breakdown of the eucalyptus leaves.

Most koalas live for 6–8 years and during that time live in a limited home range (0.1–1.35 km^2). They communicate with each other by making low-frequency calls that can travel long distances in less dense bush.

1 Use Figure 1 to describe how the range of koalas has changed over time.

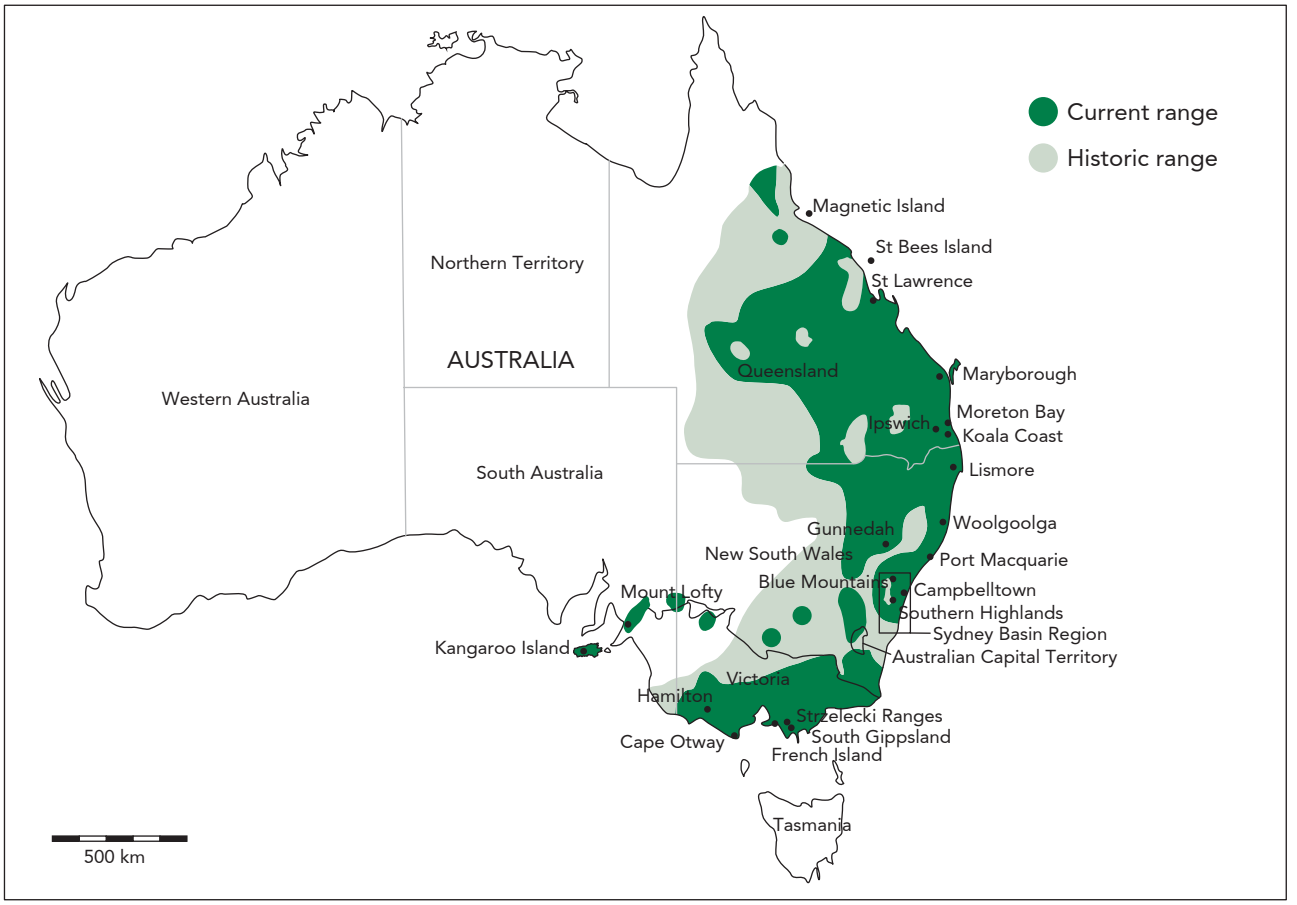

FIGURE 1 The distribution of the koala (*Phascolarctos cinereus*) on the east coast of Australia

2 Identify two possible factors that could have resulted in the change in koala range.

3 Use Figure 2 to identify the nearest ancestor(s) of the modern koala.

4 Describe how the number of koala species has changed as a result of increasing aridity and seasonality of the climate.

The mitochondrial DNA (mtDNA) of modern koalas was compared to 14 museum specimens from different places and time points. Each of the historic koalas only had four haplotypes (variations in the mtDNA), and all of these were found in the samples from modern koalas.

5 What does the mtDNA evidence suggest about the genetic diversity of the koala population? Justify your answer.

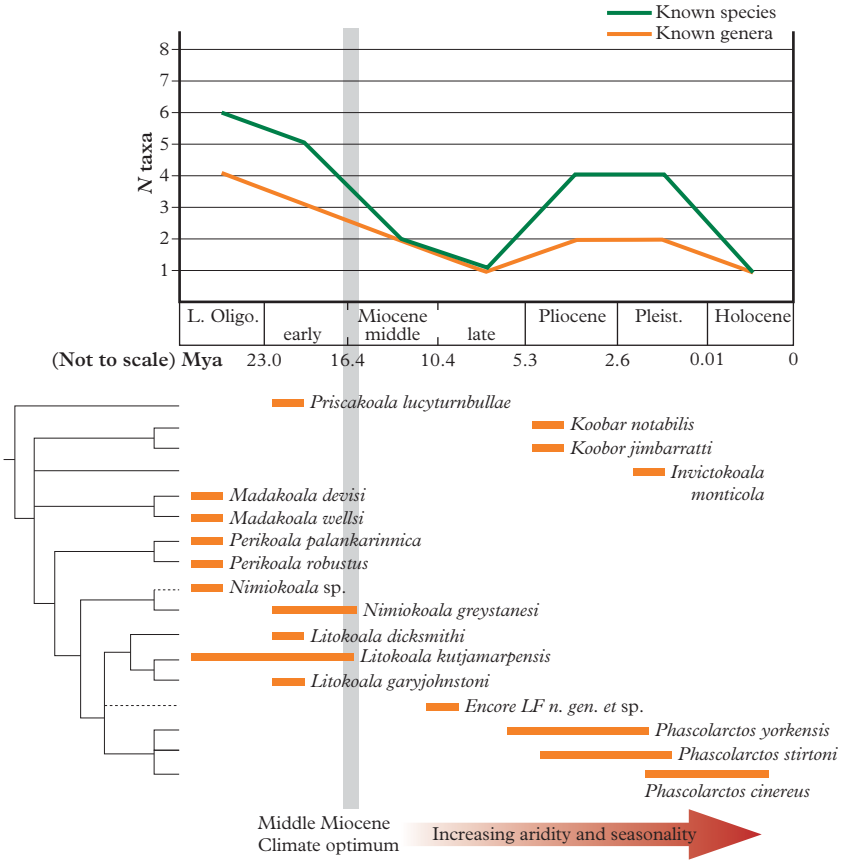

FIGURE 2 The evolution of koala species over time

In March 2018, scientists conducted an analysis of the genetic diversity of the koala populations in Sandy Point, South Gippsland, Raymond Island and Cape Otway. The results are indicated in Table 1.

TABLE 1 Genetic diversity of the koala populations

	Sandy Point	South Gippsland	Raymond Island	Cape Otway
Number of individuals sampled	11	90	50	30
Mean number of alleles detected per locus	1.7	6.5	3.3	3.3

6 Use the data in Table 1 to identify which location (Sandy Point, South Gippsland, Raymond Island or Cape Otway) had the highest genetic diversity in its koala population.

7 All the alleles present in the Sandy Point population were also found in the South Gippsland population. In contrast, the South Gippsland population had 113 alleles that were not present in the Sandy Point population. What does this suggest about the origin of the Sandy Point population?

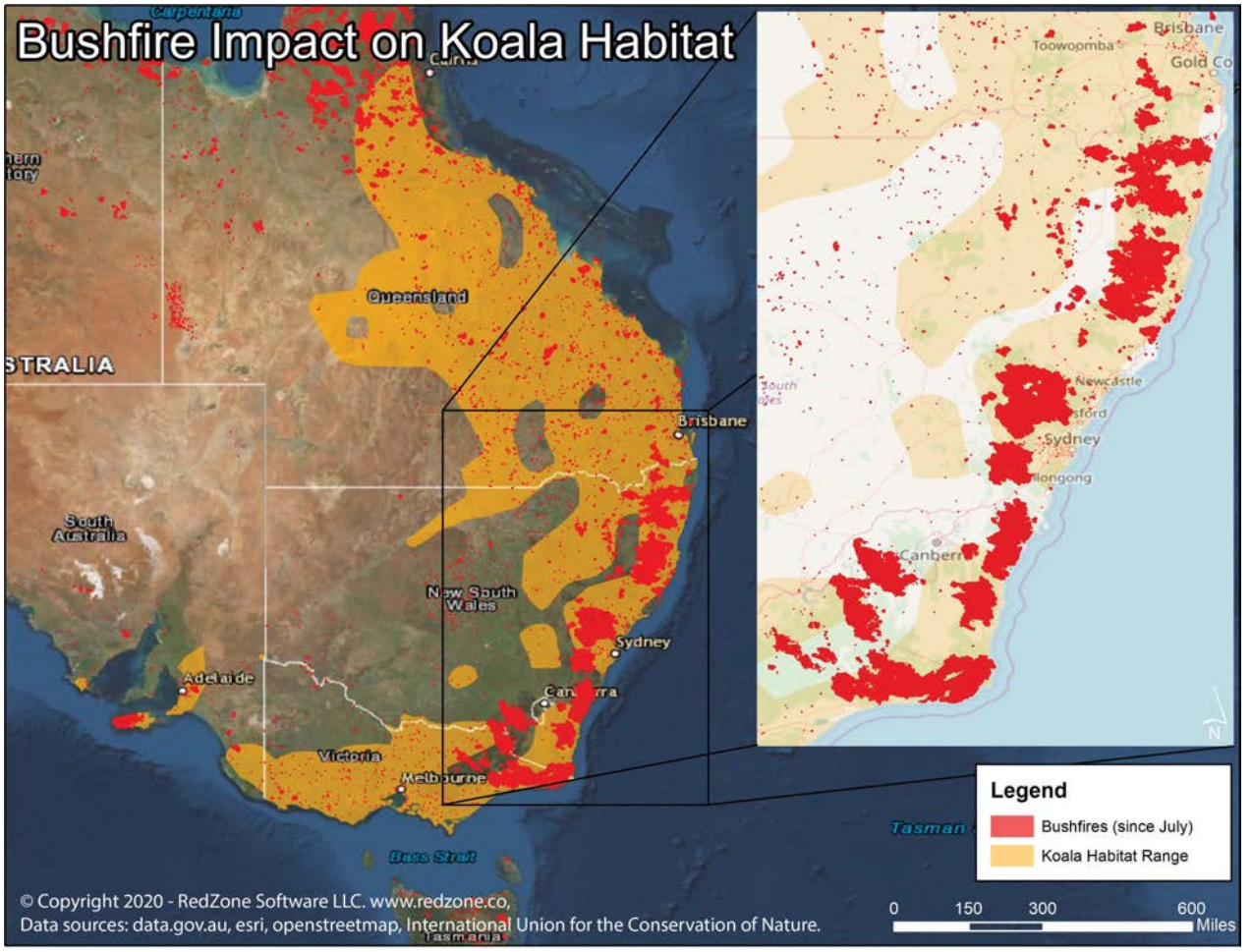

FIGURE 3 Koala habitat overlapped the bushfires in the 2019/2020 summer season.

The 2019/2020 bushfires were extensively spread over the east coast of Australia. Many of these areas overlapped the location of koala populations (Figure 3).

8 Describe the impact the bushfires will have on the genetic diversity of the koala population.

9 As the different populations of koalas become more isolated due to bushfires removing their habitat, they will become more vulnerable to genetic drift. Define 'genetic drift' and explain how it could be harmful to a koala population.

10 Small populations of koalas have developed a sexually transmitted bacterial infection called chlamydia. This can result in urinary tract infections, blindness and death. Wildlife workers usually treat the infection with antibiotics; however, this has led to antibiotic-resistance in the chlamydia bacteria.

Describe how the use of antibiotics can increase the population of antibiotic-resistant bacteria.

FIGURE 4 Koala populations are under threat.

Area of Study 3

Student-designed scientific investigation

A student-designed scientific investigation involves using key science skills (stating an aim, formulating a hypothesis and planning and carrying out a controlled experiment) to answer a science question based on your biological knowledge. Your investigation could involve using an experiment from Unit 3 or 4 and extending it to examine new factors, or alternatively you may want to develop a new experiment for yourself. The information below is a step-by-step guide on how to tackle this Area of Study 3 SAC.

Use a logbook to record the details and date of each step in your investigation.

Step 1: Ask a scientific question

Consider the topics and experiments that you have done throughout the year. Do you have any questions that remain unanswered? Could you modify a prior experiment to answer that question?

It is important to phrase your question carefully. For example, asking 'How does fertiliser A affect plant growth?' is not the best approach since you will not be able to examine the mechanism of each of the individual chemicals in the fertiliser. Instead, it might be better to ask, 'Does fertiliser A cause an increase in the number of leaves on a specific plant?' The second question here would help when designing your own experiment.

FIGURE 1 Thinking up a testable scientific question is no easy feat.

Step 2: Develop a valid, reproducible test

Most experiments need a reliable, reproducible test as their basis. You will have used a variety of these tests during your Biology course. These may include:

- the success of genetically modifying bacteria
- the rate at which a plant uses carbon dioxide (uses oxygen) during photosynthesis
- the rate of cellular respiration during yeast growth
- the rate at which an enzyme produces a product (or uses up a reactant)
- the ability of a fruit to resist infection by a fungus
- modelling of disease transmission
- the effectiveness of antibacterial substances
- modelling the rate of evolution.

There are many variables in these tests that can become a new independent variable. Changing this variable could affect the results (the dependent variable) of the test. For example, changing the temperature could affect the rate of photosynthesis, or diluting an antibacterial substance could affect how well it is able to inhibit the growth of bacteria. For each of these tests, you will need to check that they will answer the original question that you asked in Step 1.

If you are using a new test, you will need to repeat the test (unchanged) three or more times to check that the results are reliable and reproducible.

Step 3: Form a hypothesis

Once you have a reliable test, and a variable you want to change (independent variable), you will need to form a hypothesis. A hypothesis is a statement that can be tested experimentally. There are two parts to the hypothesis:

- how the independent variable (IV) will change the dependent variable (DV), and
- the scientific reason why this will occur.

This can be written as:

If the <DV> is affected by the <IV>, then the <DV> will increase/decrease when the <IV is increased or decreased.

Step 4: Design a controlled experiment

When designing your experiment, you will need to consider all the possible factors that could affect the outcome. Using the reproducible test from Step 2 will limit these factors. Consider all the possible sources of error that may occur. How will you improve your accuracy? (e.g. What equipment will you need to calibrate?) How could you improve your precision? (e.g. How big will your sample size be? How many times will you repeat your experiment?) Copy the flowchart diagram from Figure 3 on the next page into your logbook and complete the details.

Step 5: Record the results of your experiment

Make sure you record any changes that you made to your original test. Record the amounts and concentrations of each material that you use, and the steps you took to minimise any errors. Include the results of every part of the experiment, both quantitative (numbers) and qualitative (descriptions) in your logbook. Your final poster might not include all these details, but it is useful to have them just in case. Draw up tables for your data. See Table 1 on the next page as a guide to how to produce a good table.

Identify any outliers (any measurement that is very different from the other data points) and attempt to explain them.

Draw a graph to show the relationship between the independent variable and the dependent variable. Refer to your Biology toolkit for graphing tips.

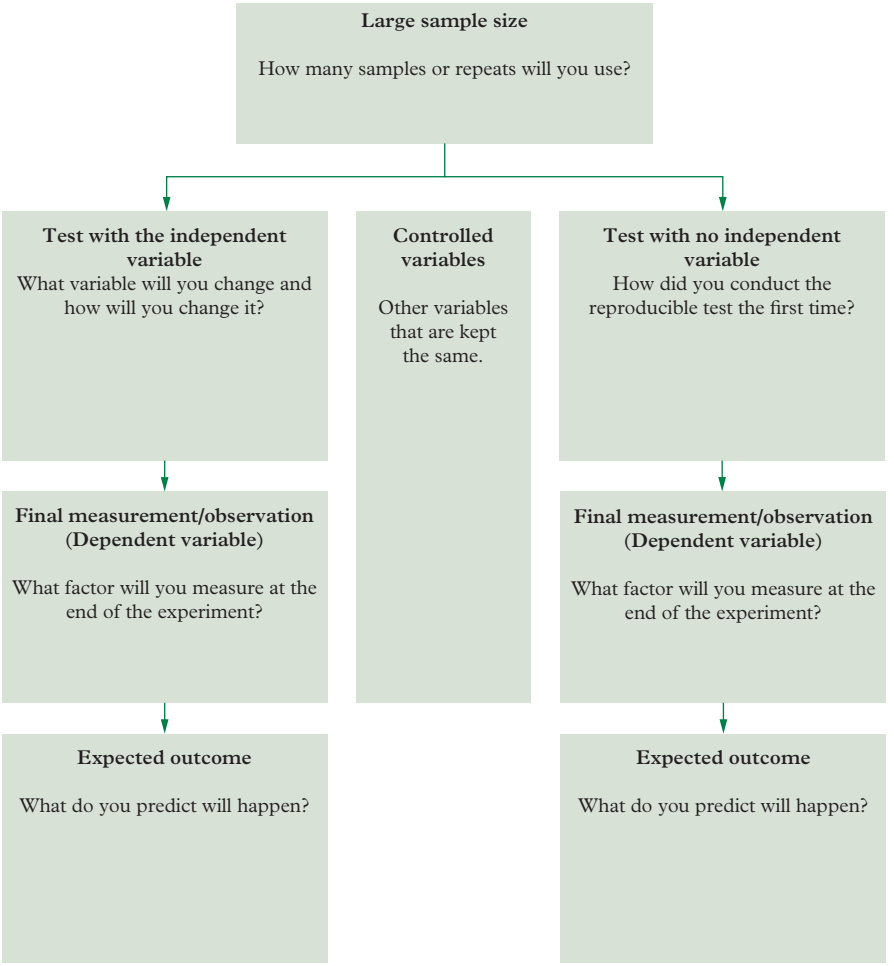

FIGURE 2 Flowchart to help you design your experiment

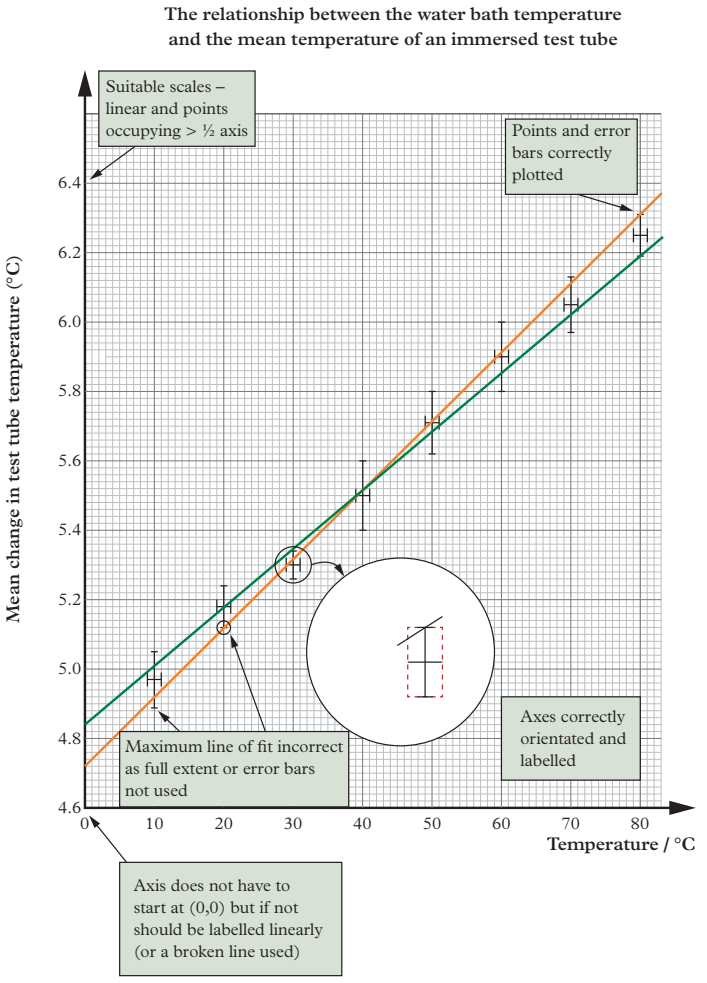

FIGURE 3 A graph of the independent and dependent variables including error bars

TABLE 1 Average change in temperature of test tube when placed in a water bath for 5 minutes

Temperature of water bath °C	Change in temperature test 1 °C	Change in temperature test 2 °C	Mean temperature change °C
10	4.89	5.05	4.97
20	5.12	5.24	5.18
30	5.26	5.34	5.30
40	5.40	5.60	5.50
50	5.62	5.80	5.71
60	5.80	6.00	5.90
70	5.97	6.13	6.05
80	6.19	6.31	6.25

Consistent significant figures in each column

Headings given with units

Systematic presentation

Uncertainty to 1–2 significant figures max

Processed data to the same number of significant figures as the raw data

All raw data to the resolution of the instruments used

Step 6: Discuss your results

The discussion section is a chance to make a connection between your results, the biological concepts you have studied and the scientific question that you asked. In this section, you should answer the following questions:

- Do you have enough data to answer your question? Is this a limitation of your experiment?
- Is there any correlation/causation between the independent variable and the dependent variable?
- Is there a conclusion to be made about the relationship between the independent variable and the dependent variable?
- Do the data support the hypothesis?
- How do your results compare to the biological concepts that you have learnt?
- How would your results apply in the real world?
- Do you have any further questions as a result of your experiment?

Step 7: Prepare a poster of your experiment

Refer to your Biology toolkit and the *Biology for VCE Units 3 & 4 Student workbook* for tips on how to produce a good Area of Study 3 poster.

Remember to use the layout provided by VCAA, which is shown in Figure 4.

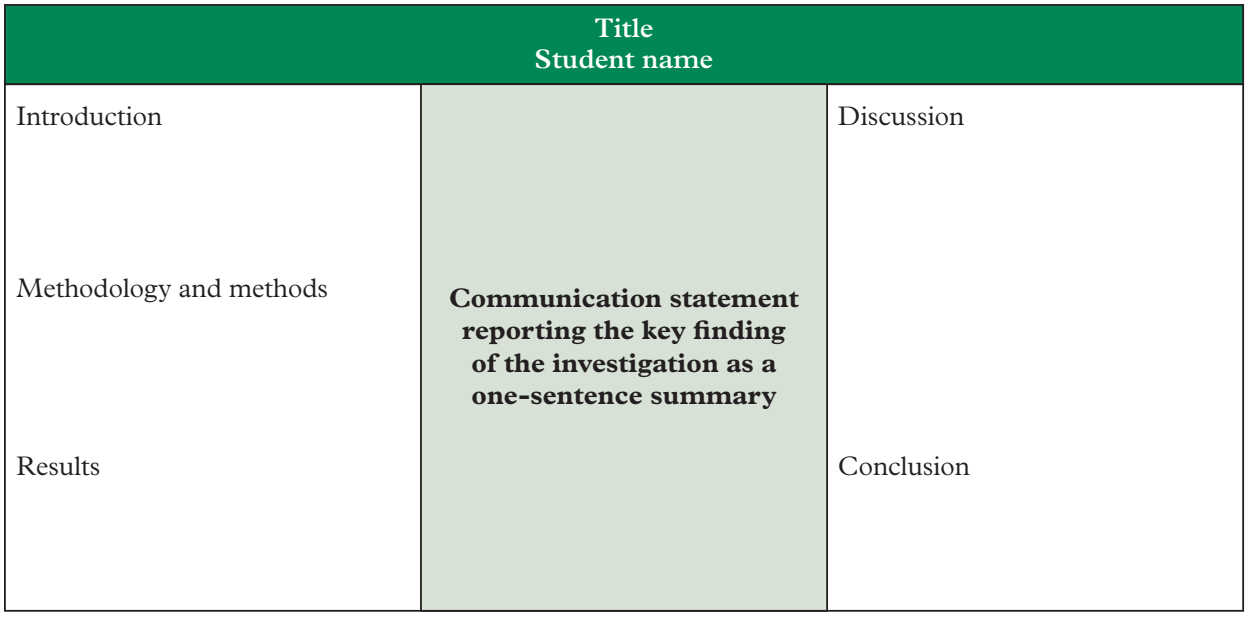

FIGURE 4 The layout for the student poster

Step 8: Celebrate!

TABLE 2 Assessment rubric

Component	Content requirement	Mark allocations (X/40)
Title	Question under investigation	
Introduction	Brief explanation or reason for undertaking the investigation, including a clear aim, a hypothesis and/or prediction and relevant background biological concepts	
Methodology and methods	Brief outline of the selected methodology used to address the investigation question	
	Summary of data generation method/s and data analysis method/s	
Results	Presentation of generated data/evidence in appropriate format to illustrate trends, patterns and/or relationships	
Discussion	Interpretation and evaluation of analysed primary data	
	Identification of limitations in data and methods, and suggested improvements	
	Cross-referencing of results to relevant biological concepts	
	Linking of results to investigation question and to the aim to explain whether or not the investigation data and findings support the hypothesis	
	Implications of the investigation and/or suggestions as to further investigations that may be undertaken	
Conclusion	Conclusion that provides a response to the investigation question	
	Identification of the extent to which the analysis has answered the investigation question, with no new information being introduced	
References and acknowledgements	Referencing and acknowledgement of all quotations and sourced content relevant to the investigation	
Logbook	Students record in their logbooks all elements of their investigation planning, comprising identification and management of relevant risks, recording of raw data, and preliminary analysis and evaluation of results, including identification of outliers and their subsequent treatment.	

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UNIT 4

Practice exam questions

Multiple choice (Total = 10 marks)

1 A non-self-molecule that causes an immune response is called:
- **A** an antigen.
- **B** an antibody.
- **C** a receptor.
- **D** a pathogen.

2 Which of the following white blood cells can be found patrolling the circulatory system when in an inactivated state?
- **A** Natural killer cells
- **B** Neutrophil
- **C** Eosinophil
- **D** Dendritic cells

3 Which two signals must a cytotoxic T-lymphocyte receive to be activated?
- **A** An antigen and an increase in temperature
- **B** An antibody and a cytokine
- **C** An antigen and a cytokine
- **D** An increase in temperature and the presence of apoptotic bodies

4 Which of the following methods would be the best at containing a measles outbreak?
- **A** Vaccinate all infected people
- **B** Stop imports of all fruit and vegetables into Australia
- **C** Store all perishable food under 4°C
- **D** Isolate all infected people

FIGURE 1 A girl with measles

5 What is an allele?
- **A** Two different genes
- **B** An alternative form of a gene
- **C** An alternative form of a protein
- **D** A trait that is inherited

6 What are the conditions of genetic equilibrium?
- **A** Large enough population, mutational equilibrium, large immigration or emigration numbers, random reproduction, natural selection
- **B** Large enough population, mutational equilibrium, no immigration or emigration, non-random reproduction, no natural selection
- **C** Small enough population, mutational equilibrium, no immigration or emigration, non-random reproduction, natural selection
- **D** Large enough population, mutational equilibrium, no immigration or emigration, random reproduction, no natural selection

7 Genetic divergence is:
- **A** when a population is separated by a geographical barrier.
- **B** another term for sympatric speciation.
- **C** where organisms with similar structures have a shared ancestry.
- **D** when a population of interbreeding organisms evolves into two or more species.

8

Human	KKA**S**KPKKAASKAPTKKPKATPVKKAKKK**L**AATPKK
Chimpanzee	KKA**S**KPKKAASKAPTKKPKATPVKKAKKK**L**AATPKK
Mouse	KKA**T**KPKKAASKAPSKKPKATPVKKAKKK**P**AATPKK
Rat	KKA**T**KPKKAASKAPSKKPKATPVKKAKKK**P**AATPKK
	A	B

FIGURE 2 A section of the *histone H1* gene for the human, chimpanzee, mouse and rat

In the amino acid sequences shown in Figure 2, the change in the sequence at **A** is conservative, whereas the change in the sequence at **B** is non-conservative because:

A S & T are biochemically similar, whereas L & P are not.

B S & T are the same amino acid, whereas L & P are not.

C S & T are biochemically different and would result in a completely different protein.

D L & P are the same amino acid.

9 The middle ear bones (malleus, incus and stapes) are found in:

A all animals.

B all mammals.

C all primates.

D all hominoids.

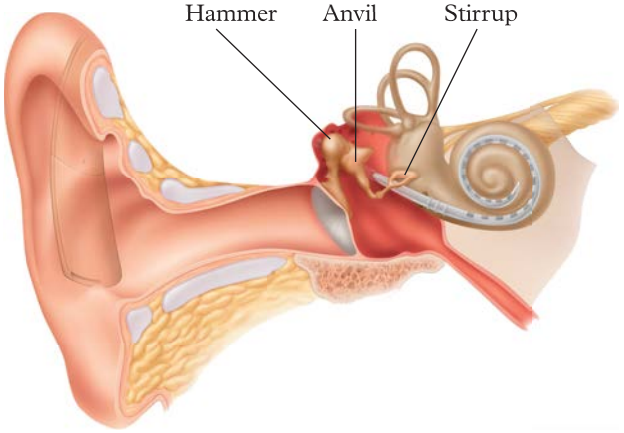


FIGURE 3 The three small bones found in the middle ear

10 A graph comparing the size of a feature found in the different species of hominin is shown in Figure 4. This feature is most likely:

A the ratio of arm size to leg size.

B the height of each hominin species.

C the cranial capacity of each hominin species.

D the size of the leg muscles.

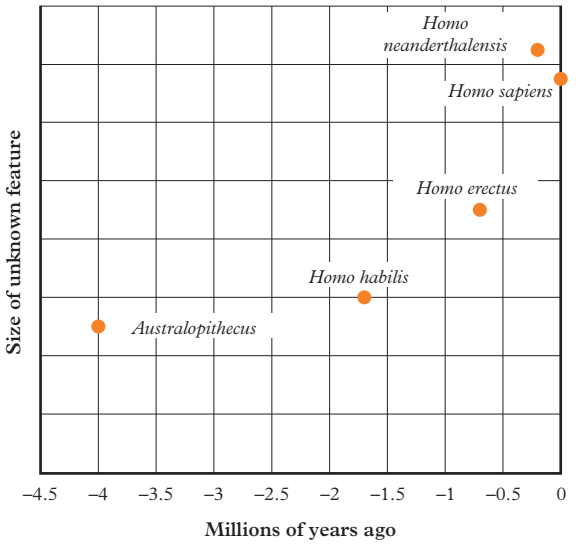


FIGURE 4 Sizes of a feature in different hominids over millions of years

Short answer (Total = 20 marks)

11 Explain why antibiotic medication is not prescribed by doctors for the flu. (2 marks)

12 Tobacco mosaic virus is a virus that infects plants. T4 bacteriophage is a virus that infects bacteria. What would happen if these viruses were introduced into a human body? (2 marks)

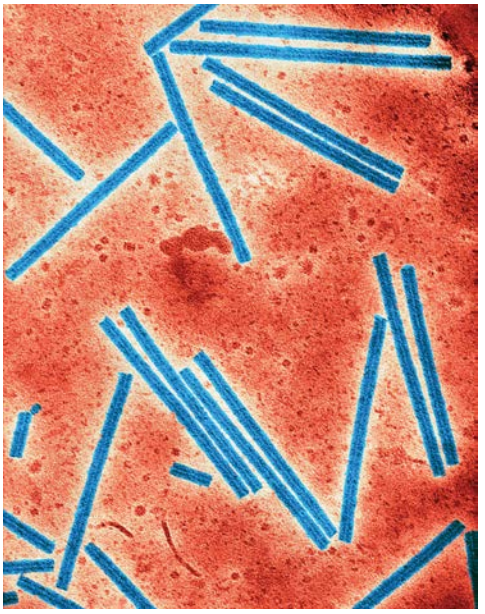


FIGURE 5 Tobacco mosaic virus

13 Write down the correct term for each of the following. (2 marks)

a One ancestral species of ant has become two different species due to a newly formed river separating two populations. 1 mark

b One ancestral species of grass has become two different species because a population of kangaroos grazed on one side of a hill and not the other. 1 mark

14 A fossil is the preserved remains or impression of an organism that is found in the rock record. (5 marks)

a Explain how a fossil is formed. 2 marks

b Why can only sedimentary rocks preserve fossils? Explain why igneous and metamorphic rocks cannot preserve fossils. 1 mark

c Explain the two different methods of dating a fossil. 2 marks

FIGURE 6 The fossilised remains of *Tyrannosaurus rex*

15 Use the phylogenetic tree in Figure 7 to answer the questions that follow. (7 marks)

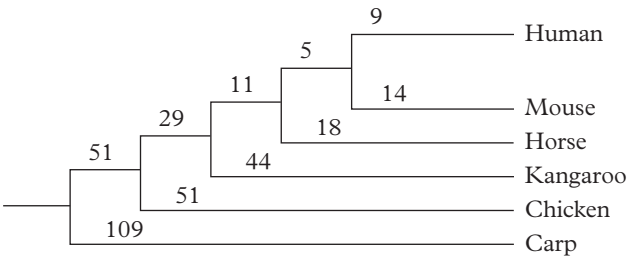


FIGURE 7 A phylogenetic tree of the relatedness of different animals

a Which animal is most closely related to the human? 1 mark

b Why are all the branches different lengths? 1 mark

c What does the line where all organisms branch off from represent, and what is it called? 2 marks

d Explain, using the different physical features of each species, why this phylogenetic tree is shaped the way it is. For example, humans have legs, but carp do not. 2 marks

e Identify the outgroup. 1 mark

16 Who are thought to be the First Peoples to inhabit Australia? Use evidence to support your answer. (2 marks)

UNITS 3 & 4

Practice exam questions

Multiple choice (Total = 20 marks)

1 Exons and introns differ in that:
- **A** mature mRNA consists of only exons.
- **B** RNA polymerase only transcribes the introns.
- **C** only introns are translated into proteins.
- **D** exons are found outside the coding genes.

2 Translation of the mRNA transcribed from the *trp* operon will result in:
- **A** one enzyme molecule.
- **B** five enzyme molecules.
- **C** seven enzyme molecules.
- **D** eight enzyme molecules.

3 A 55-year old woman is diagnosed with breast cancer after a routine mammogram. It is found she has a mutated *BRCA2* gene (BRCA2 is a protein involved with DNA repair). This increaseds her chance of developing breast cancer by 50–80%. The other women in her family decide to be tested for a mutated *BRCA2* gene as well, so they can monitor their health and take preventative measures. The DNA fragments for a mutated *BRCA2* gene are larger than for a normal *BRCA2* gene. Using the information in Figure 1 above, explain which family members are more likely to develop breast cancer.
- **A** Daughter 1 and granddaughter
- **B** Daughter 2, sister and niece
- **C** Both daughters
- **D** Sister, niece and granddaughter

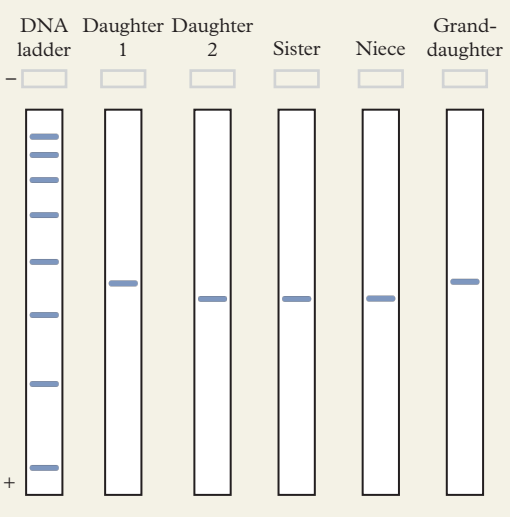


FIGURE 1 Gel electrophoresis results for family members with DNA ladder.

4 Golden rice is considered a transgenic organism because:
- **A** it was developed for commercial purposes.
- **B** the parent organisms were selected for their desirable traits.
- **C** it has DNA from another organism transferred into its genome.
- **D** it has undergone a series of block mutations.

5 The enzymatic breakdown of protein molecules into amino acids during digestion is an example of:
- **A** an anabolic reaction
- **B** a catabolic reaction
- **C** cycling of coenzymes
- **D** denaturing of enzymes

6 In a low carbon dioxide environment, the rate of photosynthesis will:
- **A** decrease.
- **B** increase.
- **C** stay the same.
- **D** be zero, because photosynthesis will not occur.

7 Q fever is a bacterial infection caused by the bacterium *Coxiella burnetii*. This bacterium spreads from cattle, sheep and goats to humans. Symptoms of infection include high temperatures, chills, sweats, headaches and extreme fatigue. When a person infected with Q fever recovers, they become immune to further infections. This is an example of:

A natural active immunity.
B natural passive immunity.
C artificial active immunity.
D artificial passive immunity.

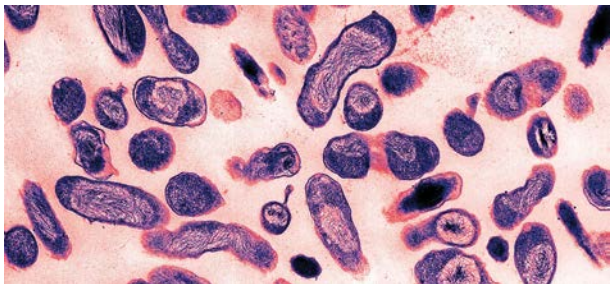


FIGURE 2 *Coxielle burnetii* causes Q fever.

8 An R^0 number refers to:

A the percentage of the population that must be vaccinated for herd immunity.
B the amount of time an infected person should be quarantined.
C the number of people that can be infected by a single contagious person.
D the virulence of a pathogen.

9 In 1883 on the islands of Krakatoa, off the coast of Java and Sumatra in Indonesia, a massive volcanic eruption 13 000 times more powerful than an atomic bomb destroyed every plant and animal species nearby. One year later, the island was recolonised by simple plants and insects, and 2 years after that by larger trees, birds and mammals. Genetic diversity was limited in these populations. This is due to:

A the bottleneck effect.
B reproductive isolation.
C the founder effect.
D selective breeding.

10 Who was 'mitochondrial Eve'?

A The first female *Homo sapiens* to mate with *Homo neanderthalensis*
B The first female *Homo sapiens* with mitochondria
C The female *Homo sapiens* from which all human beings descended (according to mitochondrial DNA)
D The first female *Homo sapiens* that left Africa

11 What is meant by the term 'degeneracy'?

A Codon nucleotides degrade quickly if not used.
B Codon nucleotides degrade slowly if not used.
C Only one codon may code for a single amino acid.
D More than one codon may code for a single amino acid.

12 Transcription of the structural genes of the *trp* operon will result in:

A one mRNA strand.
B five mRNA strands.
C seven mRNA strands.
D eight mRNA strands.

13 In the CRISPR-Cas9 complex, Cas9 has been identified as a type of:

A DNA polymerase.
B DNA ligase.
C endonuclease.
D reverse transcriptase.

14 Which of the following does not affect the rate of enzyme reaction?

A Changing the temperature
B Changing the pH
C Changing the size of the container
D Changing the concentration of the substrate

15 Fill in the blanks in the following sentence from the choices below:

The light-dependent reaction of photosynthesis occurs in the _____ while the light-independent reaction, also known as the _____ occurs in the: _____.

A thylakoid sacs, Calvin cycle, stroma

B thylakoid sacs, Krebs cycle, stroma

C chloroplast, Calvin cycle, mitochondria

D leaves, Benson cycle, roots

16 What role does MHCI play in the immune system?

A It is a glycoprotein that allows cells of the immune system to recognise it as self.

B It is a receptor on the surface of immune cells that recognises foreign cells.

C It is an antigen that initiates an overreaction of the immune system.

D It is a molecule found on the surface of bacterial cells.

17 Which cells are responsible for the production of antibodies?

A Red blood cells

B B-lymphocytes

C T-lymphocytes

D Macrophages

18 What happens to the immune system in an autoimmune disease?

A The immune cells die.

B The immune system produces too many cells.

C The immune system attacks the body's own tissues.

D The immune system shuts down.

19 A scientist divides up a population of fruit flies in a lab and grows them for several generations under different conditions (light, temperature, food source, etc.). After several months they reintroduce the populations, but it is found that they can no longer interbreed. This is an example of:

A allopatric speciation.

B sympatric speciation.

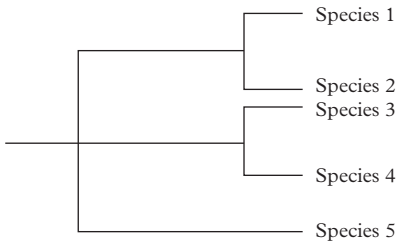
C genetic convergence.

D sexual selection.

20 Which phylogenetic tree would represent the following DNA sequences?

Species 1	AGA TCA GAT CAG ATC CAG TTT ACA GTC ATC GAT
Species 2	AGA TCA GAT CAG ATC CAG TTT ACA GTC ATC GAT
Species 3	AGA TCA GAC CAG ATC CAG TTT ACA GTC ATC GAT
Species 4	AGA TCA GAC CAG AGC CAG TTT ACA GTC ATC GAT
Species 5	AGA TCA GAC CAG AGC CAG TTT ACA GTC ATC GAT

A



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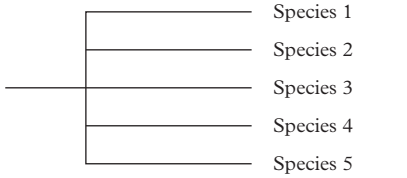

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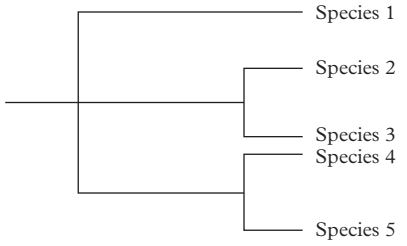

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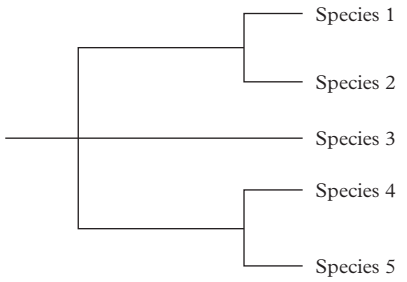

Short answer (Total = 40 marks)

21 Compare the structure of DNA and RNA using the table below. (3 marks)

	DNA	RNA
Relative length		
Nitrogenous bases		
Base pairing		
Stability		
Sugar		
Strands		

22 Explain why the proteome of a eukaryote cell would be larger than its genome. (2 marks)

23 Most plants are classified as C_3, where the process of photosynthesis occurs within the same space. C_4 and CAM plants evolved in hot, dry conditions and have slightly different reactions. (6 marks)

a How does the process of photosynthesis in C_4 plants differ from those in C_3 plants? 2 marks

b How does the process of photosynthesis in CAM plants differ from those in C_3 plants? 2 marks

c Where is RuBisCo found in the photosynthesis pathways of C_3, C_4 and CAM plants? 2 marks

FIGURE 3 The cactus is a type of CAM plant.

24 Summarise the following barriers to infection in humans listed below. (6 marks)

a Chemical barriers 2 marks

b Physical barriers 2 marks

c Microbiota barriers 2 marks

25 Explain why the barriers listed in question 24 are referred to as 'non-specific'. (1 mark)

26 Carbon-14 has a half-life of 5730 years, decaying to nitrogen-14. A fossil sample is tested and found to be 25% carbon-14 and 75% nitrogen-14. Calculate the age of the fossil. (2 marks)

27 Identify one vestigial structure in a mammal of your choosing. (1 mark)

28 Use the information in the table below to answer the questions that follow. (5 marks)

Enzyme	Organism from which derived	Target sequence (cut at *) 5' → 3'
AvaI	*Anabaena variabilis*	G*C/TCGA/GG
BamHI	*Bacillus amyloliquefaciens*	G*GATCC
BglII	*Bacillus globigii*	A*GATCT
EcoRI	*Escherichia coli* RY13	G*AATTC
EcoRII	*Escherichia coli* R245	*CCA/TGG
HaeIII	*Haemophilus aegyptius*	GG*CC
HhaI	*Haemophilus haemolyticus*	GCG*C
HindIII	*Haemophilus influenzae* RD	A*AGCTT
HpaI	*Haemophilus parainfluenzae*	GTT*AAC
KpnI	*Klebsiella pneumoniae*	GGTAC*C
MboI	*Moraxella bovis*	*GATC
PstI	*Providencia stuartii*	CTGCA*G
SmaI	*Serratia marcescens*	CCC*GGG
SstI	*Streptomyces stanford*	GAGCT*C
SalI	*Streptomyces albus* G	G*TCGAC
TaqI	*Thermus aquaticus*	T*CGA
XmaI	*Xanthomonas malvacearum*	C*CCGGG

a Write the complementary strand to the following DNA sequence. 1 mark

DNA	ATC	CTG	CAG	AGC	TGG	AGC
Complementary strand						

b Which restriction enzyme would you use to cut the DNA sequence? Mark the cut site on the sequence in Question **a**. 2 marks

c The CRISPR sequence of the CRISPR-Cas9 complex is usually about 20 nucleotides long. How does the CRISPR-Cas9 complex compare to the use of bacterial restriction enzymes? 2 marks

29 High-performing athletes tend to have a higher vital capacity (VC) in their lungs; that is, their lungs are able to take in more air. An athlete and a non-athlete compete in a marathon. At the end of the race, the non-athlete is breathing heavily and the next day wakes up with stiff legs. The athlete does not. (5 marks)

a Explain why these symptoms did not affect the elite athlete as much. 3 marks

b The athlete decides to help the non-athlete with their training. In the marathon the following year, they both compete and finish in a similar time. The non-athlete is no longer breathing heavily and their legs are not stiff the next day. Explain what has occurred over the year of training. 2 marks

FIGURE 4 An elite athlete and a non-athlete have different vital lung capacities.

30 In the application of CRISPR-Cas9, how does targeted knock-down differ from targeted knock-in and knock-out? (2 marks)

31 Cancer immunotherapy is a more targeted treatment for cancer than surgery, chemotherapy and radiotherapy. (4 marks)

a Explain the difference between non-specific and specific immunotherapy. 2 marks

b Would cancer vaccines, such as the HPV vaccine, be an example of a non-specific or specific immunotherapy? Explain your answer. 2 marks

32 Describe the difference, using examples, between alleles and genes. (2 marks)

33 Explain how the bottleneck effect can occur in a population. (1 mark)

CHAPTER 15

Practical work

To complete VCE Biology, you will need to complete at least 10 hours of practical activities and scientific investigations for each of Units 3 and 4.

The investigations may be practical investigations that generate primary data, or research investigations that involve the collation of secondary data. All investigations that are undertaken as part of your course, as well as School-assessed Coursework (SACs), should be written in a logbook that will be monitored and submitted to teachers. Before undertaking an investigation for the first time, ethical concerns should be considered, including the importance of sociocultural, economic, political and legal factors that may arise from science-related decision-making.

Practical investigation can be assessed in two of your SACs:

1. Comparison and evaluation of biological concepts, methodologies and methods, and findings from three student practicals (Outcomes 1 and 2 – Unit 3 or 4)
2. Area of Study 3: Design and conduct a scientific investigation related to cellular processes and/or how life changes and responds to challenges, and present an aim, methodology and method, results, discussion and a conclusion in a scientific poster (Outcome 3 – Unit 4)

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SAFETY IN THE LABORATORY

This chapter will highlight key safety concerns for each practical, though there are some general safety concerns to be considered before completing all practical work.

- → Hair should be tied back.
- → Do not eat or drink in the lab.
- → Always be aware of your peers and act in a way that will not cause harm.
- → Wear a lab coat, safety glasses, closed-toed shoes and gloves.
- → Review the school's safety procedures and location of the eyewash, shower, spill kits and first aid kits.
- → Handle all chemicals with care and consult your teacher and risk assessments for the hazards involved with each particular chemical.
- → Keep open flames away from flammable materials.
- → Handle hot material with appropriate equipment (i.e. heat-resistant gloves and tongs).
- → Always check that electrical equipment is not damaged and that there are no exposed wires before use.
- → Fieldwork should be completed in groups, with a full risk assessment completed before any excursion.

It is the responsibility of the teacher and school to conduct a risk assessment before any practical covered in this book.

FIGURE 1 Pear rust is a type of fungal infection. A fungal infection may occur through coincidence or be an opportunistic infection.

UNIT 3 PRACTICALS

PRACTICAL	**2.1** Extracting DNA from strawberries
PRACTICAL	**2.5** Exploring protein structures
PRACTICAL	**3.1** Digital endonuclease digestion
HIGH-TECH PRACTICAL	**3.4** The pGLO plasmid digestion and gel electrophoresis
PRACTICAL	**4.2** Enzymes in washing detergent
PRACTICAL	**5.1** Testing for photosynthesis using alginate balls with *Chlorella*
PRACTICAL	**6.3** Factors that affect the rate of aerobic cellular respiration
HIGH-TECH PRACTICAL	**7.1** Micropipette skills and knocking-in a gene

UNIT 4 PRACTICALS

PRACTICAL	**8.1** Plant defence mechanisms
PRACTICAL	**9.1** Blood typing
PRACTICAL	**10.2** Testing the effectiveness of antibacterial substances
NO-TECH PRACTICAL	**11.1** Genetic changes over time
NO-TECH PRACTICAL	**12.1** Absolute age
NO-TECH PRACTICAL	**13.2** Molecular differences between species
NO-TECH PRACTICAL	**14.4** Modelling the migration of *Homo sapiens*

2.1 PRACTICAL Extracting DNA from strawberries

Practical worksheet
2.1 Extracting DNA from strawberries

Practical demonstration
2.1 Extracting DNA from strawberries

Risk assessment
2.1 Extracting DNA from strawberries

Lab tech notes
2.1 Extracting DNA from strawberries

Aim

To extract DNA from strawberries.

Materials

- 4 × big strawberries
- Knife
- Chopping board
- Blender or Bamix® stick blender
- Container for ice bath (500 mL or larger)
- 10 mL and 100 mL measuring cylinder
- 100 mL, 250 mL and 400 mL beaker
- Hot plate
- Thermometer
- Timer
- Ice-cold ethanol
- Soap flakes
- Electronic balance
- Protease enzyme
- Chux® cloth
- Glass rod with hook on the end
- Specimen bottle

Method

1. Cut four large strawberries, using a sharp knife and cutting board, into very small pieces.
2. Add the strawberry pieces to the blender with 100 mL of warm tap water. Blend for 10–15 seconds.
3. Place the blended strawberries and water in a 250 mL beaker. Add 3 g of soap flakes and stir.
4. Heat the mixture to 65°C and hold at this temperature for 8 minutes, stirring gently.
5. Cool the mixture to 40°C by placing it in an ice-water bath (use a 400 mL beaker) until the required temperature is reached. Stir gently during the cooling process. *Cooling the strawberry mixture slows the breakdown of DNA.*
6. Add 5 mL of protease enzyme and stir gently for 5 minutes.
7. Filter the mixture through a double thickness of 'Chux' cloth into a 100 mL beaker. Squeeze the material in the 'Chux' cloth to get all the juice out of it.
8. Place 10 mL of the liquid strawberry filtrate into the specimen bottle.
9. Add 10 mL of ice-cold ethanol to the specimen bottle, pouring slowly and carefully down the side of the specimen bottle so that the ice-cold ethanol sits on top of the filtrate. It might be easier to add the ice-cold ethanol to the strawberry filtrate using a pipette.
10. Let the mixture sit for 2 or 3 minutes. The DNA is not soluble in ethanol and should precipitate out of solution near the boundary between the strawberry filtrate and the ethanol.
11. Gently swirl the DNA using the narrow glass rod with a hook on the end. Swirl the glass rod so the hook is in the strawberry filtrate just below the ethanol and gently lift it up through the ethanol. DNA should be on the glass rod near the hook. Repeat this swirling action several times to accumulate a good amount of DNA. (DNA looks like white mucus – the clearer it is, the fewer impurities are present.)

Results

Record your observations of what the DNA looks like.

Discussion

1. What was the independent and dependent variable for this experiment?
2. What other variables need to be controlled in this experiment?
3. Why were the strawberries blended?
4. Why were the strawberries heated and then cooled?
5. Can you see any DNA? If you can't, consider what went wrong and comment on how you would change the experiment if you were to repeat it.

Conclusion

Explain how the DNA was separated from the strawberry.

2.5 PRACTICAL Exploring protein structures

Practical worksheet
2.5 Exploring protein structures

Practical demonstration
2.5 Exploring protein structures

Risk assessment
2.5 Exploring protein structures

Lab tech notes
2.5 Exploring protein structures

Context

You have studied transcription, translation and the primary, secondary, tertiary and quaternary structure of proteins.

Answer these questions before you complete this activity.

1 Use the following words to describe the structure of DNA: nucleotide, phosphate, deoxyribose sugar, nitrogenous base, complementary, antiparallel, double helix.
2 Describe the process of transcription.
3 Describe two ways the mRNA is modified before leaving the nucleus.
4 Describe how mRNA, tRNA and rRNA are involved in producing a polypeptide.
5 What is the difference between a polypeptide and a protein?

Aim

To identify primary, secondary and tertiary structures in a 3D diagram of the BRCA1 protein.

Materials

- Computer

Method

1 Navigate to the following link: https://www.ncbi.nlm.nih.gov/protein.
2 Type '1Y98' into the search bar. Click on the BRCA protein ribbon diagram.
3 Select 'full-featured 3D viewer'. This will open another window that will provide you will a rotating 3D image of the BRCA protein. You will be able to hold the left mouse button down and manually rotate the protein to examine it from all angles.

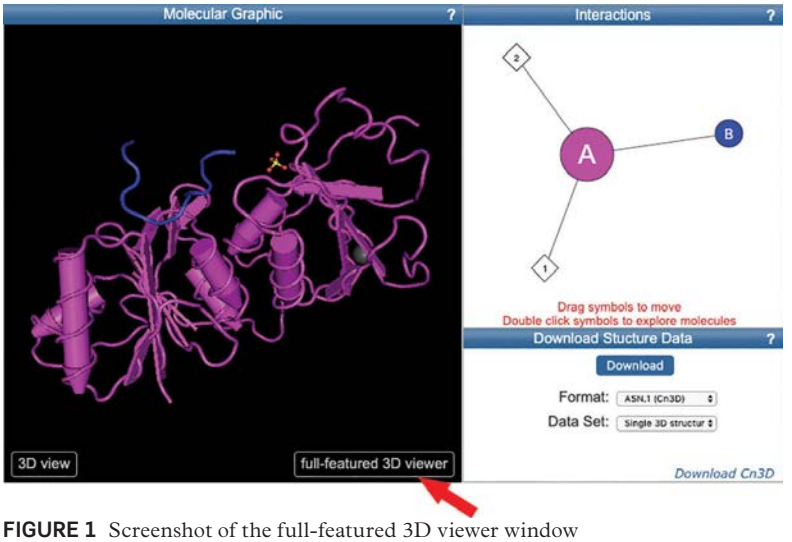


FIGURE 1 Screenshot of the full-featured 3D viewer window

4 Hover the arrow over the 'Style' tab and select one of the options from the 'Proteins' menu. This allows you to see different versions of the protein structure.

5 When you have finished exploring, select the 'Ribbons' version of the protein.

6 At the top of the screen, select the 'Color' tab. From the dropdown menu, select 'Secondary structures' and then 'Sheet in Yellow'. Both the helix and strand objects point in an amino to carboxyl direction (i.e. the point towards the end of the protein). Now answer questions 1–3 in the Results section.

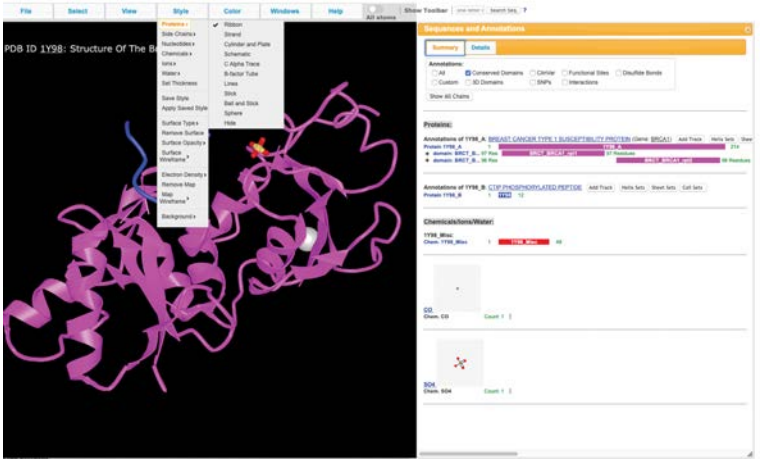


FIGURE 2 Screenshot of the Style tab

7 After answering questions 1–3, change the colour back to purple by selecting 'Chain' in the 'Color' tab.

FIGURE 3 Screenshot of the Color tab

8 Select the 'Details' window on the yellow panel to the right of the window. This will allow you to examine the amino acid sequence of the BRCA protein. The viewer shows the one-letter abbreviation for the amino acid sequence of the protein.

FIGURE 4 Screenshot of the Details window

9 Select one of the letters that represents an amino acid. This should cause the letter to be highlighted in yellow. The corresponding section of the protein should also be highlighted in yellow. You may need to rotate the protein to identify the highlighted amino acid.

FIGURE 5 Screenshot of the letters that represent an amino acid in the Details window

10 The blue section is part of a different protein to which BRCA1 normally binds to do its job of repairing DNA. This protein is called CtIP. To help visualise the CtIP protein, click on the 'Protein 1Y98_B' sequence in the bottom left corner of the Details window.

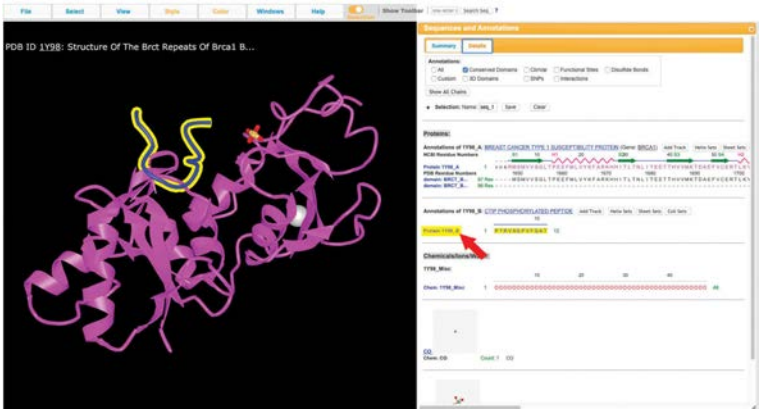


FIGURE 6 Screenshot of the Protein 1Y98_B

11 Hover the arrow over the pink letters in the 'Details' window that represent each amino acid in the 'Protein 1Y98_A' chain. The number of each amino acid will appear. Scroll across the sequence until you find location 130 (amino acid M: methionine). Select the amino acid. The corresponding amino acid will be highlighted on the protein structure in yellow.

FIGURE 7 Screenshot showing the location of pink nucleotide letters in the Details window

12 Click on the 'Select' tab and choose 'by Distance'.

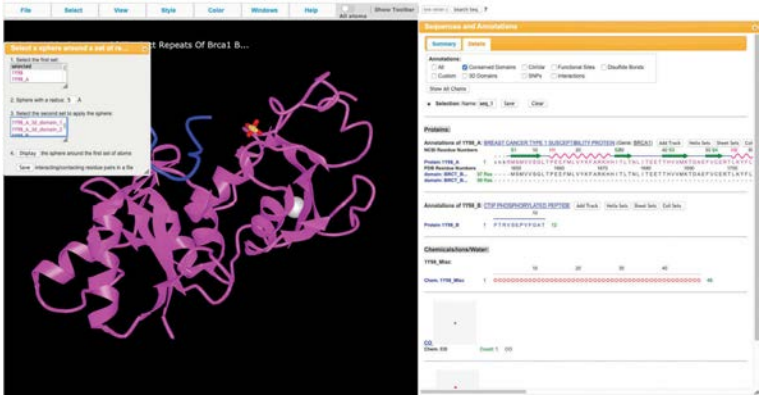


FIGURE 8 Screenshot of the 'Sphere with a radius' box

13 Make sure the 'Sphere with a radius' box is set to 5 and select 'Display'. This will highlight the residues of the CtIP protein in yellow that are within 5 angstroms (5×10^{-10} m) of this M1775R mutation.

14 You should be able to see an area of the CtIP protein is also highlighted in yellow. Click on the 'View' tab and select 'Zoom in Selection'. The closeness of these two sections suggests there is a bond between these two amino acids. Now answer Question 4 in the Results section.

Results

1 What secondary protein structures are highlighted in yellow?

2 What secondary protein structures are highlighted in red?

3 What colour are the random coils?

4 How will a mutation at this point in the protein affect the bond with the CtIP protein?

Discussion

1 Is the BRCA1 protein a tertiary or quaternary structure? Define these terms to explain your answer.

2 Are random coils the same or different in all BRCA1 proteins? Discuss.

3 How does a change in a single amino acid affect the structure of the BRCA1 protein?

4 The BRCA1 protein is responsible for repairing damage to DNA. Suggest one possible consequence for an individual who has a BRCA mutation.

Conclusion

How does the structure of a protein affect its function?

3.1 PRACTICAL Digital endonuclease digestion

Practical worksheet
3.1 Digital endonuclease digestion

Practical demonstration
3.1 Digital endonuclease digestion

Risk assessment
3.1 Digital endonuclease digestion

Lab tech notes
3.1 Digital endonuclease digestion

Context

The *Aequorea victoria* jellyfish (common name crystal jelly) produces a unique protein that emits a low-energy green light as a result of bioluminescence. This green fluorescent protein (GFP) is one of a group of proteins that are used as markers by molecular biologists.

In 1992, the *GFP* gene was reverse transcribed (from mature mRNA to cDNA) and DNA polymerase was used to make the single-stranded cDNA into double-stranded DNA. This gene was then placed into a plasmid.

The plasmid containing the *GFP* gene (pGLO) also contains other genes including:

- *ampR*: gene for ampicillin resistance
- *araC*: gene that induces (switches on) the expression of the GFP when in the presence of arabinose sugar.

Molecular scissors called endonucleases (or restriction enzymes) can be used to digest (cut) the plasmid into sections. Each endonuclease has its own specific recognition sequence. The endonuclease enzyme moves along the DNA helix until it binds to the specific sequence, where it then cuts the DNA to form either a blunt end or a sticky end.

Many scientists use virtual modelling to determine the location and size of each segment generated by an endonuclease.

FIGURE 1 The *Aequorea victoria* jellyfish hosts the GFP.

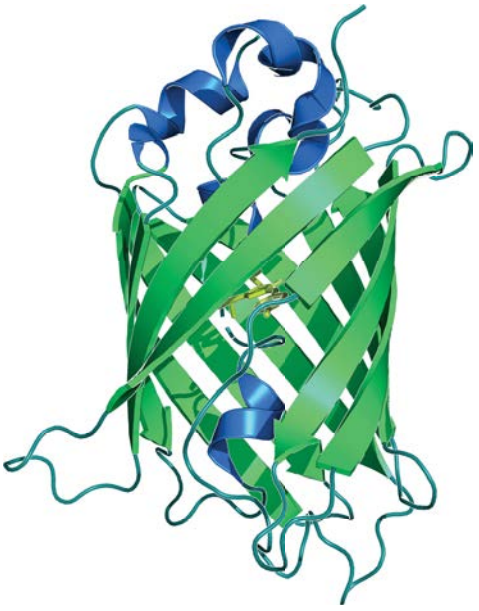


FIGURE 2 The structure of the green fluorescent protein (GFP)

Aim

To investigate the accuracy of predictions with regards to the number and size of DNA segments produced from the digestion of a pGLO plasmid.

Materials

- Computer
- pGLO sequences (obook)

Method

Part A: Pre-lab work

Before starting this practical, answer questions 1–4 in the Discussion section.

Part B: Practical work

1 Open the document called 'pGLO sequence' located on your obook pro.
2 Highlight the entire sequence in the document (including the numbers) and copy it (Ctrl+C).
3 Go to the website: http://restrictionmapper.org/.
4 Paste the nucleotide sequence onto the 'Sequence Info' box.
5 In the 'Include' box under 'Select Individual Enzymes' select the endonuclease HindIII. Select 'Virtual Digest' to activate the program. This will take you to a page with the cut sequences. You may need to scroll down to see each segment.
6 Record the length of the segments in Table 1.
7 Repeat the Virtual Digest procedure for the other endonuclease (EcoRI).

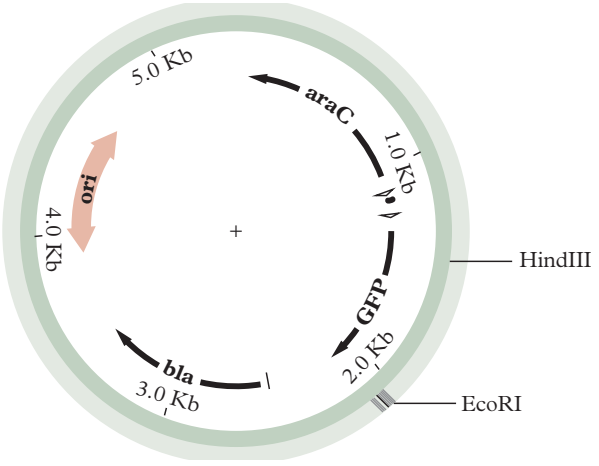


FIGURE 3 A diagram of the pGLO plasmid

Results

Record your results in the table below.

TABLE 1 Predicted digestion of DNA by endonucleases

Endonuclease	Number of segments	Length of segments
HindIII		
EcoRI		

Discussion

1 The gene for the GFP was found in a eukaryote. Describe the protein that would be produced if this gene was placed (unchanged) into a prokaryote.
2 What is a plasmid?
3 Would a blunt end or a sticky end be more likely to reconnect? Explain your reasoning.
4 Use Figure 3 to predict the number of fragments that will be produced with each endonuclease. **Hint:** The pGLO plasmid is circular.
5 Many factors can affect the rate at which the DNA sequence is cut by an enzyme. List the factors that can affect the rate of reaction involving enzymes.
6 Why do EcoRI and HindIII cut the pGLO plasmid at different sites?
7 The endonuclease PvuI cuts the pGLO plasmid in the middle of the *ampR* gene. If another gene was inserted in this site, and then the plasmid was inserted into a bacterial cell, what effect would it have on the cell's ability to survive exposure to ampicillin?

Conclusion

How does an endonuclease locate and cut DNA at a specific recognition sequence?

3.4 HIGH-TECH PRACTICAL
The pGLO plasmid digestion and gel electrophoresis

Practical worksheet
3.4 The pGLO plasmid digestion and gel electrophoresis

Practical demonstration
3.4 The pGLO plasmid digestion and gel electrophoresis

Risk assessment
3.4 The pGLO plasmid digestion and gel electrophoresis

Lab tech notes
3.4 The pGLO plasmid digestion and gel electrophoresis

Context

Part A: Enzyme digestion

See Practical 3.1 to gain context for the enzyme digestion you are about to complete.

Part B: Gel electrophoresis

Gel electrophoresis is used to separate DNA strands according to their size. The negatively charged DNA is attracted to the positive terminal of the gel and will therefore migrate through the gel from the negative terminal where it is loaded towards the positive electrode. The agarose gel consists of a matrix-like structure that makes it difficult for larger molecules to move through. Short molecules of DNA move quickly along the gel, while longer sections of DNA move more slowly. The bands of DNA can then be compared against a known DNA ladder to determine their length in base pairs (bp).

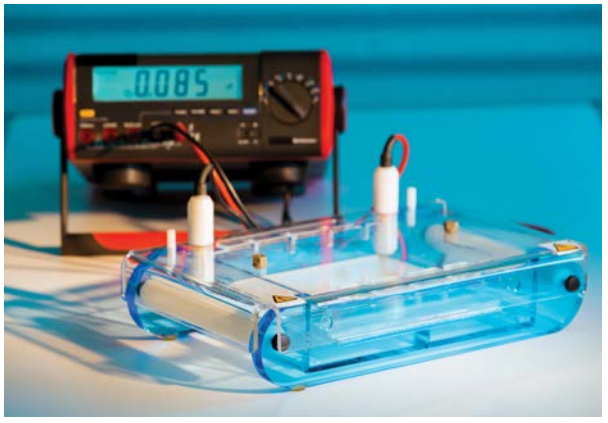


FIGURE 1 A gel electrophoresis box. **Note:** You can use any gel electrophoresis box when running this practical.

Aim

To use gel electrophoresis to separate DNA sequences of different lengths.

Materials

- White spotting tile
- 5 mL water with coloured food dye
- 37°C water bath
- Microcentrifuge
- 2–20 µL micropipette
- 2 × Eppendorf tubes
- Eppendorf tube rack
- Container of ice
- Permanent marker
- Timer
- Waste container
- 7 µL pGLO plasmid on ice
- 10 × 7 µL restriction buffer (with green dye) on ice
- 3 µL FASTDIGEST™ HindIII enzyme on ice
- 3 µL FASTDIGEST™ EcoRI enzyme on ice
- 1 mL sterile water
- 30 mL TBE buffer
- 2–20 µL micropipette
- Bluegel™ electrophoresis tank
- 10 µL DNA ladder
- 2% agarose gel with dye

Method

Part A: Enzyme digestion

1 Label the Eppendorf tubes Undigested pGLO, HindIII digest and EcoRI digest.

2 Using a fresh micropipette tip for each reagent and sample, pipette (in the correct order) the sterilised water, the buffer, the pGLO plasmid, and the enzymes into each tube according to Table 1.

TABLE 1 Components in each Eppendorf tube

Tube	Sterilised water	Buffer	pGLO plasmid	EcoRI	HindIII
Undigested pGLO	16 µL	2 µL	2 µL	-	-
HindIII	14 µL	2 µL	2 µL	-	2 µL
EcoRI	14 µL	2 µL	2 µL	2 µL	-

3 Tightly close the lids of all the tubes and mix the components by gently flicking the tubes with a finger. Pulse spin the tubes in a micro-centrifuge to collect the contents on the bottom.

4 Incubate the reactions for 15 minutes in the 37°C water bath.

5 Store the tubes on ice while preparing for the gel electrophoresis procedure.

Part B: Gel electrophoresis

1 Slowly pour 30 mL of buffer solution into the gel electrophoresis tank.

2 Slowly place the gel into the electrophoresis chamber.

3 Load 5 µL of the DNA ladder into a well of the gel.

4 Load 13 µL of each DNA sample into the corresponding wells of the gel, as shown in Figure 2. Ensure you use a fresh tip for each sample.

5 Place the clear lid on top of the chamber and press the power button to run the gel for 20–30 minutes. Place the dark cover over the gel electrophoresis chamber. **Note:** Be sure to check the voltage that is appropriate for your gel electrophoresis unit.

6 After 20 minutes turn on the UV lamp and examine the gel through the hole in the dark cover. Fluorescent bands should be visible under UV light.

Results

1 Draw the banding that is visible under the UV light.

2 Identify the lengths of each DNA fragment.

FIGURE 2 An example of an agarose gel with samples loaded in the wells at the top of the gel prior to electrophoresis. Note that the negative and positive terminals have been labelled.

Discussion

1 Explain why the restriction enzyme reactions are stored on ice between procedures.

2 If the current is run through the gel electrophoresis for too long, what will happen to the DNA fragments?

3 Why is buffer added to the DNA samples before the restriction enzyme is added?

4 Why do the DNA strands move towards the positive terminal of the gel?

5 Do long DNA fragments or short DNA fragments move further along the gel?

Conclusion

How does gel electrophoresis separate the different DNA strands?

4.2 PRACTICAL
Enzymes in washing detergent

Practical worksheet
4.2 Enzymes in washing detergent

Practical demonstration
4.2 Enzymes in washing detergent

Risk assessment
4.2 Enzymes in washing detergent

Lab tech notes
4.2 Enzymes in washing detergent

CAUTION: Amylase solution can be a health hazard if inhaled.

Context

Dirty clothes can have a variety of stains, including carbohydrates such as sugar and flour. These molecules are made up of simple sugars such as glucose joined in long chains to form starch. Many modern detergents used to wash clothing contain enzymes that digest any starch that may be part of the stain. One example is amylase in the formula below:

$$\text{Starch} \xrightarrow{\text{Amylase}} \text{Glucose}$$

Iodine can be used to test for the presence of starch. Iodine (usually a pale yellow/brown) becomes black/purple in the presence of starch. In the presence of glucose, the iodine remains a pale yellow/brown.

A biochemical company is interested in adding enzymes to their detergent. They are aware that there are many factors that may impact the effectiveness of the enzyme.

Aim

To determine the effectiveness of amylase in digesting starch, when added to a detergent.

Materials

- 15 mL 1% amylase
- 15 mL 0.5% starch solution
- Iodine/KI solution (suitable for testing starch)
- 5 mL of each buffer (pH 4, 7, 10)
- Different liquid laundry detergents (potentially with different pH values)
- 9 × test tubes
- 10 mL measuring cylinder
- 3 × white spotting tiles
- Timer
- pH meter
- Plastic disposable pipettes
- Permanent marker
- Water bath (40°C)
- Thermometer

FIGURE 1 A white dimple tile/spotting plate

Method

1. Place a single drop of iodine solution in each dimple on the white tile.
2. Add a single drop of the starch solution to the iodine on the first dimple. Note the colour change.
3. Add a single drop of the amylase solution to the iodine on the second dimple. Note any colour change.
4. Add a single drop of the pH 7 buffer to the iodine on the third dimple. Note any colour change.
5. Label three of the test tubes: 'S' (starch), 'A' (amylase) and 'pH7' (buffer).
6. Measure 5 mL of starch solution and add it to the starch test tube.
7. Measure 5 mL of amylase solution and add it to the amylase test tube.
8. Measure 5 mL of pH 7 buffer and add it to the buffer test tube.

9 Place all three test tubes in the 40°C water bath for 5 minutes.

10 When all three solutions have reached the correct temperature, add the amylase solution to the buffer and mix thoroughly. Return the mixture to the water bath for 1 minute.

11 Add the starch solution to the amylase/buffer mixture and mix thoroughly.

12 Add one drop of the mixture to the first dimple on the next row of the white tile, and immediately return the test tube to the water bath. Make sure that the mixture in the pipette is returned to the test tube so that the temperature change is minimal. Note any colour change.

13 After 30 seconds, add another drop of the starch/amylase/buffer mixture to the next dimple containing iodine. Note the colour change.

14 Repeat Step 13 every 30 seconds until the iodine no longer becomes blue/black. This represents the time taken for all the starch to be broken down.

15 Clean all the equipment and repeat steps 4–14 for pH 4.

16 Clean all the equipment and repeat steps 4–14 for pH 10.

17 Use the pH meter to test the pH of each of the detergents.

Results

Record your results in the table below.

TABLE 1 The time taken for the starch to be digested by the amylase in different pH buffers

Amount of 0.5% starch (mL)	Amount of 1% amylase (mL)	5 mL of pH buffer added	Time taken for starch to be digested
5	5	pH 7	
5	5	pH 4	
5	5	pH 10	

TABLE 2 pH of different washing detergents

Washing detergent	pH

Discussion

1 Explain the purpose of steps 1–3 in the method.

2 Explain why each of the test tubes were heated to 40°C in the water bath before being mixed together.

3 Explain why the buffer was mixed with the amylase and returned to the water bath for 1 minute before the starch was added.

4 Which buffer produced the fastest rate of starch digestion by amylase?

5 Describe, at a molecular level, why the other buffers produced a slower rate of reaction.

6 Use the results from Table 2 to suggest which of the washing detergents would be able to have the amylase enzyme added.

Conclusion

Describe the effect of pH on the action of an enzyme.

5.1 PRACTICAL Testing for photosynthesis using alginate balls with *Chlorella*

Practical worksheet
5.1 Testing for photosynthesis using alginate balls with *Chlorella*

Practical demonstration
5.1 Testing for photosynthesis using alginate balls with *Chlorella*

Risk assessment
5.1 Testing for photosynthesis using alginate balls with *Chlorella*

Lab tech notes
5.1 Testing for photosynthesis using alginate balls with *Chlorella*

Context

Part A: Preparing the alginate balls with *Chlorella*

Chlorella is a single-celled alga that photosynthesises to produce glucose for energy. The glucose is used to produce other molecules, including proteins, fats and vitamins. It is this that led scientists in 1946 to research the possibilities of using *Chlorella* instead of animals in protein production. When dried, *Chlorella* contains up to 50% protein. Complications with the harvesting of the algae, together with the inability of humans to digest the cell walls has resulted in *Chlorella* only being used in a limited range of products.

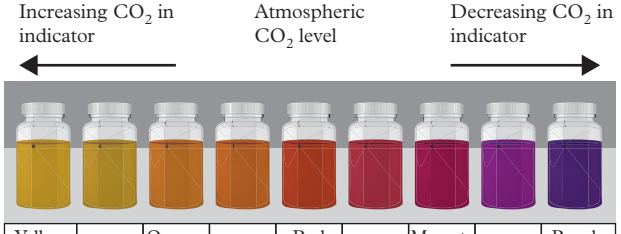
Part B: Testing for photosynthesis and cellular respiration

In this experiment, you will use *Chlorella* trapped in alginate balls to measure the rate of photosynthesis and cellular respiration. You will incubate them in a CO_2 indicator solution that is sensitive to changes in pH caused by gaseous CO_2 dissolved in water to form carbonic acid.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+(\text{acid})$$

The indicator that you will use detects the level of carbonate (CO_3^{2-}) in the solution.



Yellow		Orange		Red		Magenta		Purple
pH 7.6	pH 7.8	pH 8.0	pH 8.2	pH 8.4	pH 8.6	pH 8.8	pH 9.0	pH 9.2

FIGURE 1 Indicator colours for detecting carbonate in solution

Aim

To determine factors that affect the rate of photosynthesis.

Materials

Part A: Preparing the alginate balls with *Chlorella*

- A sample of *Chlorella*
- Warmed alginate solution
- 3 × plastic pipettes
- 50 mL beaker
- 2 × 100 mL beaker
- Scissors
- 50 mL calcium chloride
- Distilled water
- Tea strainer

Part B: Testing for photosynthesis and cellular respiration

- 20 × alginate balls
- 12 mL carbonate indicator solution
- 4 × test tubes
- Test-tube holder
- Aluminium foil
- Timer

Method

Part A: Preparing the alginate balls with *Chlorella*

1 Carefully pipette 3 mL of *Chlorella* into a 50 mL beaker. Allow the *Chlorella* to settle to the bottom of the container before extracting the sample.

2 Use a clean pipette to add 3 mL of warmed alginate solution to the beaker. Record the temperature of the solution.

3 Carefully mix the two solutions. Avoid adding air bubbles into the mixture.
4 Place 50 mL of calcium chloride solution in a new 100 mL beaker.
5 Cut the tip from the end of a clean disposable pipette to allow larger droplets of the alginate mixture to pass through.
6 Collect the alginate mixture in the cut pipette. Use the pipette to allow single drops of the alginate mixture to drop into the calcium chloride solution.
7 Allow the alginate balls to sit in the beaker of calcium chloride for 5–10 minutes.
8 Tip the alginate balls into the tea strainer placed over an empty 100 mL beaker.
9 Gently rinse the alginate balls with tap water to remove all traces of calcium chloride solution.

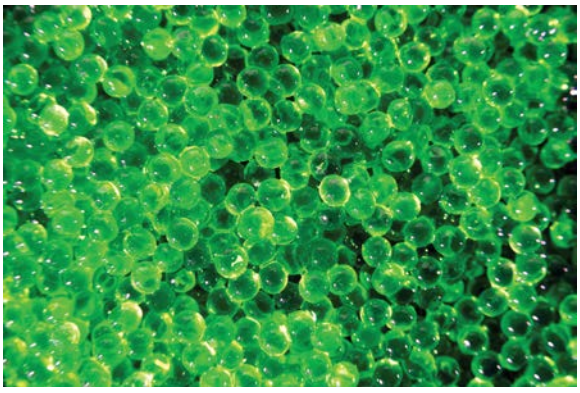


FIGURE 2 Your alginate balls will come out looking something like these.

Part B: Testing for photosynthesis and cellular respiration

1 Place 3 mL of carbonate indicator solution into each of the four test tubes.
2 Use a permanent marker to label the test tubes:
- 'Light negative control'
- 'Light test'
- 'Dark negative control'
- 'Dark test'

3 Place 10 alginate balls (rinsed with distilled water) in the 'Light test' tube and 10 alginate balls into the 'Dark test' tube.
4 Wrap the 'Dark negative control' and the 'Dark test' in aluminium foil.
5 Place all four test tubes in front of the light source for 15 minutes.

Note: The strength of the light will determine how long the *Chlorella* needs to be left before a noticeable colour change will occur.

Results

Record your results in the table below.

TABLE 1 Test-tube results

Test tube	Indicator colour (time = 0)	Indicator colour (time = 15 minutes)
Light negative control		
Light test (with alginate balls)		
Dark negative control		
Dark test (with alginate balls)		

Discussion

1 When CO_2 is released, what colour will the indicator become?
2 When the *Chlorella* cells absorb CO_2, what colour will the indicator become?
3 Describe the colour change that resulted from exposure of *Chlorella* to light.
4 Use your knowledge of photosynthesis to explain the change described in Question 3.
5 Describe the colour change that resulted from exposure of *Chlorella* to a lack of light.
6 Use your knowledge of photosynthesis to explain the change described in Question 5.
7 A student claimed that cellular respiration and photosynthesis are interdependent processes. Explain why the student was correct.

Conclusion

What factors affect the rate of photosynthesis?

6.3 PRACTICAL Factors that affect the rate of aerobic cellular respiration

Practical worksheet
6.3 Factors that affect the rate of aerobic cellular respiration

Practical demonstration
6.3 Factors that affect the rate of aerobic cellular respiration

Risk assessment
6.3 Factors that affect the rate of aerobic cellular respiration

Lab tech notes
6.3 Factors that affect the rate of aerobic cellular respiration

Context

Yeast (*Saccharomyces cerevisiae*) are single-celled organisms that are classified as fungi. Like all organisms, the growth rate of yeast is affected by the availability of energy in the form of ATP. Cellular respiration is represented by the chemical equation:

$$C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l) + 36 \text{ or } 38 \text{ ATP}$$

As the yeast reproduce, they undergo aerobic cellular respiration and produce carbon dioxide gas. The gas produced can be collected by a balloon. The rate at which the diameter of the balloon increases is an indication of the rate of carbon dioxide production.

This aerobic cellular respiration is controlled by many different enzymes. Multiple factors can affect these enzymes, including the type and concentration of nutrients, pH and temperature.

Aim

To determine factors that affect the rate of aerobic cellular respiration.

Materials

- 16 × 125 mL Erlenmeyer (conical) flasks
- 16 × balloons
- Sucrose (table sugar)
- Fructose, lactose and glucose
- pH indicator
- Permanent marker
- Masking tape
- Packets of dried yeast
- Vinegar
- Ammonia
- Ice
- White spotting tile
- Stopwatch
- 40°C water bath
- 80°C water bath
- Scales
- 100 mL measuring cylinder
- Pipettes
- Thermometers

Method

Part A: Temperature

1. Label four flasks A through to D.
2. Add 80 mL of tap water (neutral pH only) to each flask.
3. Place each flask at different temperatures:
   - Flask A: Ice
   - Flask B: Room temperature
   - Flask C: 40°C water bath
   - Flask D: 80°C water bath
4. Use the scales to weigh 5 g of sucrose and place in Flask A. Repeat for each of the other flasks so that each flask has an equal amount of sucrose.
5. Use the scales to weigh 4 g of dried yeast, add it to Flask A and stir gently. Repeat for each of the other flasks so that each flask has an equal amount of dried yeast.
6. Place a balloon over the lip of the neck on each flask and seal it with masking tape.
7. Every few minutes, spin each flask slowly to mix the contents.
8. Measure the diameter of the balloon every 2 minutes for 20 minutes. Record your observations in an appropriate table.

Part B: Sucrose concentration

1. Label four flasks E through to H.
2. Add 80 mL of 40°C tap water (neutral pH) to each flask.
3. Use the scales to weigh and place the following amounts of sucrose into each flask:
   - Flask E: No sucrose
   - Flask F: 5 g sucrose
   - Flask G: 30 g sucrose
   - Flask H: 50 g sucrose

4 Use the scales to weigh 4 g of dried yeast and add it to Flask E and stir gently. Repeat for each of the other flasks so that each flask has an equal amount of dried yeast.
5 Place a balloon over the lip of the neck on each flask and seal it with masking tape.
6 Every few minutes, spin each flask slowly to mix the contents.
7 Measure the diameter of the balloon every 2 minutes for 20 minutes. Record your observations in an appropriate table.

Part C: pH

1 Label four flasks I through to L.
2 Add 80 mL of tap water (neutral pH only) to each flask.
3 Add vinegar or ammonia to adjust the pH as described below. Place a single drop of universal pH indicator in each dimple of a white spotting plate. Test the pH of the mixture in each flask by placing a drop of the mixture in with the universal indicator.
   - Flask I: Add vinegar until the pH = 3.
   - Flask J: Add vinegar until the pH = 5.
   - Flask K: Add vinegar or ammonia until the pH = 7.
   - Flask L: Add ammonia until the pH = 10.
4 Use the scales to weigh 5 g of sucrose and place in Flask I. Repeat for each of the other flasks so that each flask has an equal amount of sucrose.
5 Use the scales to weigh 4 g of dried yeast and add it to Flask I and stir gently. Repeat for each of the other flasks so that each flask has an equal amount of dried yeast.
6 Place a balloon over the lip of the neck on each flask and seal it with masking tape.
7 Every few minutes, spin each flask slowly to mix the contents.
8 Measure the diameter of the balloon every 2 minutes for 20 minutes. Record your observations in an appropriate table.

Part D: Nutrient type

1 Label four flasks M through to P.
2 Add 80 mL of tap water (neutral pH only) at 40°C to each flask.
3 Use the scales to weigh 5 g of each of the sugars and add to the appropriate flask as shown below. Mix to dissolve the sugars.
   - Flask M: 5 g fructose
   - Flask N: 5 g glucose
   - Flask O: 5 g sucrose
   - Flask P: 5 g lactose
4 Use the scales to weigh 4 g of dried yeast and add it to Flask M and stir gently. Repeat for each of the other flasks so that each flask has an equal amount of dried yeast.
5 Place a balloon over the lip of the neck on each flask and seal it with masking tape.
6 Every few minutes, spin each flask slowly to mix the contents.
7 Measure the diameter of the balloon every 2 minutes for 20 minutes. Record your observations in an appropriate table.

Results

Draw an appropriate table for each experiment.

Discussion

1 At what stage of aerobic cellular respiration (glycolysis, Krebs cycle, electron transport chain) is carbon dioxide produced?
2 Which flasks showed the largest amount of CO_2 production in each experiment?
3 In what conditions did the yeast have the highest carbon dioxide production?
4 What conditions were the least favourable for carbon dioxide production?
5 Use your understanding of enzymes to explain why the following conditions affect the rate of aerobic cellular respiration:
   - Low temperatures
   - High temperatures
   - Presence of lactose
   - Extremes of pH
6 Explain what would happen to the rate of carbon dioxide production if the level of oxygen was too low.

Conclusion

Explain how temperature, pH, type of nutrient and concentration of nutrient affects the rate of aerobic cellular respiration.

7.1 HIGH-TECH PRACTICAL

Micropipette skills and knocking-in a gene

Practical worksheet
7.1 Micropipette skills and knocking-in a gene

Practical demonstration
7.1 Micropipette skills and knocking-in a gene

Risk assessment
7.1 Micropipette skills and knocking-in a gene

Lab tech notes
7.1 Micropipette skills and knocking-in a gene

Context

In genetic engineering, to knock-in refers to inserting a new gene into the genetic material of an organism. If the gene is from a different species, the genetically modified organism is called a transgenic organism.

Knocking-in a new gene must be done carefully to make sure that no other important genes are affected. Often CRISPR is used to locate a specific sequence in the DNA, while the enzyme Cas9 is used to cut and insert the specific gene.

This experiment involves knocking-in a gene for ampicillin resistance. The gene is located in a plasmid called the pGLO plasmid.

Calcium chloride ($CaCl_2$) is used to attract the negatively charged plasmid to the membrane of the *Escherichia coli* cells. When the cells and plasmid mixture are moved from ice to heat for a short time (heat shock), the membrane of the bacterial cell will allow the plasmid to enter. Once inside, the cell will use the plasmid DNA to make the enzymes that allow it to survive on ampicillin agar.

Aim

To knock-in a gene for ampicillin resistance into a bacterial cell.

Materials

- 2 × sterile Eppendorf tubes
- 10 × sterile plastic pipettes
- Crushed ice in insulated container
- 10 mL 0.05 M $CaCl_2$, sterile, on crushed ice
- 5 × disposable inoculation spreaders (sterile)
- 20 mL sterile water
- *E. coli* colonies on an agar plate
- 100 µL GFP plasmid containing the ampicillin resistance gene on crushed ice
- Permanent marker
- 2 × Petri dishes of LB agar
- 2 × Petri dishes of LB agar + ampicillin
- 2–20 µL micropipette
- 20–200 µL micropipette
- Micropipette tips (sterile)
- 42°C water bath
- Foam Eppendorf tube rack (floats in water bath)
- Stopwatch
- 250 µL Luria broth
- Tape (to seal plates)
- Microbiological waste bag
- Thermometer
- Microcentrifuge
- Bunsen burner
- Bench mat wiped with antibacterial detergent
- Incubator (able to be set at 30°C)

Method

Part A: Skill practice – Using a micropipette

A micropipette is used to measure and transfer small amounts of liquid. Micropipettes come in different sizes and it is important to never exceed their upper or lower limits.

A window on the micropipette usually contains three digits to represent the volume that it has been set to. These values can be changed by turning the dial at the bottom of the plunger clockwise to increase and anticlockwise to decrease.

Each micropipette needs to have a plastic tip placed on the end. It is important to never turn the micropipette upside-down since this will cause the liquid to move into the base of the micropipette and provide potential contamination in the future.

1 Place a tip on the end of the micropipette.
2 Gently press down the plunger to the *first* stop.

FIGURE 1 A micropipette

3 Place the end of the micropipette tip into the food dye coloured water.
4 Slowly release the plunger so that the coloured water is pulled into the micropipette tip.
5 Remove the micropipette from the coloured water and place the tip above the first dimple of the spotting tile.
6 Gently press the plunger to the *second* stop so that all the coloured water is placed on the spotting tile. Check that all the coloured water has been removed from the tip.
7 Remove the micropipette from the spotting tile before releasing the plunger to prevent the coloured water being taken up by the micropipette again.
8 Repeat steps 2–7 several times, checking that exactly the same sized drop of coloured water is distributed each time.

Part B: Knocking-in a gene

Once you are confident in using a micropipette, continue with the knocking-in a gene component of this practical.

1 Label one Eppendorf tube '+ plasmid' and the other tube '− plasmid'. Place the tubes upright on crushed ice. Keep the tubes capped at all times.
2 Use the 20–200 µL micropipette (set to 125 µL) to add a total of 250 µL of ice cold $CaCl_2$. Return all tubes to the ice.
3 Place the Bunsen burner on the bench mat. Light the Bunsen burner and put it on a blue flame. Place the agar plate with colonies of *E. coli* on the bench mat. The area under the Bunsen burner flame is considered a sterile area.
4 Use a sterile inoculation loop to remove a single *E. coli* colony from the agar plate, taking care to keep all equipment under the Bunsen burner flame. Place the tip of the inoculation loop containing the *E. coli* colony into the '+ plasmid' tube and spin the loop rapidly to dislodge the bacteria into the liquid. Close the lid of the tube and return to the ice.
5 Use the same inoculation loop to repeat Step 4, this time placing the *E. coli* bacterial colony in the '− plasmid' tube. Place the inoculation loop in the designated waste container when finished.
6 Keep the lids of the tubes closed tightly and use your finger to tap the base a few times to mix the bacteria through the liquid. The liquid should become milky with no lumps or particles present. Return the tubes to the ice.
7 Use the microcentrifuge to spin down the contents of the plasmid solution in a short pulse. Be sure to balance the contents of the centrifuge before turning it on.
8 Use the 2–20 µL (set to 10 µL) to transfer 10 µL of the plasmid solution to the bacterial suspension in the '+ plasmid' tube only. (NOT the '− plasmid' tube.) Place the tip in the waste container.
9 Securely close the lid of the tube and use your finger to tap the base of the tube to mix the plasmid containing the gene through the *E. coli* solution. Use the microcentrifuge to gather the contents of the tube at the base.
10 Return the tube to the ice. Incubate both tubes for 15 minutes in the ice.
11 While waiting, label the four agar plates as:
   - LB plates labelled: 'LB agar + plasmid' and 'LB − plasmid'
   - LB/Amp plates labelled: 'LB/Amp + plasmid' and 'LB/Amp − plasmid'
12 Removed the two tubes from the ice and place in the floating foam tube rack so that the liquid in the tubes will be exposed to heat. Place the floating rack in the 40°C water bath for 90 seconds.

13 Immediately remove the tubes and return to the ice.

14 Use the 20–200 µL micropipette (set to 125 µL) to transfer 250 µL of Luria broth to each tube. Remember to change the micropipette tip each time.

15 Close the lids of the Eppendorf tubes firmly before tapping the base of the tube with your finger to mix the contents thoroughly.

16 Allow the contents of the tubes to rest at room temperature for 10 minutes.

17 Set out the agar plates (agar side down) under the Bunsen burner flame. The 'LB − plasmid' and 'LB/Amp − plasmid' should be placed next to the '− plasmid' tube, and the 'LB + plasmid' and 'LB/Amp + plasmid' tube should be placed next to the '+ plasmid tube.

18 Use the 20–200 µL micropipette (set to 100 µL) to add 100 µL of the '− plasmid' mixture to each of the 'LB − plasmid' and 'LB/Amp – plasmid' plates. Use a separate sterile spreader for each plate to spread the liquid evenly over the agar.

19 Use the 20–200 µL micropipette (set to 100 µL) to add 100 µL of the '+ plasmid' mixture to each of the 'LB + plasmid' and 'LB/Amp + plasmid' plates. Use a separate sterile spreader for each plate to spread the liquid evenly over the agar.

20 Place the lids on the agar plates and seal with the tape.

21 Place the agar plates in a 30°C incubator for 24–48 hrs.

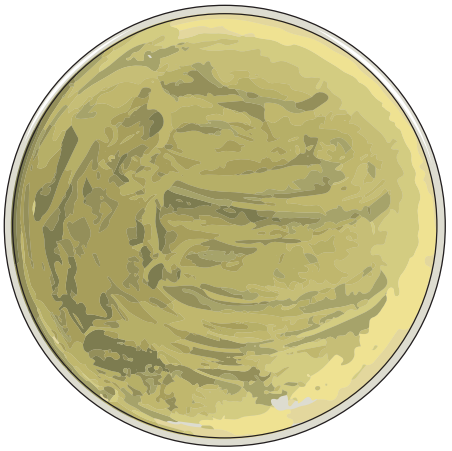


FIGURE 2 A sealed agar plate

22 Inspect the growth of the bacteria on the agar plates after incubation.

Results

Record your observations of each of the four agar plates.

TABLE 1 Agar plate results

Agar plate	Observation
'LB agar + plasmid'	
'LB − plasmid'	
'LB/Amp + plasmid'	
'LB/Amp − plasmid'	

Discussion

1 Are the bacteria genetically modified and/or transgenic?

2 Define the term 'knock-in gene'.

3 What is a plasmid?

4 Explain how heat shock allowed the plasmid to move through the membrane of the bacterial cells.

5 What is the purpose of the 'LB agar + plasmid', 'LB − plasmid' and 'LB/Amp − plasmid' agar plates?

6 Discuss the purpose of adding the ampicillin to the 'LB/Amp + plasmid' plate.

7 Explain the pattern of bacterial growth on each agar plate.

Conclusion

Explain how the technique of knocking-in a gene could be used to improve anaerobic fermentation in yeast.

8.1 PRACTICAL Plant defence mechanisms

Practical worksheet 8.1 Plant defence mechanisms
Practical demonstration 8.1 Plant defence mechanisms
Risk assessment 8.1 Plant defence mechanisms
Lab tech notes 8.1 Plant defence mechanisms

CAUTION: Do not consume the fungus-infected fruit.

Context

Like animals, plants are vulnerable to infectious diseases. Many different types of fungi can infect leaves, stems, roots and fruit and cause damage to the plant. As a result, plants have developed primary and secondary defence mechanisms to protect themselves. This may include hair and waxy cuticles to prevent the fungi from reaching the cells and a thick skin on the fruit.

FIGURE 1 A fungus-infected fruit can be any mouldy fruit such as a strawberry left in the fridge for too long.

Finding fungus on leaves or fruit does not prove that the fungus is the cause of symptoms such as rot. The fungus may be there by coincidence, or could be an opportunistic infection. Before a claim that a fungus is the cause of a symptom can be made, it must be supported by evidence.

Koch's postulates can be used to investigate the link between a fungus and observed symptoms. This includes the following steps.

1 Observe symptoms on infected fruit.
2 Take a sample of fungus from the infected fruit.
3 Use the fungus to infect a second (healthy) fruit.
4 Observe any symptoms that appear on the second fruit.

If the symptoms on the two fruits are the same, it can be concluded that the fungus is the cause of the disease.

Aim

To examine Koch's postulates in fruit infected with fungi.

Materials

- Disposable gloves and facemasks
- Healthy fruit with solid skin (e.g. strawberry or apple)
- Fungus-infected fruit inside a plastic bag (e.g. mouldy strawberry or apple – this should be the same as the healthy fruit)
- Paper towel
- Wooden skewer
- Permanent marker
- Clean plastic bag

FIGURE 2 An uninfected apple

Method

1 Use plastic gloves to cover your hands. Place a facemask over your mouth and nose.
2 Identify the healthy fruit. Use paper towel to wipe its surface clean.
3 Gently apply pressure to a small area on one side of the healthy fruit to bruise it, but do not break the skin.
4 Repeat Step 3 on the opposite side of the healthy fruit.
5 Gently push a clean wooden skewer into each bruise to create a small hole approximately 1 cm deep.
6 Use a permanent marker to write a letter 'C' on the fruit next to one of the holes. This will be the control side.
7 Write a letter 'I' next to the other hole. This will be the infected side.
8 Observe the infected fruit inside the plastic bag. Record your observations.
9 Carefully open the plastic bag. Do not remove the fruit from the bag. Use the wooden skewer to pick a small sample of fungus from the infected fruit. Immediately reseal the bag.
10 Use the skewer to place the fungus into the hole on the infected side of the healthy fruit.
11 Place the healthy fruit into a new clean plastic bag. Seal the bag and label it with your name. **Note:** Dispose of your gloves and facemask into the general rubbish.
12 Incubate the bag on a windowsill at room temperature for 2 weeks. Do not reopen the bag. Observe the development of symptoms. Record your observations.

Results

Record your results in the table below.

TABLE 1 Results of infected and control fruit

Fruit location	Observation before infection	Observation 2 weeks after infection
Control side		
Infected side		

Discussion

1 What symptoms were observed on the infected fruit at the start of the activity?
2 What symptoms were observed on the fruit with the two holes after the incubation period?
3 What conclusions can be made after comparing the symptoms of the two fruits?
4 Suggest why it was important that the infected fruit and the healthy fruit were the same type of fruit (e.g. both apples rather than an apple and a pear).
5 The owner of an orchard has noticed symptoms of brown rot on a tree. The other trees are healthy. Suggest how the owner could prevent the spread of brown rot to their healthy trees.
6 Taking a sample of pathogen from infected tissue and using it to infect healthy tissue is a technique used to help identify diseases in plants. Suggest why the same technique is not used in humans.

Conclusion

How do Koch's postulates provide evidence that a pathogen is the cause of an infection?

9.1 PRACTICAL Blood typing

Practical worksheet
9.1 Blood typing

Practical demonstration
9.1 Blood typing

Risk assessment
9.1 Blood typing

Lab tech notes
9.1 Blood typing

CAUTION: Hydrochloric acid and sulfuric acid are corrosive; silver nitrate is an irritant and is toxic to aquatic life. Handle and dispose of these chemicals safely.

Context

Red blood cells are one of the most common cells in the blood. Their main role is to carry the oxygen needed for aerobic cellular respiration from the lungs to the rest of the body. Red blood cells (RBC) are produced by stem cells in the bone marrow. Immature RBCs contain a nucleus with a full set of chromosomes.

In humans, chromosome 9 contains the *ABO* gene that is responsible for blood type. This gene produces an enzyme (glycosyltransferase) that modifies the sugars found on cell membrane glycoproteins. There are three versions (alleles) of this gene.

Blood group O is caused by a deletion of guanine in the gene. This frameshift mutation results in a non-functioning enzyme. The enzyme is unable to add the final sugar onto the chain (Figure 1). This shortened sugar is not recognised by the immune system.

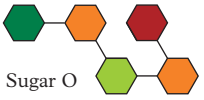


FIGURE 1 Sugar O found on the red blood cell

The gene responsible for blood group A produces the enzyme A-transferase. The role of this enzyme is to add an acetyl-galactosamine group to the sugar chain, forming sugar A.

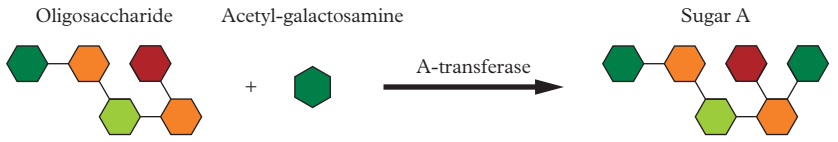


FIGURE 2 Sugar A results from the addition of acetyl-galactosamine

The gene responsible for blood group B has two nucleotide substitution mutations. The resulting enzyme, B-transferase, adds a galactose sugar to the sugar chain to form sugar B.

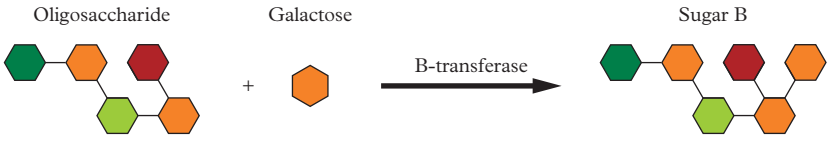


FIGURE 3 Sugar B results from the addition of galactose

Some individuals inherit a copy of the A version of the *ABO* gene from their mother, and a copy of the B version of the *ABO* gene. This means they can produce both A-transferase and B-transferase enzymes, causing both sugar A and sugar B (blood type AB) to be produced. As the RBCs mature, they lose their nucleus; however, the chain of sugars remains on their plasma membrane.

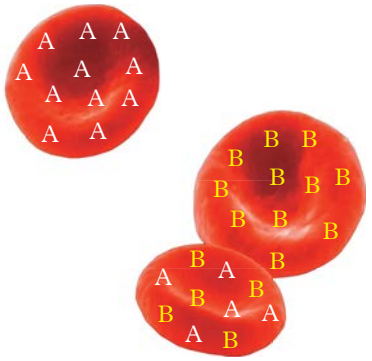


FIGURE 4 People with blood type AB have both A and B sugars on their red blood cells

Knowing the type of sugar present on the surface of the RBCs is important to prevent a reaction during a blood transfusion. A person with blood type A will react to sugar B as though it is an antigen. This means the person's immune system will react, activating helper T-cells and B-cells. As a result, the B-cells will differentiate into plasma cells and start producing antibodies against the B sugar. The anti-B antibodies cause the transfused red blood cells (RBC) to clot (agglutinate). The symptoms of ABO incompatibility include fever, breathing difficulty, muscle aches, nausea and chest or abdominal pain.

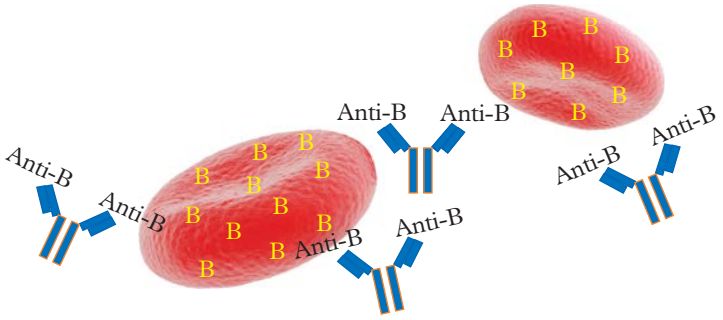


FIGURE 5 Production of antibodies

Complete the following table by predicting the blood type and antibodies that could be produced for each of the ABO sugars below.

TABLE 1 Predicted blood types and antibodies present in each blood type

Blood type	Sugars present on RBC	Antibodies produced
	A	
	B	
	AB	
	Neither A nor B	

The second most important blood group system (after ABO) is the Rhesus (Rh) antigens. A person who is Rh positive (Rh^+) has a membrane transport protein on the surface of their RBC. This protein is involved in the transport of carbon dioxide across the RBC plasma membrane. A person who is Rh^- does not have this transport protein on the plasma membrane. There is no disadvantage to this since carbon dioxide is able to diffuse freely across the membrane.

The immune system of a person who is Rh^- will recognise the Rh protein as an antigen. As a result, their immune system will produce anti-Rh antibodies, with fatal consequences.

For each blood type below, predict the expected agglutination result from mixing the blood with each antibody.

TABLE 2 Predicted agglutination of blood testing

Blood type	Anti-A	Anti-B	Anti-Rh
A+			
A−			
B+			
B−			
AB+			
AB−			
O+			
O−			

Aim

To identify the blood type of four different synthetic blood samples using antisera.

Materials

- Anti-A solution (2M hydrochloric acid solution)
- Anti-B solution (2M sulfuric acid solution)
- Sample O blood (distilled water)
- Sample A blood (0.1M silver nitrate solution)
- Sample B blood (0.1M barium nitrate solution)
- Sample blood AB (a 50:50 mix of 0.1M silver nitrate and 0.1M barium nitrate solution)
- Spotting tiles
- 6 × pipettes (one for each solution)
- Food dye coloured water for pipette practice

Method

1 Once you are confident in pipetting, place two drops of blood O sample in the first wells of the first two rows of a clean spotting tile.

2 Using a fresh pipette, place two drops of sample blood A in the second well of each row of the spotting tile.

3 Repeat for the remaining blood samples in the third and fourth wells of each row, respectively (see Figure 7).

FIGURE 6 White spotting tile

4 Add a drop of anti-A solution to each of the wells in the first row of the tile.

5 Add a drop of anti-B solution to each of the wells in the second row of the tile.

6 Observe the agglutination of some of the samples. Record the results in Table 3.

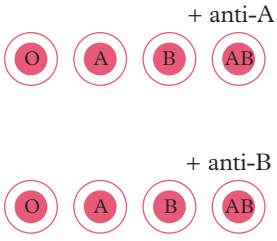


FIGURE 7 Distribution of blood samples

Results

Record your results in the table below.

TABLE 3 Results of blood testing

	Sample blood O	Sample blood A	Sample blood B	Sample blood AB
Anti-A				
Anti-B				

Discussion

1 Suggest an ethical reason why synthetic blood is used in this experiment instead of human or animal blood.

2 What is an antigen?

3 Why does a person with A sugars on their RBC mount an immune response to B sugars?

4 Name the cell that produces antibodies.

5 A hospital was preparing a patient (blood type A) for a heart transplant. This procedure usually requires a series of blood transfusions.

   a Why would they prefer type A blood for transfusion into the patient?

   b Using your knowledge of blood types and antibody production, what other types of blood could be used by the hospital?

6 When a woman is pregnant, the placenta forms a barrier between the mother and baby. This barrier carefully controls what can pass from the baby to the mother. For example, the RBC of a Rhesus positive baby cannot pass to a Rhesus negative mother until she gives birth.

   Why are Rhesus negative mothers not exposed to Rh^+ antigen from a Rh^+ baby until the birth?

7 During the birth, foetal RBC are often passed from the baby to the mother. Why are second-born Rh^+ babies at risk from the Rh^- mothers?

Extension question

The complication described in Question 7 can be prevented by injecting the mother with anti-Rh antibodies immediately after the delivery of the first baby. Explain why this procedure will prevent complications with the second-born Rh^+ baby.

FIGURE 8 If an Rh negative mother has an Rh positive baby, the foetus needs to be protected from the mother's antibodies.

Conclusion

Explain why donated blood should be cross-matched with the patient's blood before being transfused.

10.2 PRACTICAL
Testing the effectiveness of antibacterial substances

Practical worksheet
10.2 Testing the effectiveness of antibacterial substances

Practical demonstration
10.2 Testing the effectiveness of antibacterial substances

Risk assessment
10.2 Testing the effectiveness of antibacterial substances

Lab tech notes
10.2 Testing the effectiveness of antibacterial substances

Context

Supporters of alternative medicines have claimed that many common household products, such as essential oils, vinegar, garlic, onion and turmeric, provide protection against bacteria. It has been claimed that these products are antiseptics, detergents, bacteriostatics or bactericides.

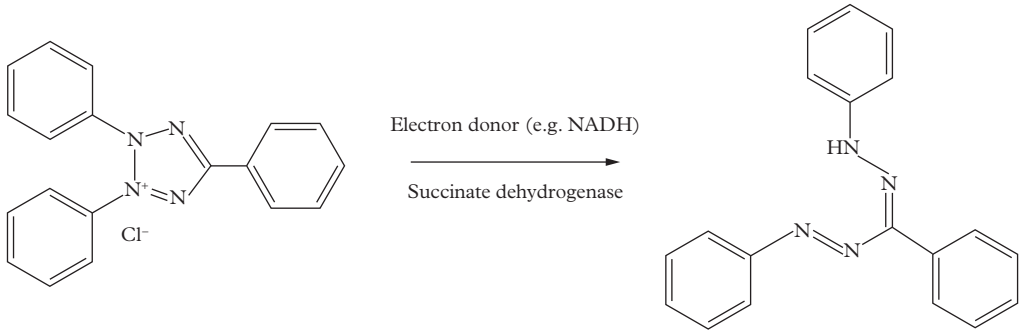
FIGURE 1 Aromatic household products have been used as antiseptic and antibacterial agents for centuries. Let's find out if they really work!

Antiseptics are used to disinfect living tissue – both prophylactically (to prevent infection) and therapeutically (to treat infection). Some antiseptics are more effective against particular microbes than others. The effectiveness of an antiseptic may be affected by factors such as dilution, temperature, pH, and the presence of detergent or organic matter.

Some products act as bactericides (kill bacteria), while others have bacteriostatic (prevent bacterial growth) properties. Other products are simply detergents that provide a slippery surface that make it difficult for bacteria to form a stable colony.

The effectiveness of each product can be tested using a prepared strip that contains a nutrient medium for culturing bacteria (petrifilm).

Escherichia coli bacteria undergo aerobic cellular respiration. The first two parts of this process (glycolysis and the Krebs cycle) results in the production of NADH. It is the NADH in the presence of the enzyme succinate dehydrogenase that converts the indicator on the petrifilm from white to red.



TPH (white colour) TPR (red colour)

FIGURE 2 Conversion of petrifilm from white to red

Aim

To determine whether detergent is effective in preventing the growth of bacteria on petrifilm.

Materials

- 3 × petrifilm
- 1000 µL micropipette and tips
- Bacterial (*E.coli* K-12 strain) working solution
- Cell spreader
- Permanent marker
- Sandwich bag
- Test solution (an antibacterial solution you want to test)
- Eppendorf tube
- Incubator set at 30–36°C

Method

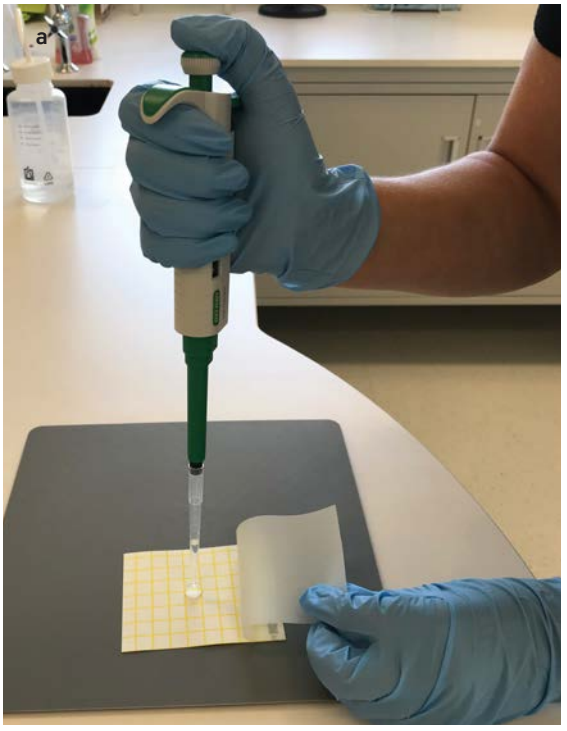
1 Label the bottom of one petrifilm sheet as the negative control.

2 Use gloves to peel back the top layer of the double-layer petrifilm. This is always done beside a lit Bunsen burner, to ensure that any unwanted bacteria are removed.

3 Use the micropipette to pipette 1000 µL (1 mL) of your test solution directly onto the centre of the petrifilm.

4 Release the clear top layer to close the petrifilm.

5 Use the cell spreader (grooved side down) to press gently down on the petrifilm to form an even circular disc. Hold the spreader down for a few seconds to make sure the solution does not spread further.



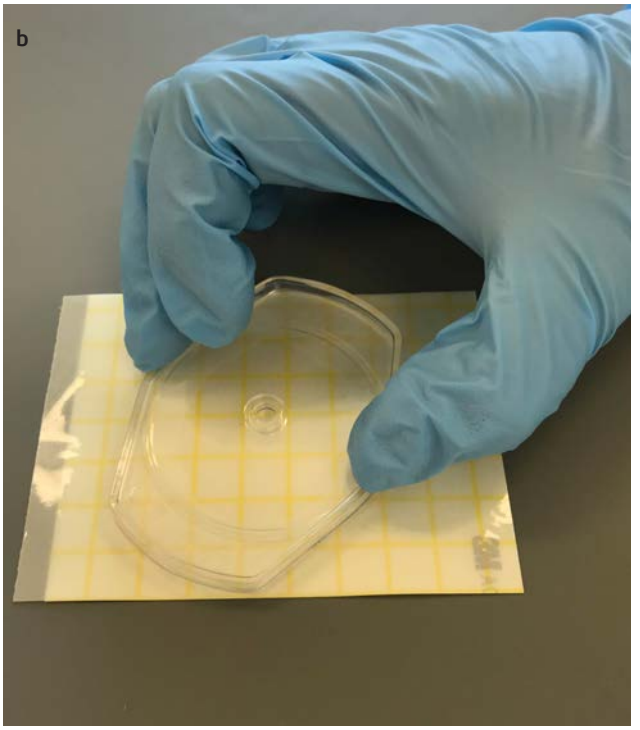


FIGURE 2 **a** Pipetting onto petrifilm; **b** using the cell spreader

6 Repeat steps 2–5 for the bacterial working solution. Label this petrifilm as your positive control.

7 Add 550 µL of the bacterial working solution to an Eppendorf tube. Use a new tip to add 550 µL of your test solution to the same tube.

8 Gently tap on the side of the tube to mix the solution.

9 Add 1000 µL of the mixed solution to a petrifilm. Label this the test solution.

10 Seal both petrifilms in a plastic sandwich bag and incubate for 24 hours at 30–36°C.

11 After 1–2 days of incubation, bacterial colonies can be seen as red dots on the petrifilm (20 cm^2). All red colonies on the petrifilm are counted, regardless of differing size and intensity.

12 If the colony count is less than 300, count the total number of colonies. If the colony count is estimated to be more than 300, determine the average number of colonies in one square (1 cm^2) and multiply it by 20 to obtain the number of colony-forming units (cfu) per mL of the test solution.

An example is shown in Figure 3:

- Total bacterial colony count: 135
- Dilution: 1 in 100
- Colony forming units: 135 × 1000 = 135 000 cfu mL^{-1}
- Colony forming units: 1.35 × 10^5 cfu mL^{-1}

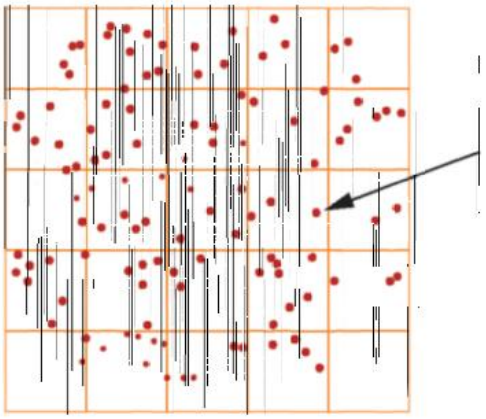


FIGURE 3 The *E. coli* colonies have converted the indicator from white to red on the petrifilm.

Results

Record your results in the table below.

TABLE 1 Observations of colony-forming units for antibacterial substances and controls

Petrifilm	Colony-forming units	Observations
Negative control		
Positive control		
Test solution		

Discussion

1 What is the purpose of the negative control?

2 What is the purpose of the positive control?

3 If there is no growth in the petrifilm with the test solution, does this mean the bacteria are all dead?

4 How could the test solution be diluted to determine its effectiveness on bacteria?

Conclusion

How effective was the test solution in preventing bacterial growth?

11.1 NO-TECH PRACTICAL

Genetic changes over time

Practical worksheet
11.1 Genetic changes over time

Practical demonstration
11.1 Genetic changes over time

Risk assessment
11.1 Genetic changes over time

Lab tech notes
11.1 Genetic changes over time

Context

The appearance of individuals in a population is governed by the genes (alleles) that they inherit from their parents. Many factors can affect which individuals are able to breed in each population. Random events, the availability of food, predators and suitable mating partners can affect the proportion of phenotypes in each succeeding generation.

Aim

To model the process of natural selection, gene flow, founder population, genetic drift and genetic bottleneck.

Materials

- 30 × coloured counters (at least five colours)
- Coloured paper (A3) – identical to one of the five coloured counters
- Timer

Method

Part A: Modelling natural selection

1 Spread four of each colour counter over the coloured paper. Record the number of each coloured counter in Table 1 (16 counters in total).
2 Identify the brightest coloured counter on your paper.
3 Over 3 seconds, you will represent a new predator in the environment. Collect as many as possible of the coloured counters identified in Step 2.
4 Place the remaining counters into 'breeding pairs'. Each counter in a breeding pair should 'mate' with an identical-coloured counter.
5 Provide each breeding pair with a 'baby' counter of an identical colour to the parents. Record the number of each coloured counter remaining in the first generation in Table 1.
6 Spread the counters over the A3 paper.
7 Repeat steps 2–6 for the second generation.
8 Repeat steps 2–6 for the third generation.
9 Repeat steps 2–6 for the fourth generation.
10 Answer the questions for Part A in the Discussion section.

Part B: Gene flow

1 Place all the counters on your coloured paper once again.
2 Divide the counters into two groups on either side of the paper. Arrange the groups so that each population has one colour that is unique (e.g. put all the red counters on one side, and all the blue counters on the other side of the paper). Evenly spread the remaining colours between the two groups. Record the numbers of each counter colour of both populations in Table 2, Generation 1.
3 Randomly grasp five counters from each population and transfer them into opposing populations. This represents the gene flow that occurs with the migration of individuals that are capable of breeding.
4 Record the numbers of each counter colour in both populations in Table 2 in the Results section.
5 Repeat steps 3–4 for the third generation.
6 Answer questions for Part B in the Discussion section.

Part C: Founder population

1 Place all the counters to one side of the coloured paper. This is the general population.
2 Randomly select three counters to 'migrate' to the other side of the paper. This is the founding population. Over time this population will breed with each other as gene flow between the two populations is restricted.

3 Answer questions for Part C in the Discussion section.

Part D: Genetic drift

1 Add a small population of 10 counters in the centre of the coloured paper. Record the number and colour of the counters in this small population in Table 3.
2 A random event causes four counters to 'die'. Remove four counters at random from your population.
3 The remaining population undergoes a breeding season. Add one matching-colour counter for each of the remaining counters. Record the counter colours in Table 3 in the Results section.

Part E: Genetic bottleneck

1 Add 10 assorted counters to the centre of the coloured paper. Record the number and colour of the counters in this small population in Table 4.
2 A disease passes through your population of counters. Only one colour of counters is immune to the disease. Remove all other coloured counters from the page.
3 The remaining population undergoes a breeding season. Add one matching-colour counter for each of the remaining counters. Record the counter colours in Table 4 in the Results section.

Results

Record your results in the tables below.

Part A: Modelling natural selection

TABLE 1 Modelling natural selection

Counter colour	Number of counters in each generation				
	Before predation	After first generation	After second generation	After third generation	After fourth generation

Part B: Gene flow

TABLE 2 Modelling gene flow

Counter colour	Generation 1		Generation 2		Generation 3	
	Population 1	Population 2	Population 1	Population 2	Population 1	Population 2

Part D: Genetic drift

TABLE 3 Genetic drift of a small population

Counter colours	Before random event	After random event

Part E: Genetic bottleneck

TABLE 4 Population changes as a result of a genetic bottleneck

Counter colours	Before disease	After disease

Discussion

Part A: Modelling natural selection

1 Describe the process of natural selection as shown in your modelling.
2 How did the population of coloured counters evolve over the four generations?
3 What was the selection pressure in the population?
4 Would you expect the final proportion of coloured counters to vary if a different environment (different-coloured paper) was used? Use an example to illustrate your answer.

Part B: Gene flow

1 Define the term 'gene flow'.
2 Suggest two ways gene flow can be increased in a population.
3 Describe how the combination of colours in each group changed with each generation.
4 From your results, how does the phenotype of a population change as a result of gene flow?

Part C: Founder population

1 How are the colours of the counters of the new founding population different to those of the general population?
2 Have the numbers of each coloured counter changed in the general population as a result of the founding population?
3 Assuming there is no gene flow between the populations over time, describe how future generations of the founding population will change over time.
4 Define the term 'founder population'.

Part D: Genetic drift

1 Define the term 'genetic drift'.
2 What types of events would be considered random or unpredictable?
3 What effect did the random event have on the numbers of each colour in the population?
4 Did everyone in the class have the same result as your group? Explain why or why not.
5 Would you expect the same result if your starting population of counters was 30? Explain why or why not.

Part E: Genetic bottleneck

1 What colour phenotype survived the disease?
2 What colour phenotype/s did not survive the disease?
3 Did breeding change the colour phenotype of individuals after the disease?
4 What would happen to the population of counters if a second disease occurred that affected the remaining colour counters?
5 Define the term 'genetic bottleneck'.
6 It is thought that the Tasmanian devil had a genetic bottleneck in the past. The genetics of the current population are so alike that their immune systems have difficulty recognising cells from each other (their self-markers are identical). How does this affect their ability to survive the Tasmanian devil facial tumour disease that is passed from animal to animal?

Conclusion

Describe the effect of natural selection, gene flow, genetic drift and a genetic bottleneck on an established population and a founder population.

12.1 NO-TECH PRACTICAL Absolute age

Practical worksheet 12.1 Absolute age

Practical demonstration 12.1 Absolute age

Risk assessment 12.1 Absolute age

Lab tech notes 12.1 Absolute age

Context

When an organism dies, it no longer absorbs radioactive material. The remaining radioactive material contains some nuclei that are stable, and other nuclei that are unstable. Unstable nuclei will gradually decay and become stable over time. The rate at which each type of atom decays is fixed for each atom. The rate at which half the radioactive material decays to a stable form is called its half-life.

Carbon-14 has a half-life of 5730 years, which means that if 1 g of carbon-14 is present at the time of death, half of it will decay in 5730 years.

By looking at the ratio of carbon-12 to carbon-14 in a fossil and comparing it to the ratio in a living organism, it is possible to determine a fossil's age.

Aim

To model the rate of radioactive decay in a fossil.

Materials

- 10 × counters
- Permanent marker
- Paper bag

Method

1. Use the permanent marker to write 'C-14' on one side of each counter. These represent the radioactive material present.
2. Place the counters in the paper bag to represent the radioactive material present in an organism at the time of death.
3. Shake the bag and tip all the counters out onto the surface of the table.
4. Pick up the counters with the C-14 facing upwards. Count them as you return them to the paper bag. Record the number of counters.
5. Put the counters that have the unmarked side facing up to the side. These counters represent the stable form of the atoms.
6. Repeat steps 3–5 until Trial 7 or until all the unstable C-14 counters have become stable.
7. Collate the results with other groups in your class.

Results

Record your results in the table below.

TABLE 1 The decay of C-14 counters

Half-life	0	1	2	3	4	5	6	7
Number of unstable C-14 counters	10							

Discussion

1. Define the term 'half-life'.
2. Do the number of radioactive atoms present at the start affect the outcome? Explain.
3. Did each group get similar results?
4. Did any group still have radioactive C-14 remaining after Trial 7?
5. Why does the total for the combined groups provide a better indication of what happens during half-life?
6. Plot the total results on a graph with the number of C-14 counters on the vertical axis and trial number on the horizontal axis. Is the result a straight or a curved line? What does the line indicate about the nature of decay?
7. How do scientists use radioactive decay to date fossils and artefacts?

Conclusion

Describe how radioactive decay can be used to determine the age of a fossil.

13.2 NO-TECH PRACTICAL

Molecular differences between species

Practical worksheet
13.2 Molecular differences between species

Practical demonstration
13.2 Molecular differences between species

Risk assessment
13.2 Molecular differences between species

Lab tech notes
13.2 Molecular differences between species

Context

Cytochrome c is a small protein (100–104 amino acids in length) found in the inner mitochondrial cristae. It forms an essential part of the electron transport chain in aerobic cellular respiration. The sequence of amino acids in cytochrome c is highly conserved across a number of species of plants, animals and many unicellular organisms.

The greater the number of differences between the cytochrome c molecule in different species, the more time has passed since those species shared a common ancestor. These differences can be used to draw the evolutionary relationship in a phylogenetic tree.

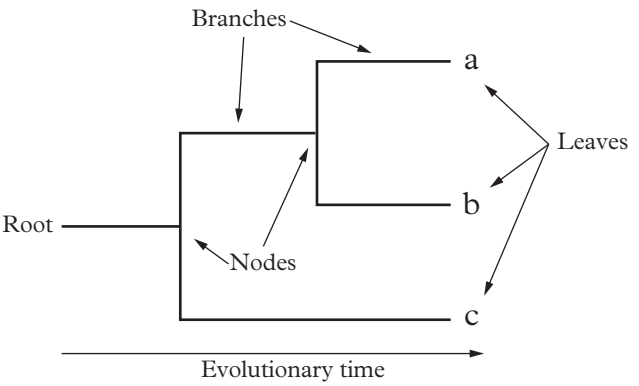


FIGURE 1 The structure of a phylogenetic tree

Aim

To relate the differences in molecular structure of the cytochrome c protein to divergent evolution.

Method

1 Use Table 1 to identify the species that is the most different to all of the other species in the table (the species with the greatest number of mutations). Write this species above Position 1 of the phylogenetic tree.

2 Compare your Position 1 species to the other species in the table. Select the species that has the least number of differences in amino acids to the Position 1 species cytochrome c. Write the name of this species at Position 2 of the phylogenetic tree (shown in Figure 2).

3 Compare the species in Position 2 to the remaining species in the table (ignoring the organisms that you have already placed in the phylogenetic tree). Select the species with the next least number of differences in amino acids compared to the Position 2 species. Write the name of this species in Position 3 of the phylogenetic tree.

4 Compare the species in Position 3 to the remaining species in the table (ignoring the organisms that you have already placed in the phylogenetic tree). Select the species with the next least number of differences in amino acids compared to the Position 3 species. Write the name of this species in Position 4 of the phylogenetic tree.

5 Repeat these steps until the phylogenetic tree is complete.

TABLE 1 Number of differences in amino acids in the cytochrome c molecule between species

Human	0					
Monkey	1	0				
Horse	12	11	0			
Rabbit	9	8	6	0		
Snapping turtle	15	14	13	14	0	
Moth	31	30	29	28	26	0
	Human	**Monkey**	**Horse**	**Rabbit**	**Snapping turtle**	**Moth**

Results

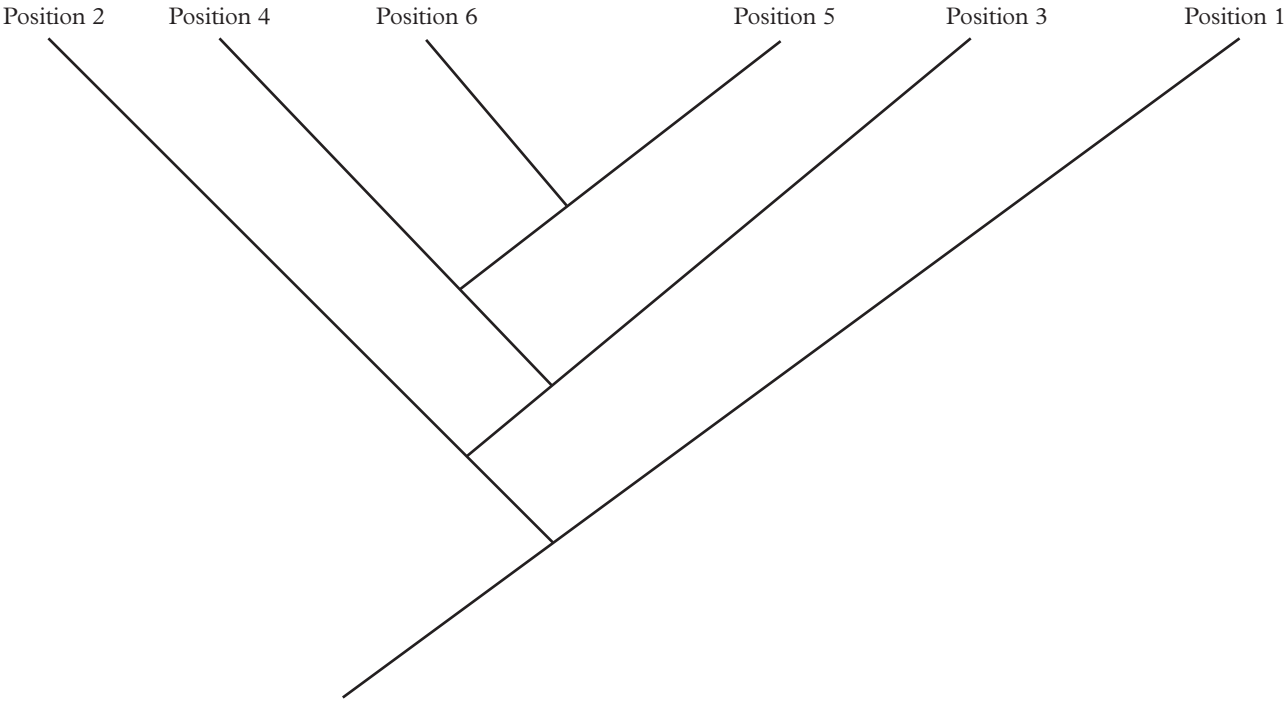


FIGURE 2 Phylogenetic tree diagram of the differences in cytochrome c in different species

Discussion

1 How have the differences in the cytochrome c molecule in different species developed?
2 What does the presence of the cytochrome c molecule in all the listed species suggest?
3 Which species had the most amino acids in common with the snapping turtle? Provide evidence from the table to support your answer.
4 What does this indicate in terms of evolutionary relationship between these two organisms?
5 Explain which organism appears to have the greatest number of differences in amino acids sequences compared to a human.
6 What does this indicate in terms of evolutionary relationship between these two organisms?
7 Scientists use the information that they find about the amino acid sequence of cytochrome c and hypothesise about a time frame for its evolution by comparing it to the amino acid sequences in animals of a known evolutionary origin. Explain why it might be difficult for scientists to find fossilised cytochrome c.

Conclusion

Explain how a phylogenetic tree can provide information on the evolutionary relationships between species.

14.4 NO-TECH PRACTICAL

Modelling the migration of *Homo sapiens*

Practical worksheet
14.4 Modelling the migration of *Homo sapiens*

Practical demonstration
14.4 Modelling the migration of *Homo sapiens*

Risk assessment
14.4 Modelling the migration of *Homo sapiens*

Lab tech notes
14.4 Modelling the migration of *Homo sapiens*

Context

Fossilised remains of early ancestors of *Homo sapiens* have been found in many locations around the world. Each fossil find has provided new evidence that has required the refinement or replacement of previous theories of human migration.

Aim

To plot the location of human fossilised remains and hypothesise the pathway of migration out of Africa.

Materials

- Map of the world
- Pencils

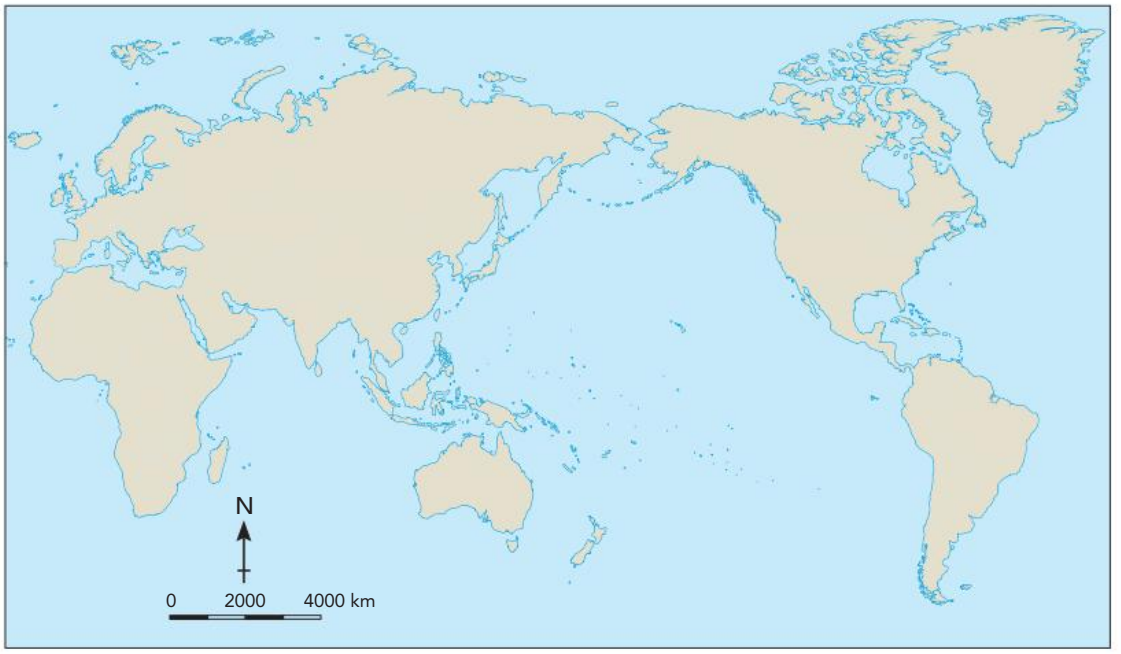


FIGURE 1 A blank map of the world, like this one, can be used for this practical.

Method

1 Plot the locations and ages of the fossils below on your map of the world.

TABLE 1 Selection of fossilised remains

Location	Fossil	Age (years ago)
Jebel Irhoud, Morocco	*H. sapiens* remains and stone tools	300 000
Gademotta, East Africa	*H. sapiens* remains	276 000
Israel	*H. sapiens* jawbone	177 000 (95 000)
Aouduma Cave, Peloponnese, Greece	*H. neanderthalensis* remains	170 000
Southern Africa and West Africa	*H. sapiens* remains	130 000
	Cooling climate almost drives *Homo sapiens* to extinction (less than 10 000 remain)	
	Climate improves	70 000
India	Genetic evidence suggests first *Homo sapiens*	65 000
Laos	*H. sapiens* remains	55 000 ± 8000
Mungo Man (Lake Mungo, NSW)	*H. sapiens* remains	50 000 ± 5000
England	*H. sapiens* remains	43 000 ± 3000
China	*H. sapiens* remains	42 000
Romania	*H. sapiens* remains	42 000
Russia	*H. sapiens* remains	40 000
Sri Lanka	*H. sapiens* remains	37 000
Okinawa, Japan	*H. sapiens* remains	32 000
Siberia, Russia	*H. sapiens* remains	24 000
	Large ice sheets expose land bridge joining Asia to America	20 000
Southern California, USA	*H. sapiens* remains	13 000
Northern Victoria, Australia	*H. sapiens* remains	13 000
Queensland, Australia	*H. sapiens* remains	10 000
Washington, USA	*H. sapiens* remains	9000
Mexico	*H. sapiens* remains	8000
New Zealand	Early bones of rats that could only have travelled on boat with *H. sapiens*	700

2 Use the data to plan a possible migration pathway for *Homo sapiens* to all parts of the world.

Results

Annotate the map of *Homo sapiens* migration.

Discussion

1 Describe the Out-of-Africa hypothesis of human evolution.

2 Describe the process scientists used to determine the date of fossilised remains.

3 Suggest why Aboriginal and Torres Strait Islander Peoples are considered the oldest living culture outside Africa.

Conclusion

Describe the pathway early Aboriginal and Torres Strait Islander Peoples used to reach Australia.

GLOSSARY

α-helix
a type of secondary structure of a polypeptide chain with a coiled, spiral structure

ß-pleated sheet
a type of secondary structure of a polypeptide chain with a sheet structure

5′ cap
an altered nucleotide at the 5′ end of mature mRNA

A

Aboriginal and Torres Strait Islander Peoples
the original inhabitants and owners of the land now known as Australia, inhabiting this land for over 65 000 years

absolute dating
a technique to determine the age of a fossil based on the decay of radioactive isotopes

accuracy
a comparison of the experimental data to the true value (the closer the data to the true value, the more accurate it is)

acetaldehyde
an organic compound that forms as an intermediate compound between pyruvate and ethanol during alcoholic fermentation

acetyl-CoA
the substrate that enters the Krebs cycle

active immunity
occurs when an organism produces antibodies in response to an antigen

active site
the portion of an enzyme in contact with the substrate; this site has a specific shape that corresponds to the shape of at least a portion of the substrate molecule

adaptive radiation
an evolutionary process in which organisms diversify rapidly from an ancestral species into several divergent forms

adjuvant
an ingredient used in some vaccines that initiates an inflammatory response, increasing the subsequent immune response

aerobic cellular respiration
the complete conversion of glucose to carbon dioxide and water in the presence of oxygen, resulting in a large release of energy (net gain of 36 ATP)

aim
the main purpose of the practical investigation

airborne
transported by air

allele
an alternative form of a gene

allergen
a normally harmless molecule recognised by the immune system that initiates an overreaction or hypersensitivity reaction

allopatric speciation
the process of speciation as a result of a permanent barrier separating the ancestral species

allosteric repressor
a type of transcription factor produced by regulatory genes that controls transcription by changing shape, enabling it to attach or release from the operator of an operon

allosteric site
a site on an enzyme that allows an effector molecule to bind in a location away from the active site

amino acids
the building blocks of all proteins

anaerobic fermentation
cellular respiration in the absence of oxygen, resulting in a net gain of 2 ATP and lactic acid (in animals), ethyl alcohol and carbon dioxide (in plants and fungi) or other products (in bacteria)

analogous structures
structures with a similar function but which have not arisen due to common ancestry

antibiotic resistance
when bacteria develop the ability to survive in the presence of an antibiotic

antibody
a large, Y-shaped protein produced by plasma cells; by the immune system to neutralise pathogens (also known as an immunoglobulin)

anticodon
a sequence of three nucleotides on tRNA that corresponds to a complementary codon on mRNA

antigen (Ag)
a molecule or part of a molecule that initiates a response by the immune system

antigenic drift
small regular mutations that result in gradual changes in the antigen particles on a virus

antigenic shift
a major change in the structure of a virus that can result from a reassortment of two viruses in a single host cell

antigen-presenting cells
cells that present antigens to B- and T-lymphocytes to initiate the adaptive immune response (commonly macrophages, neutrophils and dendritic cells)

antiparallel
where the two strands of DNA run in opposite directions to each other

artificial active immunity
active immunity initiated by a vaccine containing antigens, which then causes the production of antibodies and memory cells to that antigen

artificial immunity
occurs when an organism is intentionally exposed to a pathogen and develops immunity to the pathogen

artificial passive immunity
occurs when antibodies are passed from one individual to another, usually by intravenous injection

artificial selection
another term used to describe selective breeding

ATP (adenosine triphosphate)
a compound consisting of an adenosine molecule bonded to three phosphate groups; a high-energy molecule

attenuated
a vaccine that contains a viable pathogen with reduced virulence

autoantibodies
antibodies produced by B plasma cells that target ‘self’ markers on body cells

autoimmune disease
when the immune system attacks the body’s own cells

B

backbone
the carboxyl-amine backbone of a polypeptide, without the exposed R-groups

beneficence
the ethical principle regarding a commitment to minimising risk and doing good

benign
not spreading throughout the organism

binary fission
when a prokaryote duplicates its chromosome asexually and then splits into two cells

biochemical pathway
a series of linked enzymatic reactions

biodiesel
the liquid fuel diesel that has been produced by the breakdown of plant and animal lipids

bioethanol
ethanol produced by the anaerobic fermentation of organic matter in plants and animals

biofuel
the liquid or gas fuel formed from biomass

biogas
the combination of gases, including methane, produced by the anaerobic fermentation of organic matter found in plants and animals

biological vector
a molecule or virus that is capable of carrying genetic material into another cell

biology
the science of living things divided into different fields that cover the morphology, physiology, anatomy, behaviour, origin and geographic distribution of organisms

biomass
the mass of living organisms in a measured area; used as a measure of a solid renewable energy source (e.g. wood logs) in the production of electricity

biosynthetic pathway
a series of ordered reactions controlled by enzymes that produce an organic substance (e.g. the amino acid trp is produced this way)

block mutation
chromosomal mutations that alters large sections of a chromosome

blunt end
the double-stranded end of the nucleotide chain that is generated when some endonucleases cut strands of DNA

bone marrow
spongy tissue in bones that contains stem cells for the development of red and white blood cells and blood platelets

bottleneck effect
where a small number of individuals survive a catastrophic event, leading to a small population with reduced genetic diversity

branch
part of the phylogenetic tree that represents a lineage from a divergent event

C

C_3 plant
a plant that uses the carbon fixation pathway as the only mechanism to convert carbon dioxide into an organic molecule, 3-phosphoglyceric acid

C_4 plant
a plant that has another pathway in a different cell, before the Calvin cycle initiates, to maximise the efficiency of photosynthesis

Calvin cycle
the cyclic reactions of the light-independent phase of photosynthesis

CAM plant
a plant that has another pathway in the dark, before the Calvin cycle initiates during daylight hours, to maximise the efficiency of photosynthesis

cancer
a disease in which abnormal cells divide uncontrollably

cancer vaccine
a vaccine that can prevent the development of a cancer or treat an existing cancer

Cas9
a specific enzyme complex that is able to cut the nucleotides of viral (and other) DNA

catalyst
a substance that increases or decreases the rate of reaction without being used up

cell-mediated immunity
the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen

cellular pathogen
a disease-causing organism made of cells (such as fungi, protozoa or bacteria)

cellular respiration
the breakdown of glucose in cells to release energy; may occur with oxygen (aerobic) or without oxygen (anaerobic)

chaperone protein
a protein that sometimes assists in tertiary protein folding

checkpoint inhibitor
a drug that promotes an immune system response to cancer cells

chlorophyll
a pigment in photosynthetic organisms that facilitates photosynthesis

chloroplast
a small photosynthetic organelle in green plants, containing chlorophyll in membranous grana and enzymes in liquid stroma

chromatin
the condensed, coiled nucleosome complexes of eukaryotic DNA

chromosomal mutation
mutation that alters entire sections of chromosomes

clade
a group of taxa and their common ancestor

cladistics
a method of grouping organisms that uses evolutionary lines of descent instead of structural similarities

cladogram
an unscaled phylogenetic tree

clonal proliferation
the selection and reproduction of only one type of cell

codon
a sequence of three nucleotides on mRNA that codes for a specific amino acid

coenzyme
an organic, non-protein compound needed for an enzyme to function

coenzyme A
a coenzyme used in biochemical reactions inside the cell (including during the Krebs cycle in cellular respiration)

clonal proliferation
the selection and reproduction of only one type of cell

comparative anatomy
comparing structures between organisms to determine relatedness

competitive inhibitor
a substance that occupies the active site of an enzyme and prevents the normal substrate from binding

complement protein
a complex system of more than 30 proteins that act together to help eliminate pathogens

complete anaerobe
an organism that gains its ATP exclusively through fermentation (also called an obligate anaerobe)

conservative substitution
an amino acid substitution that doesn't cause a change in the protein

conserved gene
a gene that has remained relatively unchanged throughout evolution

contagious
where a disease is able to spread from one individual to another individual

convergent evolution
the independent development of similarities between species as a result of them having similar ecological roles and selection pressures

core skills
key 'employment skills' required in most careers

Country
an area (not just geographical) that is traditionally owned and looked after by an Aboriginal (and sometimes Torres Strait Islander Peoples) language group or community; a place that is of spiritual meaning with deep feelings of connection and attachment

cranial capacity
the size of the internal volume of a skull

CRISPR
clustered regularly interspaced short palindromic repeats; a short section of naturally occurring viral DNA that is inserted into a bacterial chromosome and used to prevent future infections

cristae
the folds formed by the inner membrane of a mitochondrion

cytokines
a large group of chemicals secreted by cells of the immune system – such as interferon, interleukin, and growth factors – that act as chemical messengers to stimulate other cells to respond to pathogens

cytotoxic T-lymphocyte
a type of T-cell that kills cancer cells and virus-infected cells

D

degeneracy
the redundancy of the genetic code, meaning more than one codon codes for the same amino acid

denaturation
the destruction of a protein (including enzymes) caused by high temperature, pH changes and other factors

dendritic cell
an antigen-presenting cell that processes antigen material and presents it on the cell surface to the T-cells of the immune system

deoxyribonucleic acid (DNA)
a type of nucleic acid that stores the genetic instructions for all life processes

derived characteristic
a feature that sets members of that clade apart from other individuals

desensitisation
a treatment process that gradually reduces the immune response to allergens

diploid
having two complete sets of chromosomes within a cell; somatic cells contain a diploid set of chromosomes

direct evidence
evidence-based or direct observation without any assumptions

disease
anything that interrupts the normal functioning of an organism

DNA ligase
an enzyme that facilitates bonding between two fragments of DNA

DNA methylation
chemical tags attached to histone proteins, coiling the nucleosomes either tightly or far apart, allowing gene expression to be controlled

DNA polymerase
an enzyme that facilitates covalent bonding of the sugar–phosphate backbone when replicating DNA

DNA profiling (fingerprinting)
when common nucleotide sequences are used to identify key characteristics such as hair or eye colour

E

electron transport chain
a series of enzymes embedded in the inner membrane of the mitochondria that enable the transfer of electrons from coenzymes; this is the final stage in aerobic cellular respiration in which most ATP molecules are formed

emerging disease
a disease that has been recognised in a human host for the first time

endemic
a disease that is restricted to a certain place or community and which exists at low levels

endonuclease
an enzyme (isolated from prokaryotes) that is used to cut DNA at specific recognition sequence sites

endoplasmic reticulum
an organelle involved in the production, modification and packaging of materials such as proteins, lipids and steroids

enzyme
a biological catalyst that increases the rate of a chemical reaction

eosinophil
a type of white blood cell responsible for fighting multicellular parasites

epidemic
the rapid spread of an infectious disease in a community in a short period of time

epidemiology
a branch of medicine that investigates the incidence, distribution and control measures relating to disease and health-related events

epigenetic factor
any external factor that modifies DNA that may influence gene expression or repression

epigenome
the set of chemical modifications that affects which regions of DNA are expressed

ethanol
C_2H_5OH produced from the anaerobic breakdown of glucose in plants and yeast

eukaryotic
a cell containing a nucleus and other membrane-bound organelles

exocytosis
active transport of substances out of a cell using a vesicle

exon
a region of DNA expressed during protein synthesis

external examination
a test written by external assessors that determines an individual's knowledge and skills in a subject

extracellular
found outside a cell

extracellular threat
pathogens that enter the host organism and survive outside of the host's cells

F

faunal (fossil) succession
the observation that fossils found in sedimentary rock strata succeed each other vertically in an orderly manner

fermentation
an anaerobic process of breaking down glucose in the absence of oxygen

first line of defence
the physical and chemical barriers that protect an organism from infection (such as intact skin and mucus)

foramen magnum
a large oval opening at the base of the human skull through which the spinal cord connects with the brain

fossil
the preserved remains, impression or trace of a once-living organism

fossil record
the record of organisms over geological time as inferred from fossil evidence

founder effect
where a small number individuals become isolated from an original population to become the founding members of a new population with reduced genetic diversity

frameshift point mutation
the addition or deletion of a nucleotide that alters the entire sequence of amino acids from the point of mutation onwards

fungus
a eukaryotic organism whose cells have organelles, a cell wall and hyphae, which needs to consume nutrients for cellular respiration (an example is yeast); plural fungi

G

gametic
involving gametes, or germline cells with half the usual number of chromosomes

gel electrophoresis
a process that separates negatively charged fragments of DNA according to size

gene
a section of DNA that has a specific function

gene cloning
a process in which a gene is inserted into a plasmid, placed into a host cell, and the cell reproduced in a medium

gene expression
the transcription and translation of a gene into a polypeptide chain

gene flow
the movement of genes between populations of a species due to migration of individuals

gene mutation
mutation that occurs within the DNA sequence of genes within a chromosome

gene pool
the entire set of genes and their allele combinations in any given population

gene regulation
the process of controlling gene expression and therefore the amount and type of proteins produced

genetically modified organism (GMO)
an organism that has had its genome intentionally changed by humans

genetic divergence
evolution that leads to descendants becoming different in form from their common ancestor due to different selection pressures

genetic drift
random changes in the allele frequencies of small isolated populations

genetic equilibrium
when the allele frequencies of a population remain stable over time

genetic isolation
when a population is completely isolated and there is no gene flow

genome
the complete set of genes for an organism

genotype
the genetic make-up of an individual

geographic isolation
when a population is separated due to a geographical barrier

geological timescale
the sequence of events in Earth's history based on the geological rock record

germline cell
a cell in the gonads that gives rise to gametes (ovum and sperm)

glycolysis
the first stage in cellular respiration; occurs in the cytoplasm and results in the net gain of 2 ATP and 2 pyruvate molecules

Golgi apparatus
an organelle involved in the packaging and secretion of cell products

granum
stacked portions of membranes in chloroplasts; site of the light-dependent reaction for photosynthesis

graph
a way of representing data to visually identify the relationship between the variables

H

half-life
the time taken for a quantity of a radioactive isotope to decay to half of its initial value

helper T-lymphocyte
a type of T-cell that plays an important role in the immune system; stimulates the activity of other immune cells by releasing cytokines

herd immunity
when the majority of the population are immunised against an infectious disease, indirectly protecting those who are not immunised

histone modification
change to histone proteins that enables gene expression to be controlled through altering the coiling of DNA (and therefore availability of a gene for transcription)

histone protein
a type of protein that eukaryotic DNA coils around to form nucleosomes

hominins
a classification group that includes modern humans and all their extinct ancestors

hominoids
a classification group that includes modern humans and the great apes

homologous structures
similar structures indicating shared ancestry, but may have different functions

homology directed repair (HDR)
a mechanism that repairs double strand breaks in DNA when template DNA is absent

humoral immune response
immunity that is mediated by antibodies and complement proteins

hybridoma
a hybridised cell that is a fusion of a plasma cell and a tumour cell

hypothesis
a testable statement or proposed explanation for the predicted outcome of a practical investigation, based on scientific reasoning

I

immunity
the resistance of an organism to an invading pathogen and its harmful effects

immunotherapy
a treatment that uses substances to either suppress or activate the immune system to help fight cancer and other diseases

incubation period
the time between exposure to a pathogen and the first symptoms

index fossil
a distinctive, abundant fossil with a wide geographic distribution over a relatively short geological period of time

infectious disease
a disease caused by a pathogen that can be transferred between hosts

inflammation
a physical condition in which part of the body becomes red, swollen, hot, and sometimes painful as a reaction to injury or infection

innate
inheritable feature, e.g. the innate immune system is present from birth

integrity
the ethical principle regarding the commitment to the search for knowledge and being honest in the approach

interferons
a group of innate cytokines that are released by virus-infected cells or immune cells; they warn neighbouring cells of infection and activate other cells in the immune system

intracellular
found within a cell

intracellular threat
pathogens that invade host cells to survive and reproduce, e.g. viruses

intron
a section of DNA that does not code for polypeptides, but may have other regulatory functions

isotope
variations of an element that differ in the number of neutrons within their nuclei; many isotopes are radioactive forms of an element

J

justice
the ethical principle regarding ensuring a fair and equal consideration of all factors

K

key adaptation
a novel phenotypic trait that allows an organism to evolve and exploit a new niche or resource, resulting in the subsequent radiation and success of a taxonomic group

Krebs cycle
the cyclic series of chemical reactions in the mitochondria of cells in which 2 ATP are formed and coenzymes NAD^+ and FAD^+ become loaded

L

lactic acid
$C_3H_6O_3$ produced from the anaerobic breakdown of glucose in animals

leaf
part of the phylogenetic tree that represents a taxonomic group (taxon)

light-dependent reaction
a chemical reaction in the chloroplast involving the splitting of water in the presence of sunlight

light-independent reaction
the second stage of photosynthesis, in which carbon dioxide is reduced to form glucose; does not need the presence of light

lymphatic system
a network of tissues and organs that transport lymph, amongst other things, throughout the body

lymph node
a small gland of the lymph system that contains white blood cells

lymph vessel
a thin-walled vessel, like a blood vessel, that carries lymph

lysozyme
an enzyme in cells that is able to catalyse the destruction of the cell walls of certain bacteria

M

macrophage
a large phagocytic cell commonly found at sites of infection

major histocompatibility complex I (MHCI)
the group of glycoproteins on the membrane of cells assist immune cells to recognise self from non-self

malignant
cancerous; able to invade other tissues

marsupials
a subgroup of mammals that nurse their young in a pouch

mast cell
a cell commonly found in connective tissue containing many granules (including histamine); forms part of the immune system

matrix
the internal space of a mitochondrion enclosed by the inner membrane

memory B-lymphocyte
a cell that 'remembers' the antigens presented by a particular pathogen for rapid antibody production in future infections

memory T-lymphocyte
a type of T-cell that has previously encountered and responded to an antigen and retained a memory of that antigen, enabling it to respond rapidly to a subsequent infection

messenger RNA (mRNA)
a sequence of nucleotides transcribed by RNA polymerase during transcription

metabolism
the total set of biochemical reactions occurring within a cell

metastasis
the spread of cancer cells from a primary tumour to another area of the body

methodology
the approach used to plan and conduct a scientific investigation with justification

MHCII
major histocompatibility complex molecules normally found on antigen-presenting cells

microbiota
the microorganisms found in a particular environment, e.g. the skin or gut

mind map
a graphical way to represent key ideas and relationships between concepts

mitochondrial DNA (mtDNA)
DNA located in the mitochondria

mitochondrion
plural mitochondria; a membrane-bound cellular organelle with a fluid matrix and inner membrane forming folds (cristae) on which are enzymes for aerobic respiration

molecular clock
the rate of accumulation of mutations, used to determine species relatedness

monoclonal antibodies
therapeutic antibodies produced from a cloned B plasma cell targeting a specific cancer antigen

monotremes
a subgroup of mammals that lay eggs; includes the platypus and echidna

multiple-choice question
examination-style question that has four possible alternatives in which you are required to select the most appropriate option

multiregional hypothesis
a hypothesis that *Homo sapiens* evolved through the gene flow between different groups of ancestors in regions outside Africa

mutagen
a factor that increases the rate of mutation above the usual spontaneous rate

mutation
a permanent change in the genetic sequence of an organism

myeloma cells
cancerous cells that develop from B plasma cells in the bone marrow

N

naive B-cell
a B-cell that has not been exposed to an antigen; it will become either a memory B-cell or a plasma cell that secretes antibodies specific to the antigen that was originally bound to it

natural active immunity
active immunity from exposure to a pathogen that causes disease in the organism, and the production of antibodies and memory cells to that pathogen

natural immunity
occurs when an organism is exposed to a live pathogen, develops the disease, and develops immunity to the pathogen

natural killer cell
a type of white blood cell and part of the innate immune system; these cells play a major role in the host rejection of both tumours and cells infected by viruses

natural passive immunity
occurs when a mother passes her antibodies across the placenta or in breast milk to her developing child

natural selection
the differential survival and reproduction of individuals due to differences in phenotype that ultimately lead to the evolution of a species

neutrophil
a type of phagocytic white blood cell that is one of the first cell types to arrive at the site of an infection

niche
the role of an organism in an environment

node
part of the phylogenetic tree that represents the common ancestor

non-cellular pathogens
non-living pathogens, e.g. viruses or prions

non-competitive inhibitor
a substance that occupies the allosteric site of an enzyme, changing the shape of the active site, preventing the normal substrate from binding

non-conservative substitution
an amino acid substitution that results in a completely different protein

non-homology end joining (NHEJ)
a mechanism that repairs double strand breaks in DNA when template DNA is absent

non-infectious disease
a disease that is not caused by a pathogen

non-maleficence
the ethical principle to avoid harm or to decrease the amount of harm inflicted

non-self antigen
a molecule on the surface of a foreign cell or body that initiates a response by the immune system

nuclear DNA
DNA found in the nucleus of the cell

nucleosome
a complex formed when short sections of DNA coil around histone proteins

nucleotides
the building blocks of nucleic acids; comprised of a sugar, phosphate and nitrogenous base

O

occipital bulge
a prominent lump or projection found at the back (occipital area) of the skull

operon
a group of prokaryotic genes with a single promoter, operator and terminator region

opposable thumb
a thumb that can be placed opposite other fingers, allowing the organism to grasp with two digits

organelle
a specialised membrane-bound structure that carries out a specific function within eukaryotic cells

outcome
the Key Knowledge and skills needed to demonstrate a satisfactory achievement for an Area of Study

outgroup
the first taxon to diverge from the original common ancestor in a phylogenetic tree

outlier
any value that sits outside of the dataset

Out-of-Africa hypothesis
a hypothesis that *Homo sapiens* evolved in Africa and then migrated to Europe and Asia approximately 70 000 years ago

oxygen debt
the amount of oxygen required to remove lactic acid from muscle tissue

P

pandemic
an outbreak of an infectious disease that crosses international borders

partial anaerobe
an organism that can gain its ATP through aerobic or anaerobic cellular respiration, depending on the conditions (also called a facultative anaerobe)

passive immunity
where an organism receives antibodies to a pathogen rather than producing them through their own immune system

pathogen
an agent that causes disease

pathogenic
capable of causing disease

pentadactyl limb
a limb with a single upper limb bone, two long bones on the lower limb and five individual digits (fingers or toes) at the end; these digits can be fused in some animals

PEP carboxylase
an enzyme found in C_4 and CAM plants that incorporates carbon from carbon dioxide into oxaloacetate

peptide
a small polypeptide chain that is less than 50 amino acids in length

peptide bond
a strong covalent bond that joins amino acids together in a polypeptide chain

phagocyte
a type of white blood cell that can ingest foreign particles or bacteria, performing phagocytosis

phagocytosis
the ingestion of bacteria or other material; the cell extends its membrane and wraps it around the bacteria, drawing it into the cell's cytosol

phenotype
the physical expression of a genotype

phosphorylation
the process of adding a phosphate to an organic compound

photorespiration
the wasteful pathway where Rubisco incorporates oxygen with RuBP, reducing photosynthesis efficiency

photosynthesis
the process where light energy is converted to the chemical energy of glucose; requires chlorophyll, carbon dioxide, water and a suitable temperature; occurs in green plants, algae and some bacteria

phylogenetic tree
a visual representation of the likely hypothesis of evolutionary relationships between different organisms, showing the path through evolutionary time from a common ancestor to the different taxa

phylogeny
the evolutionary history of taxonomic groups

phylogram
a scaled phylogenetic tree

Place
a space confined by physical or intangible boundaries occupied and regarded as belonging to individuals or groups of Torres Strait Islander Peoples (and sometimes Aboriginal Peoples); the spaces have varying spiritual meaning to the people

placentals
a subgroup of mammals that provide nutrients and remove waste from a foetus through a placenta

plasma cell
a mature B-lymphocyte that produces large amounts of identical antibodies against a specific antigen

plasmid
small circular section of DNA found in prokaryotes that is not part of their chromosome

point mutation
mutation affecting the nucleotide sequence of a gene by the substitution, insertion or deletion of a nucleotide

poly-A tail
a chain of adenines added to the end of the mature messenger RNA during post-transcriptional modification

polymerase chain reaction (PCR)
a process that amplifies the amount of a sample of DNA

polypeptide
a chain of amino acids that forms the primary structure of a protein

polysome
a cluster of ribosomes that translates a strand of mRNA

precision
the closeness of the data in each trial

primary data
data collected by the investigator from firsthand sources

primates
a classification group of unique placental mammals that have common characteristics, including using individual fingers to grasp objects

prion
a misfolded infectious protein that can cause normal proteins to also become misfolded

prokaryotic
a cell with no membrane-bound organelles

promoter
a region of DNA that RNA polymerase binds to, initiating transcription

protein
a long chain of amino acids folded into a specific shape that determines its cellular function

proteome
the entire set of proteins of an organism

protozoan
a single-celled eukaryote that is able to move by changing the shape of its body (an example is the malaria-causing *Plasmodium*)

putative evidence
a hypothesis or logical inference made from observations

pyruvate conversion
a reaction that occurs before the Krebs cycle in the mitochondrial matrix where pyruvate is converted into acetyl-CoA

Q

qualitative
data that tends to be non-numerical and is subjective (e.g. hair colour, outfit choice)

quantitative
data expressed as a number (e.g. concentration of a solution, temperature)

quarantine
a period of isolation for infected individuals

R

radioactive element
an element that emits radiation as a result of the spontaneous degeneration of its nucleus

random coil
a type of secondary structure of a polypeptide chain that does not conform to a regular pattern

random error
an error that reduces the precision of the data due to an error in the experimental process that is unpredictable

raw data
measurements or observations of the dependent variable

reading frame
the sequence of ordered triplets within a gene that are translated into mRNA codons that result in a specific sequence of ordered amino acids that comprise a protein

recombinant
containing DNA from two different sources

re-emerging disease
a disease that reappears in a population after apparent control or elimination

regulatory gene
a gene that produces factors involved in controlling the expression of genes

regulatory T-lymphocyte
a type of T-cell that regulates or suppresses other cells in the immune system

relative age
the age of a rock determined by the ages of surrounding rocks, events and organisms; this is an estimation age or age range

relative dating
a technique to determine the geological age of a fossil or rock strata, relative to other organisms, rocks, features or events without expressing absolute age

repeatability
a measure of achieving the same set of data if the experiment was repeated in the same conditions

repressible operon
an operon that is usually expressed and is only repressed when the biochemical product is in excess

repressor
a protein that can bind to DNA and prevent transcription, inhibiting the expression of a gene

reproducibility
a measure of achieving the same set of data if the experiment was repeated with a different experimenter in a different laboratory

respect
the ethical principle that takes into consideration the value of living things and the ability of living things to make their own decision where possible

restriction fragment length polymorphisms (RFLP)
the difference in the length of DNA fragments that results from cutting DNA with endonucleases

reverse transcriptase
an enzyme used to produce complementary DNA from mature mRNA

R-group
variable side chain on amino acids that gives the amino acids their specific chemical properties

ribonucleic acid (RNA)
a type of nucleic acid involved in protein synthesis

ribosomal RNA (rRNA)
a component of ribosomes involved in the process of translation

risk assessment
a document that outlines the potential risks, hazards and subsequent control measures that should be taken to avoid harm

RNA polymerase
an enzyme that transcribes DNA into mRNA during transcription

root
the start of a phylogenetic tree, representing the ancestral lineage

rough endoplasmic reticulum
organelle involved in the synthesis, packaging and transport of proteins

Rubisco
the key enzyme needed for carbon fixation in the Calvin cycle

S

sacrum
a small triangular bone at the end of the spine formed by the fusion of vertebrae

School-assessed Coursework (SAC)
an internal assessment, written by the school, to be completed in class time that contributes towards the study score for a subject

scientific method
the experimental process of formulating a hypothesis then collecting data in order to determine whether the hypothesis is supported

secondary data
data collected from another person, not the investigator, which is relevant to the scientific investigation

selective breeding
when humans select organisms based on particular traits and interbreed them to increase the desired phenotype in the population

self-antigen
a molecule that can initiate an immune reaction in another organism but not in the parent organism

semi-conservative substitution
an amino acid substitution that may change the resulting protein

shared characteristic
a feature that all members of a group have in common

short-answer question
examination question that requires a short written response

somatic
relating to body cells that are diploid (not gametes)

speciation
the formation of a new reproductively isolated species as a result of evolution

sticky end
the overhanging nucleotides that occur when a strand of DNA is cut by some endonucleases; these ends are more likely to rejoin

strata
the layers of sedimentary rock

stroma
the fluid matrix of the chloroplast, containing enzymes for the light-independent reaction of photosynthesis

structural gene
a gene involved in the production of a protein

study score
the score out of 50 for a subject, calculated by VCAA, using School-assessed Coursework and the end-of-year examination

substitution mutation
the mutation where a nucleotide is swapped for a different nucleotide

substrate
the substance on which an enzyme acts

subunit
a polypeptide within a quaternary protein

susceptibility test
test used to determine the sensitivity of a pathogen to particular factors

sympatric speciation
when two or more descendent species evolve from a single ancestral species within a single geographical location

systematic error
an error that reduces the accuracy of the data by causing the reading to differ from the true value

T

table
a form of organising data systematically into columns

T-cell transfer therapy
a type of immunotherapy where T-cells are given to a patient to target cancerous cells

template strand
the strand used to attach complementary bases during transcription

terminator
a region of DNA at the end of a gene to stop transcription

thylakoid
the membranous, flattened, disc-shaped sac in chloroplasts on which the light-dependent reaction of photosynthesis occurs

thymus
an organ of the lymph system responsible for the maturation of T-lymphocytes

tissue
a group of cells that combine to achieve a particular function

traditional medicine
health practices, beliefs and knowledge incorporating plant-, animal- and mineral-based medications to prevent and treat disease and improve well-being

transcription
the process of copying the genetic information in DNA into mRNA

transcription factor
protein or mRNA produced by regulatory genes that help regulate gene expression

transcriptome
the entire range of mRNA molecules within a specific eukaryotic cell

transfer RNA (tRNA)
a type of RNA that continuously picks up amino acids and transfers them to the ribosomes using its anticodon to match with codons on mRNA

transgenic organism
an organism that has had DNA from another organism inserted into its genome

transitional fossil
a fossil that exhibits traits that are common to both an ancestral group and its descendent group

translation
the process of converting the codon on mRNA into a polypeptide chain of amino acids

transmission
the passing of a pathogen from an infected individual to a non-infected individual

triplet
a three nitrogenous base sequence in DNA that corresponds to codons on mRNA

***trp* operon**
a repressible operon that continually produces the amino acid tryptophan until it accumulates within the cell and the operon is switched off

true value
the value that accurately represents the measurement if the experiment was conducted perfectly

tumour
a growth of abnormal cell tissue that can be benign or malignant

U

unicellular
a living organism composed of a single cell

V

validity
a measure of whether the investigation measures what it intends to

vector
an organism that carries a disease-causing pathogen from one organism to another

vesicle
a small fluid-filled structure bound by a membrane that transports fluids around a cell

vestigial structures
structures that had functional importance in ancestral species but have reduced in size over time and have limited or no use in their present form

virulence
the ability of a pathogen to cause disease

virus
a non-living pathogen that consists of genetic material surrounded by a protein coat; it reproduces through the use of a host cell's organelles

W

World Health Organization (WHO)
an agency of the United Nations concerned with public health

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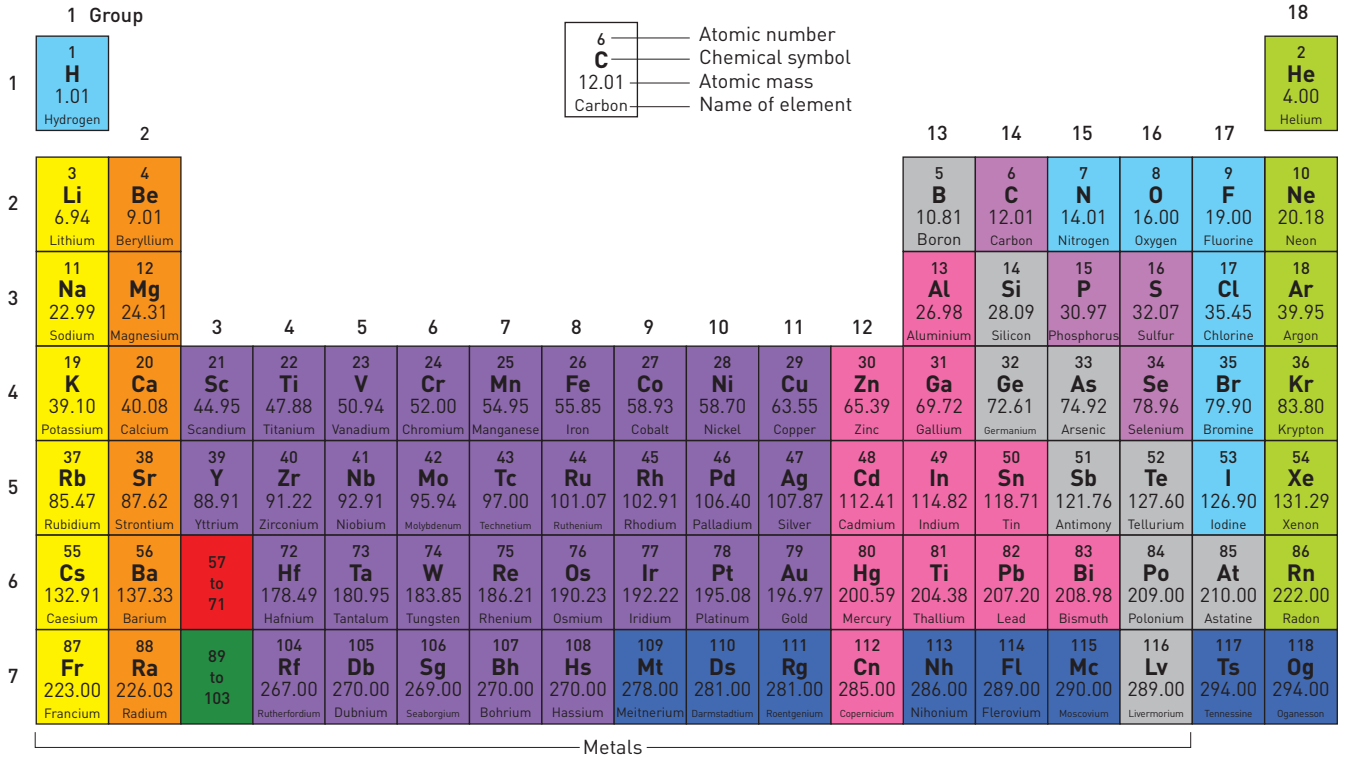
NOTES

APPENDIX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18
6
C
12.01
Carbon
Atomic number
Chemical symbol
Atomic mass
Name of element
1 H 1.01 Hydrogen
2 He 4.00 Helium
2
13
14
15
16
17
3 Li 6.94 Lithium
4 Be 9.01 Beryllium
5 B 10.81 Boron
6 C 12.01 Carbon
7 N 14.01 Nitrogen
8 O 16.00 Oxygen
9 F 19.00 Fluorine
10 Ne 20.18 Neon
11 Na 22.99 Sodium
12 Mg 24.31 Magnesium
3
4
5
6
7
8
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12
13 Al 26.98 Aluminium
14 Si 28.09 Silicon
15 P 30.97 Phosphorus
16 S 32.07 Sulfur
17 Cl 35.45 Chlorine
18 Ar 39.95 Argon
19 K 39.10 Potassium
20 Ca 40.08 Calcium
21 Sc 44.95 Scandium
22 Ti 47.88 Titanium
23 V 50.94 Vanadium
24 Cr 52.00 Chromium
25 Mn 54.95 Manganese
26 Fe 55.85 Iron
27 Co 58.93 Cobalt
28 Ni 58.70 Nickel
29 Cu 63.55 Copper
30 Zn 65.39 Zinc
31 Ga 69.72 Gallium
32 Ge 72.61 Germanium
33 As 74.92 Arsenic
34 Se 78.96 Selenium
35 Br 79.90 Bromine
36 Kr 83.80 Krypton
37 Rb 85.47 Rubidium
38 Sr 87.62 Strontium
39 Y 88.91 Yttrium
40 Zr 91.22 Zirconium
41 Nb 92.91 Niobium
42 Mo 95.94 Molybdenum
43 Tc 97.00 Technetium
44 Ru 101.07 Ruthenium
45 Rh 102.91 Rhodium
46 Pd 106.40 Palladium
47 Ag 107.87 Silver
48 Cd 112.41 Cadmium
49 In 114.82 Indium
50 Sn 118.71 Tin
51 Sb 121.76 Antimony
52 Te 127.60 Tellurium
53 I 126.90 Iodine
54 Xe 131.29 Xenon
55 Cs 132.91 Caesium
56 Ba 137.33 Barium
57 to 71
72 Hf 178.49 Hafnium
73 Ta 180.95 Tantalum
74 W 183.85 Tungsten
75 Re 186.21 Rhenium
76 Os 190.23 Osmium
77 Ir 192.22 Iridium
78 Pt 195.08 Platinum
79 Au 196.97 Gold
80 Hg 200.59 Mercury
81 Ti 204.38 Thallium
82 Pb 207.20 Lead
83 Bi 208.98 Bismuth
84 Po 209.00 Polonium
85 At 210.00 Astatine
86 Rn 222.00 Radon
87 Fr 223.00 Francium
88 Ra 226.03 Radium
89 to 103
104 Rf 267.00 Rutherfordium
105 Db 270.00 Dubnium
106 Sg 269.00 Seaborgium
107 Bh 270.00 Bohrium
108 Hs 270.00 Hassium
109 Mt 278.00 Meitnerium
110 Ds 281.00 Darmstadtium
111 Rg 281.00 Roentgenium
112 Cn 285.00 Copernicium
113 Nh 286.00 Nihonium
114 Fl 289.00 Flerovium
115 Mc 290.00 Moscovium
116 Lv 289.00 Livermorium
117 Ts 294.00 Tennessine
118 Og 294.00 Oganesson
Metals
Rare earth elements
Lanthanoid series
57 La 138.91 Lanthanum
58 Ce 140.12 Cerium
59 Pr 140.91 Praseodymium
60 Nd 144.24 Neodymium
61 Pm (145) Promethium
62 Sm 150.4 Samarium
63 Eu 151.97 Europium
64 Gd 157.25 Gadolinium
65 Tb 158.93 Terbium
66 Dy 162.50 Dysprosium
67 Ho 164.93 Holmium
68 Er 167.26 Erbium
69 Tm 168.93 Thulium
70 Yb 173.04 Ytterbium
71 Lu 174.97 Lutetium
Actinoid series
89 Ac 227.03 Actinium
90 Th 232.04 Thorium
91 Pa 231.04 Protactinium
92 U 238.03 Uranium
93 Np 237.05 Neptunium
94 Pu 244.00 Plutonium
95 Am 243.00 Americium
96 Cm 247.00 Curium
97 Bk 247.00 Berkelium
98 Cf 251.00 Californium
99 Es 252.00 Einsteinium
100 Fm 257.00 Fermium
101 Md 258.00 Mendelevium
102 No 259.00 Nobelium
103 Lr 260.00 Lawrencium
METALS
alkali metal
alkaline earth metal
lanthanide
actinide
transition metals
post-transition metals
NON-METALS
diatomic non-metals
polyatomic non-metals
noble gases
OTHER
metalloids
unknown chemical properties

This front cover shows the Gippsland lakes in Victoria where 'sea sparkle', a phytoplankton known as *Noctiluca scintillans*, turns the waters a bright, glowing blue. When the organism is disturbed in the water, a molecule called luciferin reacts with oxygen and produces its bioluminescence.

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