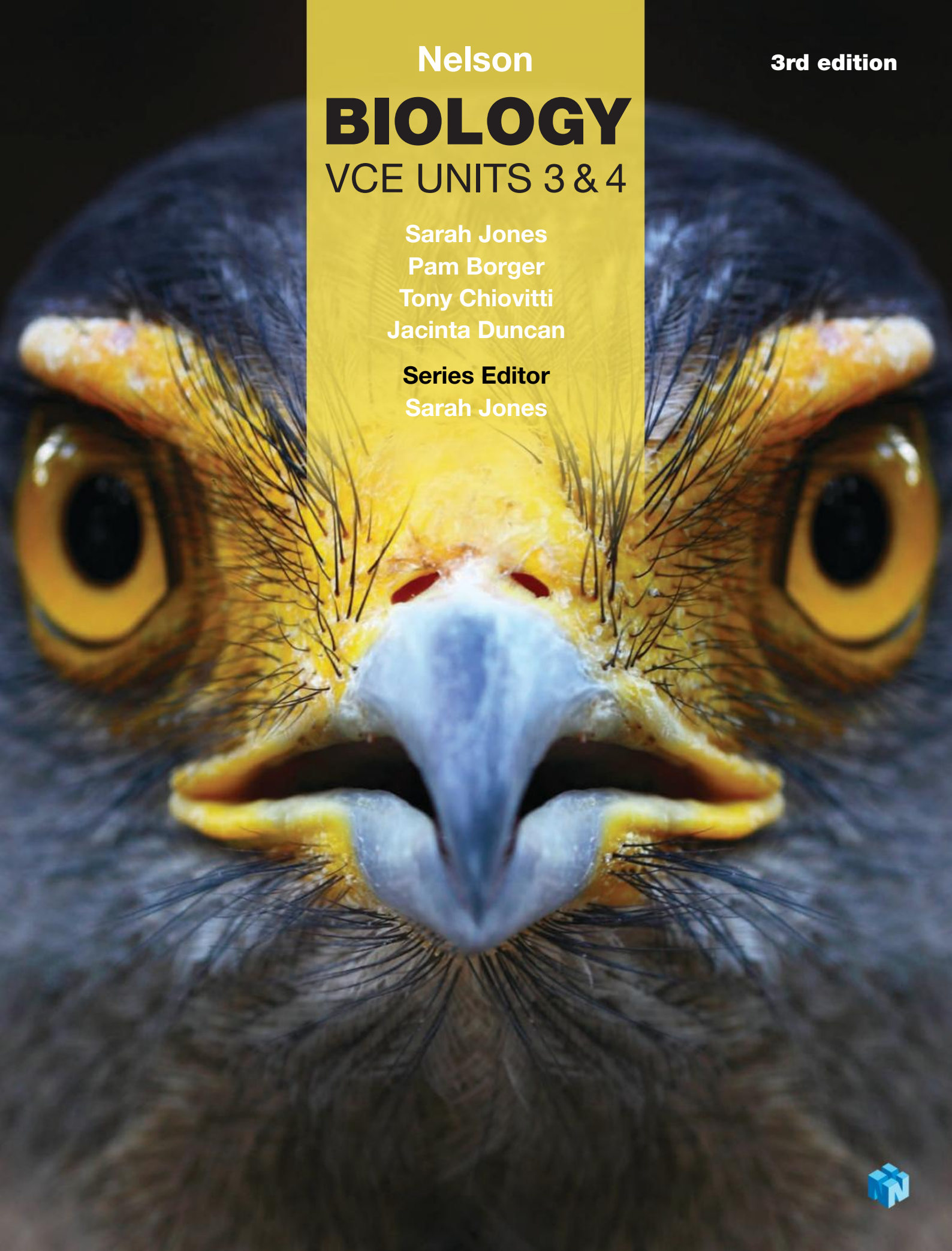

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BIOLOGY

VCE UNITS 3 & 4

3rd edition

Sarah Jones
Pam Borger
Tony Chiovitti
Jacinta Duncan

Series Editor
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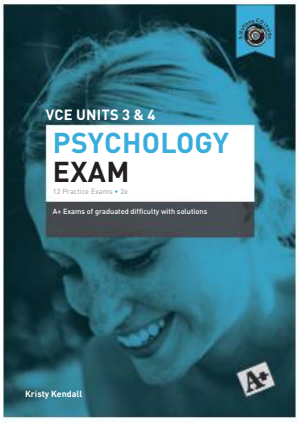

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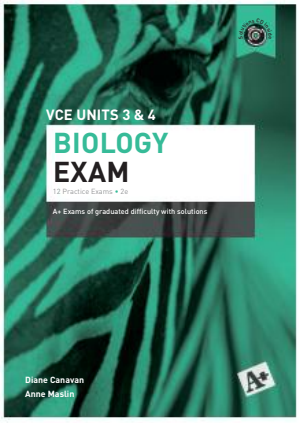

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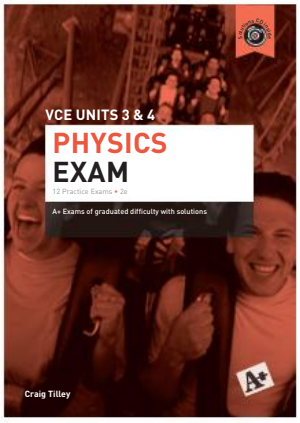

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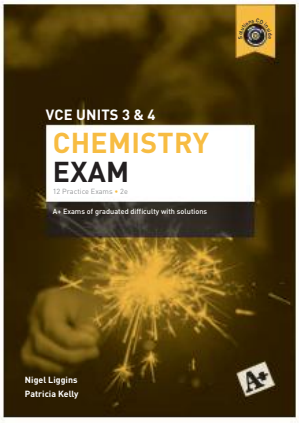

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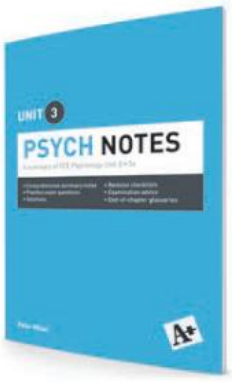

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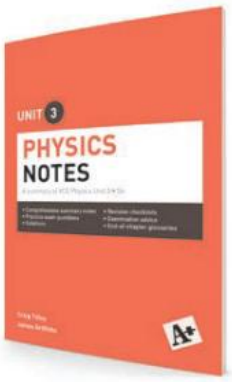

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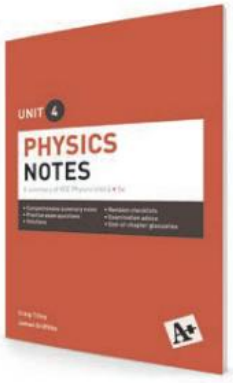

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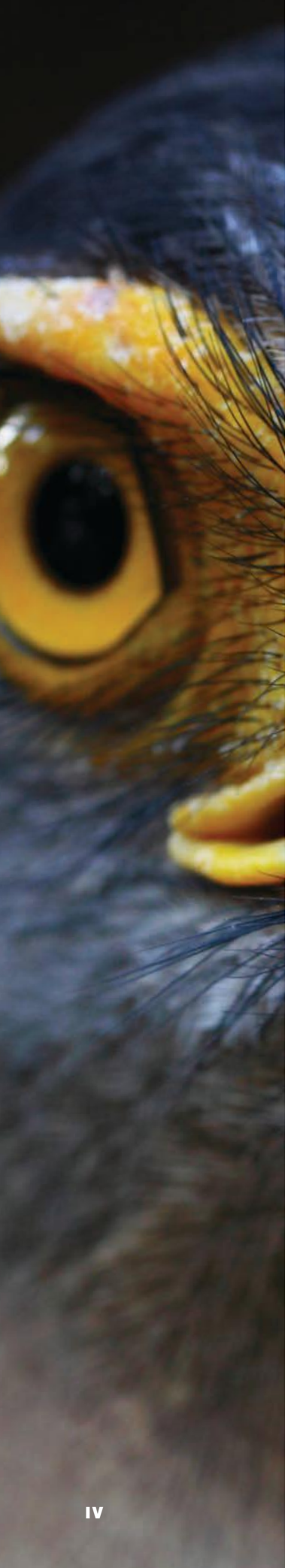

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UNIT 4 HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES OVER TIME? 226

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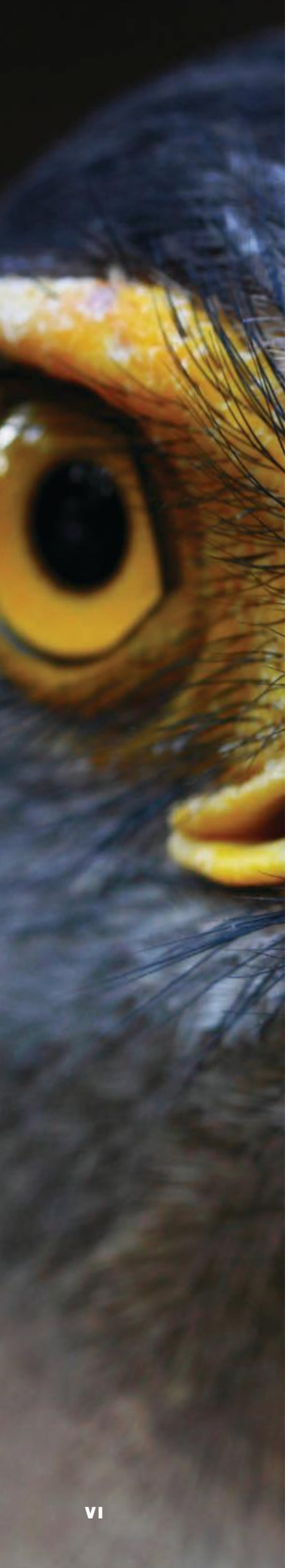

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PREFACE

This third edition of *Nelson Biology VCE Units 3 & 4* has been structured to meet the requirements of the VCAA VCE Biology Study Design 2017–2021.

The text has been written to promote a high level of understanding while also presenting content in easy-to-understand language. The rationale and aims of the VCAA VCE Study Design have formed the basis of the approach for all of our authors. We are proud to have gathered a team of highly experienced subject experts from research, academia and secondary teaching. Our authors have been chosen for their comprehensive knowledge of biology and best teaching practice in biology education at secondary and tertiary levels. They are all dedicated to science communication and lifelong learning, and together they have produced an outstanding series.

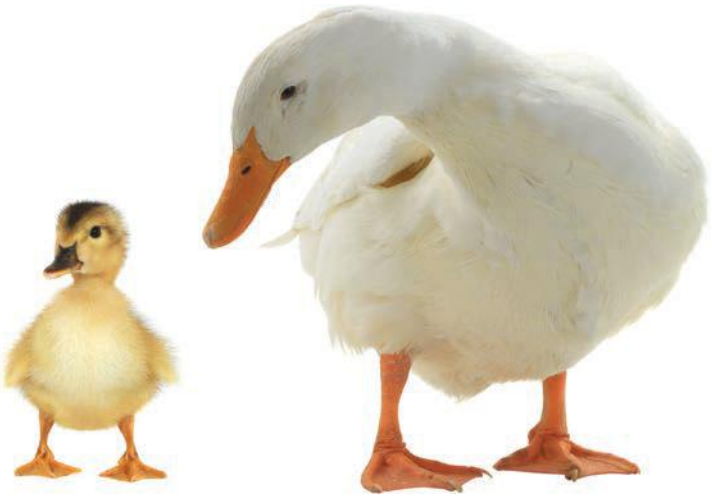
One of the goals of this text is to help students gain a wide perspective on the breadth and depth of Biology in both theory and practice. To understand the living world around us is the key role of a biologist, and a key goal of this text is to make that understanding possible. It is hoped that, through the study of living things, students will gain an appreciation of the complexity of life from the cellular level to the expansive ecosystems of the world, that they will better understand difficult bioethics situations, and will better evaluate and discuss scientific issues.

Unit 4, Area of Study 2 requires students to analyse the interrelationship between scientific knowledge and its applications in society. These important skills have been scaffolded in context throughout the text in Biological Knowledge and Society boxes. When students come to complete the assessment task they will have built up and practised the critical thinking, analysis and communication skills that they need for success.

Scientific investigation is a key skill in biology. Activities and experiments included in the text introduce, reinforce and enable students to practise the science skills. The dedicated *Scientific investigations* chapter thoroughly examines each of the key science skills and outlines how to develop, plan, conduct, analyse and communicate the results of a practical investigation task as required in Unit 4, Area of Study 3. Detailed information on how to develop and present a scientific poster according to VCAA requirements is also provided in this chapter.

Chapters 1–11 each conclude with a concept summary that pulls together all the ideas in the chapter in an easy-to-understand visual format. This summary will be invaluable to students as they try to assimilate all the concepts in the Biology course.

The third edition of *Nelson Biology VCE Units 3 & 4* provides students with the tools and knowledge to prepare fully for assessments, exams and future studies in the area. It will also give them a deeper understanding of the precious living world around them, whether or not they pursue further studies in any of the various fields of biology.



AUTHOR TEAM

Dr Sarah Jones – series editor

Sarah completed her PhD at the Walter and Eliza Hall Institute of Medical Research in Melbourne and held research positions at Harvard Medical School, Boston, and Trinity College, Dublin, before returning to Australia as a Research Fellow at Monash University. She was a medal-winning member of the Australian International Biology Olympiad Team before becoming a tutor then Acting Director of the program, designing theoretical and practical learning material and exams for Australia's highly successful Olympiad teams. Sarah is a member of, and previous Project Manager for, the Australasian Society for Immunology. She has authored multiple peer-reviewed scientific articles in leading journals and continues to research the causes of, and potential new treatments for, autoimmune diseases.

Pam Borger

Pam studied at the University of Melbourne, gaining a Bachelor of Science and a Diploma of Education. She went on to complete an Honours degree in genetics while undertaking work at the Murdoch Institute at the Royal Children's Hospital. Recently, Pam completed her Master's degree in School Leadership. She has taught Biology and developed curriculum for more than 30 years in various schools. Pam worked at the regional level as a science project officer for the Science in Schools project, followed by a leading role in regional youth transitions and pathways. Pam is currently organising and delivering science outreach activities and leading educational projects in a number of areas. She has been actively involved in VCE Biology examinations as an assessor, exam setter and expert 'vetter' for many years.

Jacinta Duncan

Jacinta began her career as a molecular biologist investigating population genetics. After 4 years as a research scientist she completed her Diploma of Education at the University of Melbourne and has been teaching VCE Biology for 15 years. She worked for 2 years as a Lecturer in Science Education for the Masters of Teaching at the University of Melbourne, including coordinating Biology Method. She is now the Director of the Gene Technology Access Centre, a specialist science centre delivering programs for students and teachers in cell and molecular biology. Jacinta is currently completing a Masters of Education by Research. She was pivotal in introducing bioinformatics tasks into Victorian schools, and designs educational resources that assist students in understanding Biology concepts.

Dr Tony Chiovitti

Tony Chiovitti attained a BSc (Hons) at the University of Melbourne in 1992. He completed a PhD at the School of Botany, University of Melbourne, in 1997, investigating the cell wall biochemistry of Australian red algae and algal evolution using gene sequences. He has 8 years of postdoctoral research experience in Australia and overseas with biochemical studies of bacteria and microalgae, including collaboration in the first phytoplankton genomes to be sequenced. He obtained a Diploma of Education in 2004 and joined the education team at the Gene Technology Access Centre (GTAC), Parkville, Victoria in 2007, and is now the Deputy Director. Tony has developed and delivered educational programs for students and professional learning programs for teachers on the themes of cell and molecular biology, health and disease, ecology and evolution.

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USING *NELSON BIOLOGY*

Nelson Biology VCE Units 3 & 4 has been crafted to enable you, the student, to achieve maximum understanding and success in this subject. Each page has been carefully considered to provide you with all the information you need without appearing cluttered or overwhelming. You will find it easy to navigate through each chapter and see connections between chapters through the use of linking icons. Practical work has been integrated within the text so you can see how conceptual and practical aspects of Biology are closely connected.

Each chapter begins with a **Chapter opener**. This presents the key knowledge, including Biological knowledge and society key knowledge and key science skills from Unit 3 and Unit 4 of the Year 12 VCE Biology curriculum, which will be covered in the chapter. The text has been written and reviewed by experienced biology teachers, academics and researchers to ensure up-to-date scientific accuracy for learners.

To optimise comprehension, a number of strategies have been applied to the preparation of our text to improve literacy and understanding. One of these is the use of shorter sentences and paragraphs. This is coupled with clear and concise explanations and real-world examples. New terms are bolded and highlighted as they are introduced, and appear in end-of-chapter glossaries as well as a consolidated end-of-book glossary.

Throughout the text, important ideas, concepts and theories are summarised in **Recall** boxes. This provides repetition and visual enhancement for improved assimilation of new ideas.

After each Recall box in a chapter, a **Recap** box appears. Recaps are a set of questions that review the recent concepts learned in the previous section.

In the study of Biology, you need to be given the opportunity to explore and discover the living world through practical activities. **Activity** boxes provide the opportunity for short, hands-on tasks to clarify or reinforce a concept. The activity can be performed either individually or in groups.

The **Experiments** introduce and reinforce the key science skills. Experiments contain guided instruction on the materials, procedure, collection and analysis of results and discussion. In some cases, open-ended investigations are presented in the experiments. You have the opportunity to design and carry out your own scientific investigation, either individually or in a group. You are prompted to consider ideas for improvement and further investigation to illustrate that science is an ongoing and improving process.

The **Risk assessment** table occurs within the experiment boxes. The table highlights the risks of the experiment and provides suggestions on how to minimise these risks but it is not comprehensive. Teachers are expected to amend this table in the case of substitutions or in the case of any additional risks. This may mean obtaining and following Material and Safety Data Sheets (MSDS) for certain chemicals. All teachers are required to follow the safety guidelines of their specific school and associated government legislation when students are in their care.


CHAPTER 1
PLASMA MEMBRANES
By the end of this chapter you will have covered the following material.
KEY KNOWLEDGE
KEY SCIENCE SKILLS

RECALL

RECAP

ACTIVITY

EXPERIMENT


Biological knowledge and society: An issue in the fish oil industry

Discovering the role of omega-3 essential fatty acids in the diet

Discovering health benefits of omega-3 fatty acids

Margin note


CHAPTER REVIEW QUESTIONS

Biological knowledge and society boxes occur in relevant chapters to address the key knowledge outlined in Unit 4 Area of Study 2. The scaffolded skills in each of these boxes will enable students to confidently complete the associated assessment task.

Full understanding of a concept is often constructed from many pieces of information. Due to the sequential nature of a book, this information cannot always be presented together as it is best placed in other chapters. Links between concepts that occur on other pages and chapters are indicated using the **Margin notes**.

The end-of-chapter review provides a number of features.

- A concept summary, a visual and text-based summary of the most important concepts presented within the chapter, will be useful when you are revising for tests and exams.
- The chapter glossary provides definitions for all the highlighted terms introduced in the chapter.
- The chapter review questions revise understanding of concepts from the chapter, grouped under the headings Remembering, Understanding, Applying, Analysing, Evaluating, Creating and Reflecting.

  Questions are ordered from lower to higher order thinking skills.

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- Study design mappings for Unit 3 and Unit 4
- Teaching programs for Unit 3 and Unit 4
- Answers to questions from the textbook

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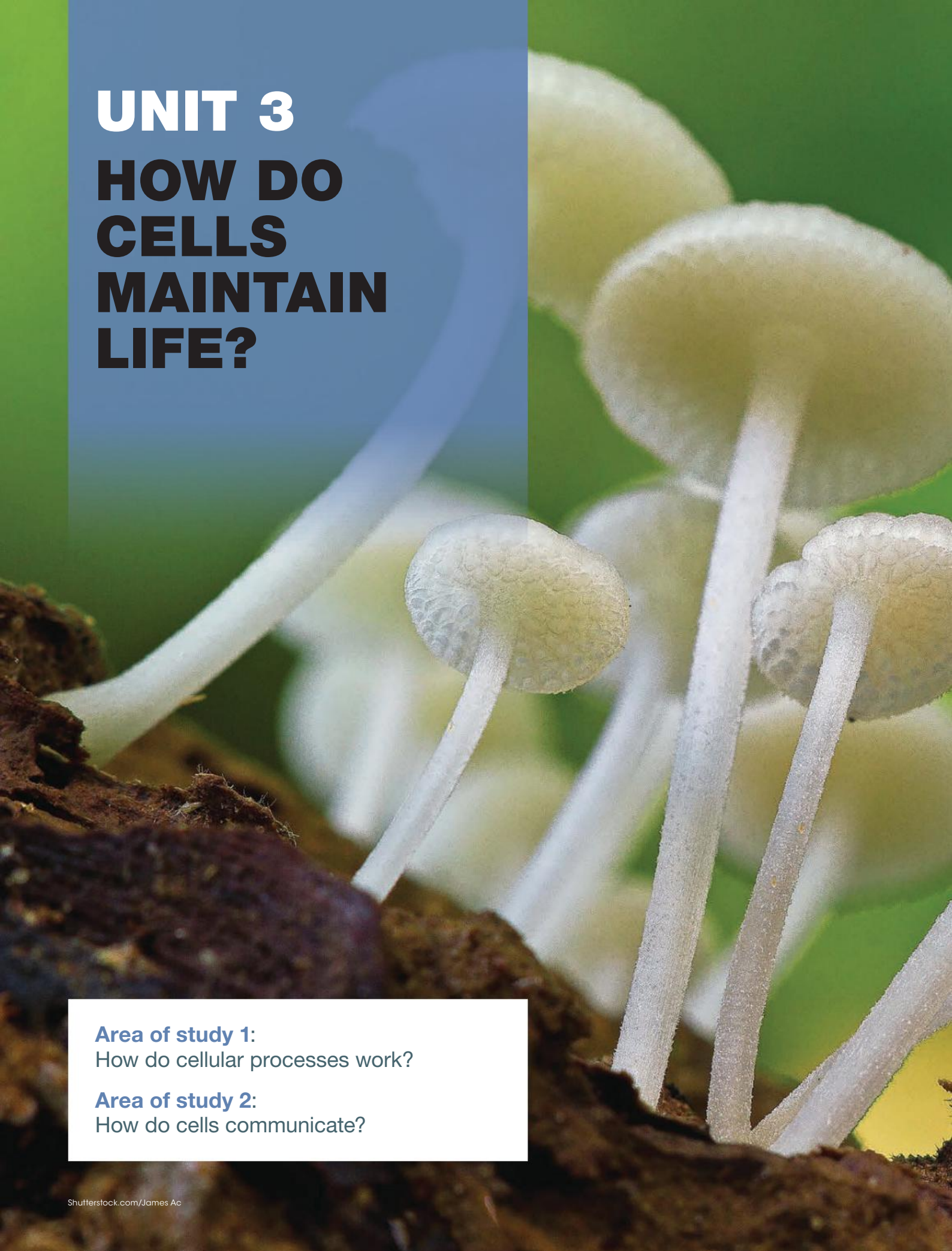
STUDY DESIGN GRID

Unit	Area of Study	Chapters											
		1	2	3	4	5	6	7	8	9	10	11	12
3	1 How do cellular processes work? Students focus on the cell as a complex chemical system.	✓	✓	✓									
	2 How do cells communicate? Students focus on how cells receive specific signals that elicit a particular response.				✓	✓	✓						
4	1 How are species related? Students focus on changes to genetic material over time and the evidence for biological evolution.							✓	✓	✓	✓		
	2 How do humans impact on biological processes? Students examine the impact of human culture and technological applications on biological processes.	*	*	*		*	*					✓ *	
	3 Practical investigation Students design or adapt an investigation related to cellular processes and/or biological change and continuity over time undertaken in either Unit 3 or Unit 4, or across both Units 3 and 4.												✓

✓ All the AOS descriptions are taken directly from the Study Design.

* Indicates chapters with Biological knowledge and society boxes related to Unit 4, Area of Study 2.

UNIT 3
HOW DO CELLS MAINTAIN LIFE?



Area of study 1:
How do cellular processes work?

Area of study 2:
How do cells communicate?

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CHAPTER 1
PLASMA MEMBRANES

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

KEY KNOWLEDGE

Plasma membranes

- the fluid mosaic model of the structure of the plasma membrane and the movement of hydrophilic and hydrophobic substances across it based on their size and polarity
- the role of different organelles including ribosomes, endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of a protein product from the cell through exocytosis
- cellular engulfment of material by endocytosis.

KEY SCIENCE SKILLS

Conduct investigations to collect and record data

- systematically generate, collect, record and summarise both qualitative and quantitative data

Analyse and evaluate data, methods and scientific models

- organise, present and interpret data using schematic diagrams and flow charts, tables, bar charts, line graphs, ratios, percentages and calculations of mean
- explain how models are used to organise and understand observed phenomena and concepts related to biology, identifying limitations of the models

Communicate and explain scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them
- use clear, coherent and concise expression

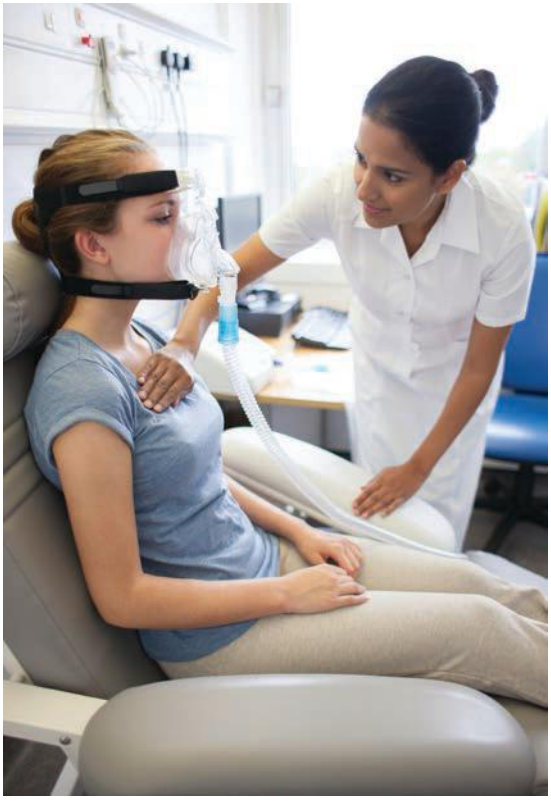

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Figure 1.1 ▶
Teen affected by cystic fibrosis. Thick mucus collecting in the airways needs to be dislodged. This is often done by physical therapy.

As small as it is, a cell in your body must be able to respond to the environment around it. At all times, water, gases, simple molecules and large macromolecules must be moved in one or both directions across the delicate yet strong plasma membrane. The membrane must be selective, keeping conditions favourable for individual cells and ultimately survival of the organism. The consequences of even just one type of change in membrane transport can be life-threatening.

Cystic fibrosis is a disease caused by a genetic mutation to the CF gene. This mutation causes abnormalities in the production and function of a protein called the cystic fibrosis transmembrane conductance regulator (CFTR), a type of protein channel. A change to CFTR disrupts the function of the chloride channel in the plasma membrane, preventing the usual flow of chloride ions and water into and out of cells. In this situation more water is absorbed into the cell, with less on the surface. Mucus is a slippery substance that lubricates and protects surfaces. With less volume of water on the surface of lung cells, mucus gets dried out, thick and difficult to displace. The result of this is that bacteria and other particles get trapped in the mucus and are not able to be swept away easily. Bacterial infections are common.

Cystic fibrosis highlights the importance of proper membrane function. When the membrane functions efficiently, critical life-sustaining processes are carried out. The cell is able to respond appropriately to its surroundings.

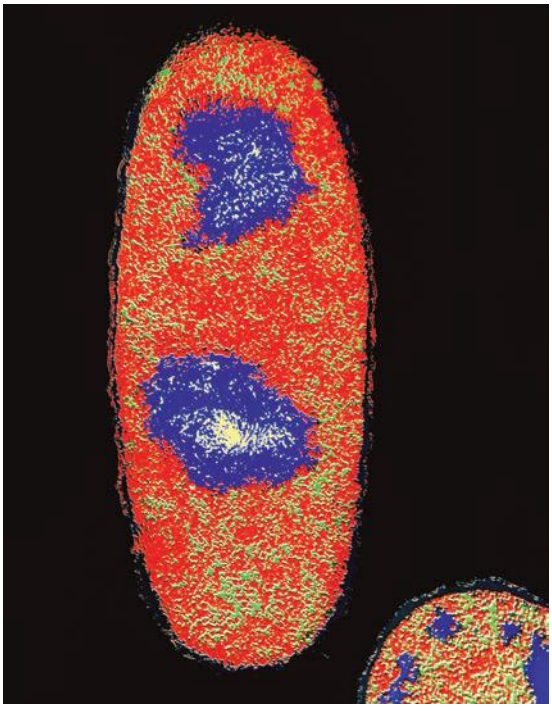

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Figure 1.2 ▲
Escherichia coli cell showing internal structure

The plasma membrane

Escherichia coli (*E. coli*) is a bacterium that inhabits the large intestines of humans. It is used as an indicator of faecal contamination at beaches. When you hear that a beach is unfit to swim in, it is usually because the *E. coli* count is over an accepted level. Being a bacterium, *E. coli* is prokaryotic in structure (see Figure 1.2).

The prokaryotic cell is surrounded by a membrane. The term 'membrane' is often used to describe any thin layer, whether in relation to living things or not. The membrane of cells is correctly called the **plasma membrane**.

As a protective boundary, the plasma membrane keeps internal contents confined in one area, preventing them moving away from each other. The plasma membrane is also important in keeping out foreign molecules that could damage or destroy the cell's components and molecules.

While the plasma membrane serves the functions mentioned, cells must also communicate with their environment to continuously monitor the external conditions and adapt to them. For example, if an *E. coli* bacterium detects a high concentration of the sugar, lactose, in the surrounding environment it responds by making proteins to take in and break down the lactose. But if the *E. coli* detects a high concentration of glucose in the environment, it responds by making different proteins to take in and break down the glucose and not waste energy producing the lactose-digesting enzymes. It also needs to release the toxic products of its metabolism. How does the *E. coli* detect these environmental differences? It depends on its proteins attached to or embedded in the membrane to gather information about the environment in various ways.

Like prokaryotic cells, eukaryotic cells are enclosed by a plasma membrane. But unlike prokaryotes, eukaryote cells have many different membrane-bound organelles. This type of organisation allows eukaryote cells to perform many different chemical processes at the one time and allows them to grow much larger than prokaryotic cells.

Specialised and unique chemical processes occur within specific cellular organelles. Mitochondria, for example, contain the enzymes for cellular respiration and ribosomes for protein synthesis. Each organelle therefore requires different inputs and outputs. The membranes of each organelle are also specialised, having different properties to regulate which substances enter and leave the organelle.

Multicellular organisms are made up of eukaryotic cells. These cells also need to exchange substances with the external environment. Each of the cells in our tissues communicates with many other types of cells about a variety of important issues, such as when it should change its behaviour, grow or differentiate to a specialised cell, or die.

Structure of plasma membranes

As early as the 1890s, Charles Overton understood that cells had an outer layer that allowed some substances into the cell and kept other substances out. He found that lipid-soluble substances readily entered the cell, which was a clue that lipid molecules may have been part of the structure of the membrane.

It was not until the invention of the electron microscope in the 1950s that details of the plasma membrane's structure were able to be seen. Since then, our knowledge of membranes and what they do has expanded.

The plasma membrane is a flexible structure so the cell can change shape easily. It is also able to grow and expand as the cell contents increase, especially during cell division. If damaged, the plasma membrane needs to be repaired quickly.

What makes up the plasma membrane to allow it to act as a regulatory boundary between the inside of the cell and the outside? How is material selected to move across the membrane? How does it reseal a puncture? To answer these questions, we need to look at the properties of the chemicals that make up the plasma membrane.

One remarkable property of plasma membranes is their ability to change shape, expand and contract. During cell division and vesicle formation, membranes can break and reassemble themselves. This is because membranes are actually two-dimensional fluids, constantly flowing and changing shape.

The plasma membrane and all membranes surrounding organelles within the cell are made up of many small phospholipid molecules and this gives the membranes great flexibility.

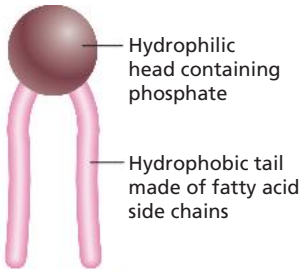


Figure 1.3 ▲
A phospholipid molecule. The hydrophilic head is attracted to water and the hydrophobic tails repel water.

The fluid mosaic model

The plasma membrane is composed of a double layer of **phospholipid** molecules, each of which can be represented by a head and two tails, as shown in Figure 1.3. The head is a **hydrophilic** phosphate group and the tail is a **hydrophobic** fatty acid. This means that the head can dissolve in water, whereas the tails are repelled from the aqueous intracellular (internal) and extracellular (external to the cell) solutions and forced to face inwards towards each other (Figure 1.4), forming a **phospholipid bilayer**.

Individual phospholipid molecules are capable of sideways movement and are highly mobile within the membrane. The lipid bilayer of the membrane is like a liquid crystal, neither solid nor liquid. A single lipid molecule can travel rapidly from one place to another. For instance, one lipid molecule in a bacterium can move from one end to the other (approximately 3.5 μm) in a second. This feature gives the membrane important flexibility, allowing the cell to change shape easily and to expand and contract without losing its integrity. It also allows the plasma membrane to break and reassemble itself during cell division.

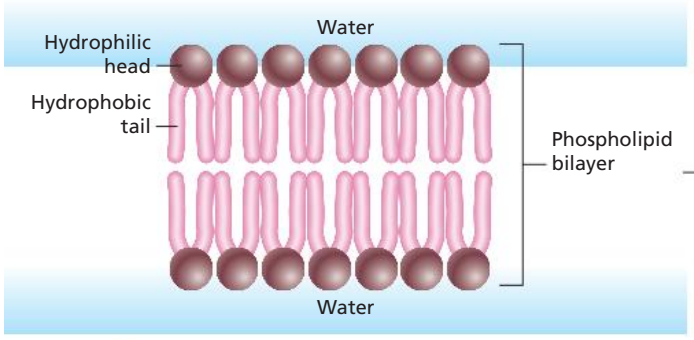


Figure 1.4 ▲
Representation of the way phospholipids form a bilayer in membranes

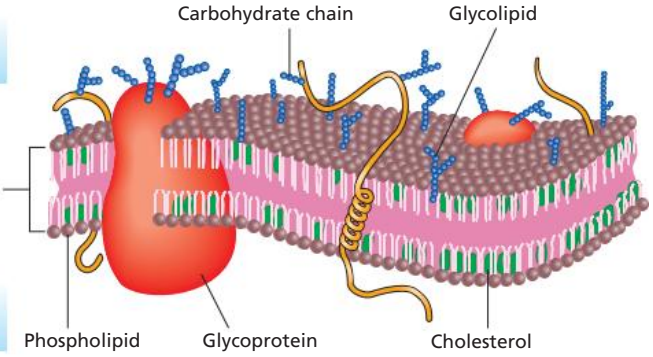
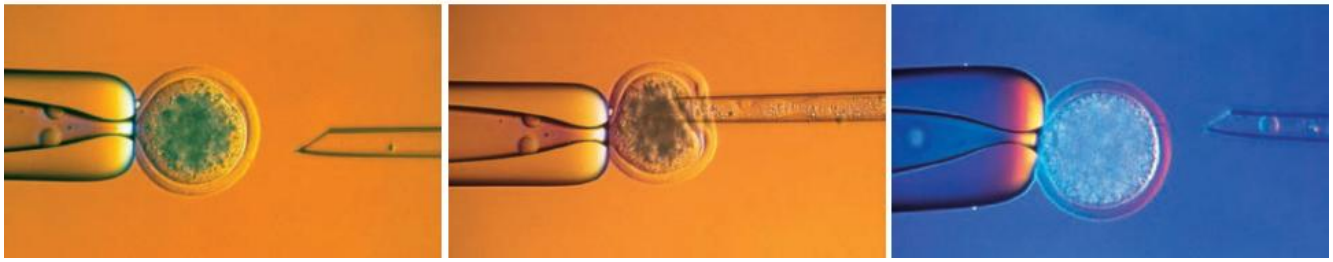


Figure 1.5 ▲
Three-dimensional view of a plasma membrane based on the fluid mosaic model

Specialised protein molecules are also embedded in the bilayer in various patterns, forming 'mosaics'. Some of these proteins can move laterally but others are fixed in position. Proteins and lipids can also flip around in the membrane. As a result of the work of SJ Singer and Garth Nicolson in the 1970s, the structure of the plasma membrane can be understood by using a '**fluid mosaic model**' (Figure 1.5).

It is the lipid components of all membranes, whether from plants, animals or bacteria, that provide membranes with the unique properties of being flexible and being able to repair themselves, change shape and grow. If the plasma membrane is punctured, some of the **cytoplasm** will leak out but the hole will quickly seal. Biotechnological procedures make use of this property when the inside of a cell needs to be accessed (Figure 1.6).

Figure 1.6 ▼
Puncturing and resealing the membrane of a cell. Microscopic sequence of removal of the nucleus of an egg



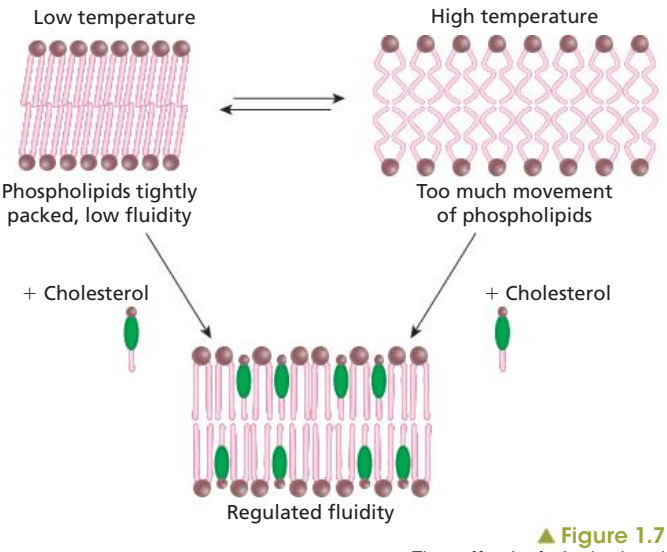
Sterols increase flexibility

Phospholipids alone do not provide the flexibility required in membranes. Strong inflexible bonds naturally form between the lipid tails. In animal cells, another type of lipid called **cholesterol** is interspersed among the phospholipid molecules. Cholesterol interferes with interactions between the lipid tails, making the membrane more flexible. In plants and bacteria, it is **phytosterol** (not cholesterol) that increases membrane flexibility.

Cholesterol has an interesting effect on membrane fluidity that depends on the temperature. At low temperatures, the phospholipid molecules in the membrane cluster together more closely as they do not have as much energy to move around. Fluidity is reduced because of this. When cholesterol is inserted between some of the phospholipid molecules, fluidity increases due to the greater distance between the molecules and their increased freedom of movement.

At high temperatures, there is more space between the phospholipid molecules because of their greater energy level. Fluidity is increased. When cholesterol is inserted between some of the phospholipid molecules in this case, the motion of the phospholipid molecule tails is reduced. This in turn decreases fluidity.

Cholesterol therefore regulates plasma membrane fluidity and acts as a buffer against fluctuations in temperature.



Adapted from: Marc Eeman & Magali Deleu, 'From biological membranes to biomimetic model membranes', Base [Online], no. 4, Vol 14 (2010), 719-736, fig. 4 URL : http://popups.ulg.ac.be/1780-4507/index.php?id=6568.

▲ **Figure 1.7** The effect of cholesterol at low and high temperatures

RECALL

- The plasma membrane forms a barrier between the internal and external environments of cells. Organelles in eukaryotic cells are surrounded by membranes.
- The plasma membrane is composed of a phospholipid bilayer. Hydrophobic tails are sandwiched between hydrophilic heads.
- The structure of the plasma membrane is explained by a fluid mosaic model.
- Cholesterol (animals) and phytosterol (plants and bacteria) increase membrane flexibility.

RECAP 1.1

1. List three functions of plasma membranes.
2. List three features of plasma membranes.
3. Recount why plasma membranes can be described by a fluid mosaic model.
4. Describe how cholesterol makes membranes more flexible.

Membrane proteins

Associated with each membrane is a set of membrane proteins that enable the membrane to carry out its distinctive activities. The types of proteins attached to a membrane vary depending on the cell type and its location. Even the two surfaces of the same bilayer, that is, the interior and exterior surfaces, differ considerably. There is also variety in the way proteins are associated with the membrane. Some proteins are bound only to the membrane surface whereas others are embedded in the phospholipid bilayer, with many penetrating from one side to the other (Figure 1.8). Surface proteins enable cell–cell interaction and communication and the exchange of substances with the external environment. Proteins on the external plasma membrane surface can be involved in signalling and communication between cells and can help to keep a cell anchored in its appropriate place. Proteins that span the membrane (called **transmembrane proteins**) can regulate the movement of substances across the membrane. For example, the concentration of ions, including sodium (Na^+), potassium (K^+), chloride (Cl^-) and calcium (Ca^{2+}), is very different from one side of the membrane to the other (Table 1.1). These differences are maintained by transmembrane ion pumps and ion channels. Some transmembrane proteins relay messages from one side of the membrane to the other when the molecule conveying the message cannot pass through the membrane.

Table 1.1 Comparison of the concentration of various ions inside and outside the cell

Type of ion	Extracellular concentration (mmol/L)	Intracellular concentration (mmol/L)
Sodium	145	15
Potassium	4.5	120
Chloride	116	20
Calcium	1.2	10

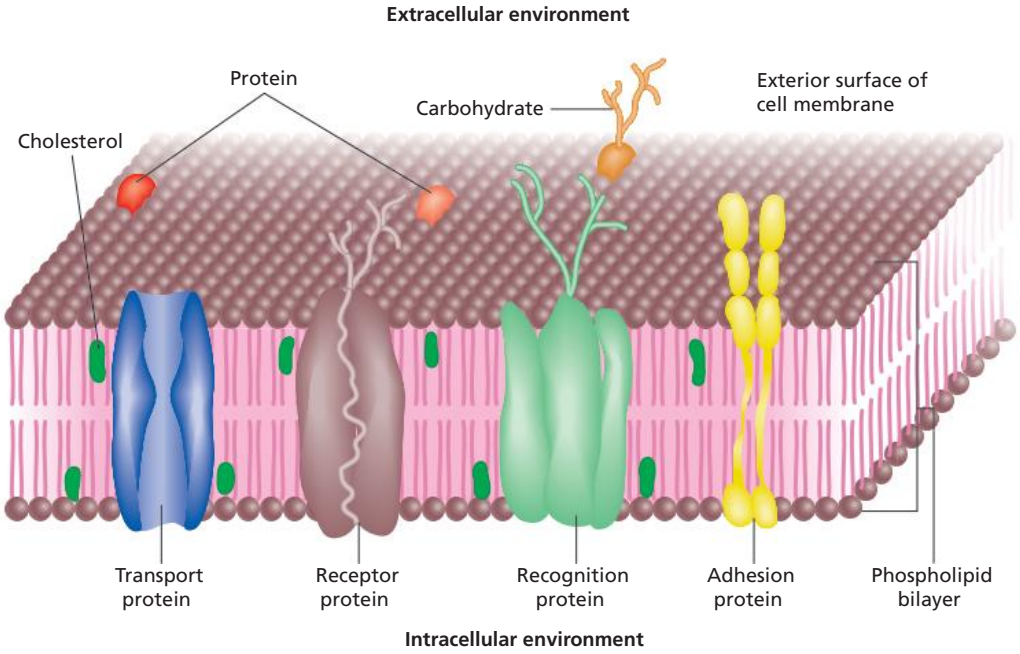


Figure 1.8 ▶ Examples of proteins associated with plasma membranes

Receptor proteins

Membrane proteins are essential for regulating cell behaviour and the organisation of cells in tissues. Proteins are also important for cellular communication. Some proteins, collectively called **receptors**, have receptor sites on their surface that detect molecules such as hormones. Each receptor is specific for a single molecule or a small number of molecules with a related structure to which the receptor binds. Molecules that bind to receptors are called **ligands** and they can include proteins, lipids, ions, carbohydrates – virtually all types of molecule. Each receptor-ligand pair is specific for each other and binds with a lock-and-key fit. Once they have bound their ligand, receptor proteins can become activated (or inactivated), usually by a change in their conformation. This may lead to various outcomes such as enzyme activation, ion channel opening or the availability of a new ligand binding site, and these outcomes can eventually control the transmission of messages within and between cells and change the behaviour of the cell.

Some membrane receptor proteins carry a carbohydrate molecule, giving them their collective name of **glycoproteins**. The addition of the carbohydrate group can give the receptor protein its particular function. It can also protect the protein core to increase the longevity of the protein in the rough extracellular environment.

Different types of cells have different receptor proteins, enabling them to carry out different functions. The specific set of receptor proteins that a cell carries is determined by the genes the cell expresses – since receptors are proteins, they are genetically encoded.

Chapter 4 discusses receptor proteins and cell signalling in more detail.

Recognition proteins

Membrane **recognition protein** molecules, called MHC proteins, act as markers that identify the cell as a normal body cell belonging to the individual. This is in contrast to a cell that has become cancerous, belongs to an invading micro-organism, or a transplant from an unrelated individual. For example, the immune system can recognise recognition proteins on 'self' cells and leave them be, but identify an invader by the **non-self** molecules it has and mount an immune response against it.

Each kind of organism has its own kind of recognition proteins (usually glycoproteins), and even different individuals of the same species can be distinguished by the proteins that they have on the surface of their cell membranes.

More detail on self-recognition and MHC proteins in the immune system is found in Chapters 5 and 6.

Adhesion proteins

In multicellular organisms, **adhesion proteins** link cells together to maintain both the three-dimensional structure and the normal functioning of tissues. Most adhesion proteins are distributed uniformly along the plasma membranes that contact other cells.

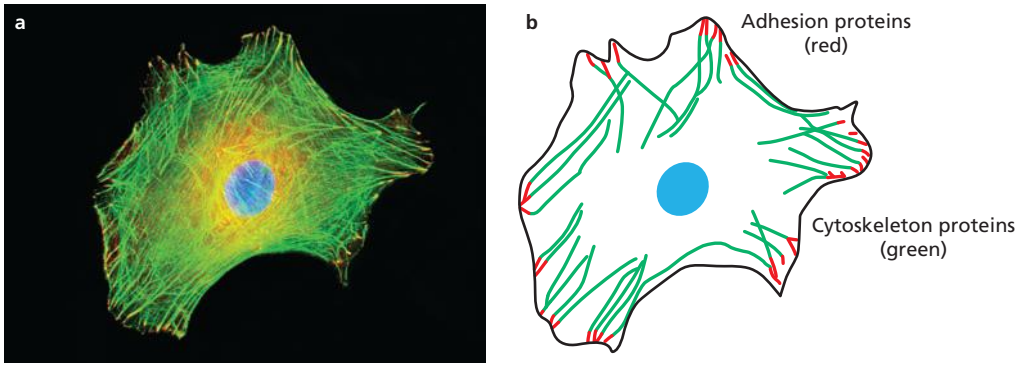


Figure 1.9 (**a**) A human lung cancer cell showing the cytoskeleton, adhesion proteins and anchor points (**b**) Corresponding schematic showing location of the nucleus, adhesion proteins (red) and cytoskeleton proteins (green)

The **cytosol**-facing end of these proteins is usually connected to parts of the internal **cytoskeleton**. The cytoskeleton is an internal skeleton of microtubules that extends throughout eukaryotic cells, giving them their shape and their ability to move and to arrange organelles (Figure 1.9). This helps to give the cell stability and to anchor the cell strongly in its environment.

Transport proteins

Lipid bilayers are impermeable to many substances, including **ions** and water-soluble molecules. **Transport proteins** allow movement of specific substances across a membrane, typically by forming a channel through it. Some transport proteins are open channels through which a substance moves on its own across a membrane. Others use energy to actively pump a substance across. The way substances move across membranes is important in cell signalling.

Cell signalling will be discussed in Chapter 4.

RECALL

- Embedded in the plasma membrane are a variety of membrane proteins that enable the membrane to carry out its distinctive activities.
- Membrane proteins allow cells to function appropriately, respond to chemical messages and recognise each other. They also link cells together and allow substances to move through the membrane.

RECAP 1.2

1 Name four types of membranes proteins.

2 State the functions of the different types of membrane proteins.

Movement through membranes

The internal environment of a cell is regarded to be all the material contained within its plasma membrane. **Extracellular fluid** bathes the outside of the plasma membrane, providing the liquid medium through which nutrients are supplied and wastes removed.

The plasma membrane enables the cell to create its own intracellular environment that is distinct and very different from the environment around it, the external environment. This boundary must be opened somehow so that the necessary exchange of substances can occur.

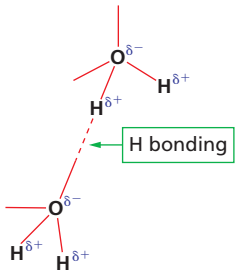
The plasma membrane is described as **selectively** or **differentially permeable** because it allows some substances through and not others. Small molecules can pass through very small openings or pores between the phospholipids, some molecules can be helped through, and some molecules are held back. The plasma membrane's permeability properties ensure that essential molecules such as glucose, amino acids and lipids enter the cell readily, necessary cell contents remain in the cell and waste compounds leave the cell. The selective permeability of the plasma membrane keeps the concentration of substances inside cells fairly constant, thus allowing the cell to maintain a constant internal environment.

Gases and small hydrophobic molecules diffuse directly across the phospholipid bilayer. Many molecules, however, are unable to move directly through. Size, charge and polarity matter.

Polarity

If we mix up a salad dressing before we pour it onto a salad, no matter how hard we shake the bottle, the oil component and aqueous (water-based) component always separate when we stop mixing. The oil sits on top and we end up with an oily dressing. An explanation for this observation lies with the chemical concept of **polarity**.

Water molecules are highly polar; the oxygen atom (one pole) of the water molecule has a negative charge and the hydrogen atoms (other pole) are positively charged. This results in the oxygen being attracted to the hydrogen atoms of a nearby water molecule and forming hydrogen bonds with it (Figure 1.10). A **hydrogen bond** is a weak intermolecular chemical bond between a hydrogen atom on one molecule and a second, more electronegative atom on another molecule or on a different part of the same molecule.



▲ **Figure 1.10** Water molecules form hydrogen bonds with each other. Each molecule forms hydrogen bonds with up to four neighbouring molecules.

Hydrogen bonding explains many of the properties of water that are essential for life. More substances dissolve in water than in any other liquid. This is due to the polarity of water molecules; it means that water molecules can interact with other charged particles, such as polar molecules or ions.

Polar molecules can form hydrogen bonds with water molecules (which are also polar) and so dissolve – they are called **hydrophilic** (water loving). However, non-polar substances will not dissolve in water because they cannot form hydrogen bonds with water molecules – they are **hydrophobic** (water-fearing).

Polar solvent (e.g. water) + polar solute → solution

Lipids are non-polar, or hydrophobic substances. They do not dissolve in water but they do dissolve in each other.

Non-polar solvent + non-polar solute → solution

Thus, for substances to form a solution, the rule is that 'like dissolves in like'. Most gases, such as oxygen and carbon dioxide, consist of very small molecules and dissolve in water because they fit between the water molecules in the spaces that are created by hydrogen bonding.

Movement of hydrophilic and hydrophobic substances across membranes

The plasma membrane bilayer can be thought of as a sandwich that surrounds the cell. The bread of the sandwich is made up of the hydrophilic heads. The non-polar hydrophobic tails of the lipid bilayer are like butter spread thickly between the pieces of the bread. The butter does not mix with water, which is a polar substance. Therefore, substances that dissolve in water cannot pass through the non-polar 'butter' of the membrane (Figure 1.11). However, molecules that are non-polar can pass through the membrane freely, as long as they are not too big.

Small polar molecules of substances such as water, ethanol and other small molecules pass through the plasma membrane, although they do so quite slowly. Simple **diffusion** of substances occurs when substances move from a region where

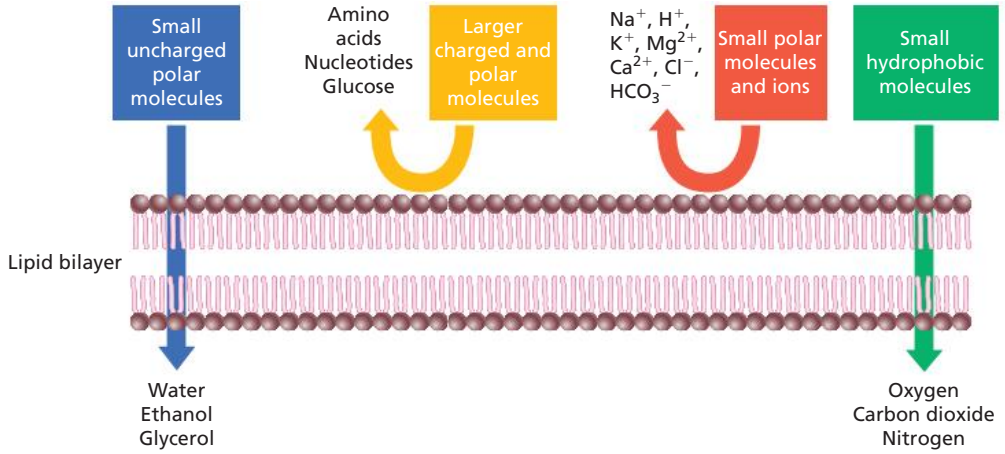


Figure 1.11 ▶
The relative permeability of a lipid bilayer to different classes of molecules

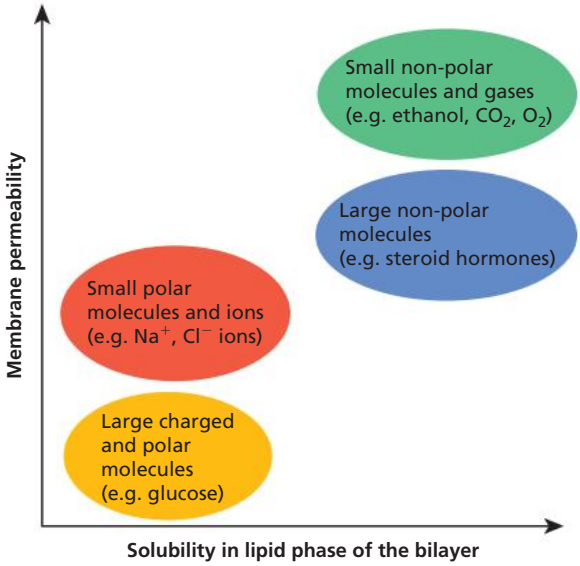


Figure 1.12 ▲
A graph showing the relationship between ability of a substance to move through the lipid phase of a membrane and the membrane's permeability to the substance

they are in a higher concentration to a region where they are in lower concentration. Small non-charged molecules such as carbon dioxide and oxygen are lipid-soluble and therefore can diffuse (dissolve) across the membrane rapidly. They are able to move between the hydrophilic heads of the phospholipids and pass through the hydrophobic tails of the membrane. The structure of the phospholipid bilayer makes it essentially impermeable to most water-soluble molecules, such as glucose and amino acids, and to charged molecules (ions). They cannot diffuse across the membrane because they are unable to enter the hydrophobic part of the lipid bilayer. Transport of hydrophilic molecules and ions across cellular membranes is assisted by transport proteins. Glucose molecules and various ions can move through channels in the membrane with assistance from transport proteins, but macromolecules cannot pass across the membrane because they are too large.

Other mechanisms move substances in bulk across the plasma membrane. **Exocytosis** involves fusion of the plasma membrane with small membrane-bound containers or sacs called **vesicles** that form inside the cytoplasm, expelling the contents of the vesicle into the extracellular environment. **Endocytosis** involves inward formation of a cavity or pouch of plasma membrane that encompasses some extracellular contents and then seals back on itself to form a vesicle in the cytoplasm containing the extracellular contents.

RECALL

- The phospholipid bilayer and embedded membrane proteins control movement of substances into and out of organelles and between the cell and its external environment.
- The plasma membrane is differentially (selectively) permeable – it allows some substances through and not others.
- The physical and chemical nature of a substance determines the way in which it will move across the plasma membranes – either freely or with assistance required.
- Polarity allows polar (hydrophilic) substances to dissolve in water (polar) but not in non-polar substances. Non-polar (hydrophobic) substances can dissolve in other non-polar substances.

RECAP 1.3

1 Distinguish between intracellular and extracellular.
2 Explain why the plasma membrane needs to be able to allow some substances to pass through it.
3 In terms of polarity, explain the expression 'like dissolves in like'.
4 Name two types of substances that can diffuse through lipid bilayers and two types of substances that cannot move directly through.

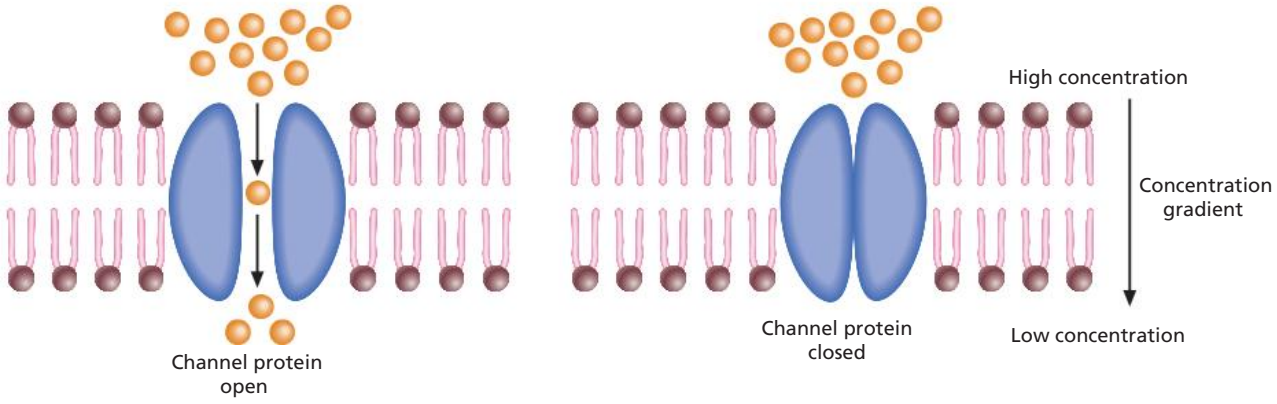
Membrane transport proteins

As seen in Figure 1.11, important large molecules such as glucose and amino acids are usually electrochemically charged and are too big to pass through the phospholipid layer directly. Even much smaller water-soluble (hydrophilic) compounds and charged particles such as sodium and chloride ions also fail to pass directly through the phospholipid layer of a membrane. How do these types of substances get through the membrane? The answer lies in channels made of transport proteins that act like gates to facilitate movement across the membrane.

Channel proteins

Channel proteins form narrow passageways through which small ions can diffuse rapidly down their concentration gradient from a high ion concentration to a lower ion concentration (Figure 1.13). Only ions of a specific size and shape can pass through a particular channel protein. Many types of channel proteins are also receptors that open only in response to a specific ligand (signal). Movement through channel proteins is a type of assisted movement across the membrane, called **facilitated diffusion**. Importantly, energy is not required for facilitated diffusion.

▼ **Figure 1.13** Facilitated diffusion through a channel protein in the plasma membrane of a cell. Movement is down the concentration gradient.



Facilitated diffusion

Carrier proteins (also known as transporters) bind to specific molecules on one side of the membrane. After binding their ligand, the protein changes shape and releases the substance on the other side (Figure 1.14). An example is the glucose transporter protein, which is located in the plasma membrane of all mammalian cell types and carries glucose in either direction, depending on the direction of the concentration gradient. Because of the required change in shape of the carrier protein, the rate of movement is lower than the rate of movement through channel proteins. Movement through carrier proteins is another example of facilitated diffusion.

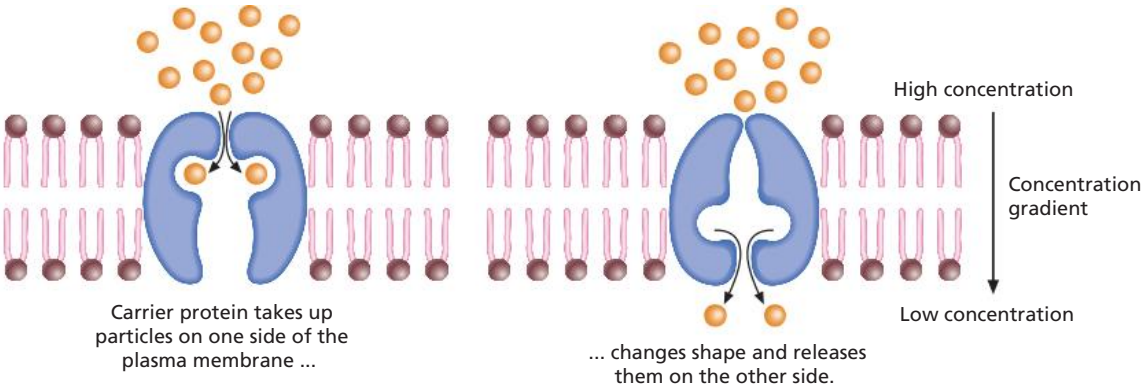


Figure 1.14 ▲
Facilitated diffusion using a carrier protein in the plasma membrane of a cell moves particles such as glucose down the concentration gradient.

ATP as a source of cellular energy is studied in more detail in Chapter 3.

Active transport

There are occasions when energy is needed to move substances across membranes. Carrier transport proteins use energy released from **adenosine triphosphate (ATP)** to power the movement of specific ions or small molecules against their chemical concentration or electric potential gradient from a region where they are in a low concentration to a region of higher concentration. As these **ATP-powered pumps** work in only one direction, they effectively act as one-way valves. An example of this occurs when ions move through the membrane of a nerve cell. This can cause a rapid change in the electric potential difference (the difference in positive and negative charges) across the membrane, and explains how the electrical charge of a nerve impulse is transmitted between nerve cells. The importance of these pumps becomes apparent when individuals, such as those suffering the disease cystic fibrosis, cannot produce them in adequate amounts. As the input of energy is required to move molecules or ions through a membrane against a concentration gradient, this movement is called **active transport**.

Figure 1.15 ▼
Active transport via a carrier protein in the plasma membrane of a cell. Energy is transferred to the carrier protein, enabling it to move the particles against a concentration gradient.

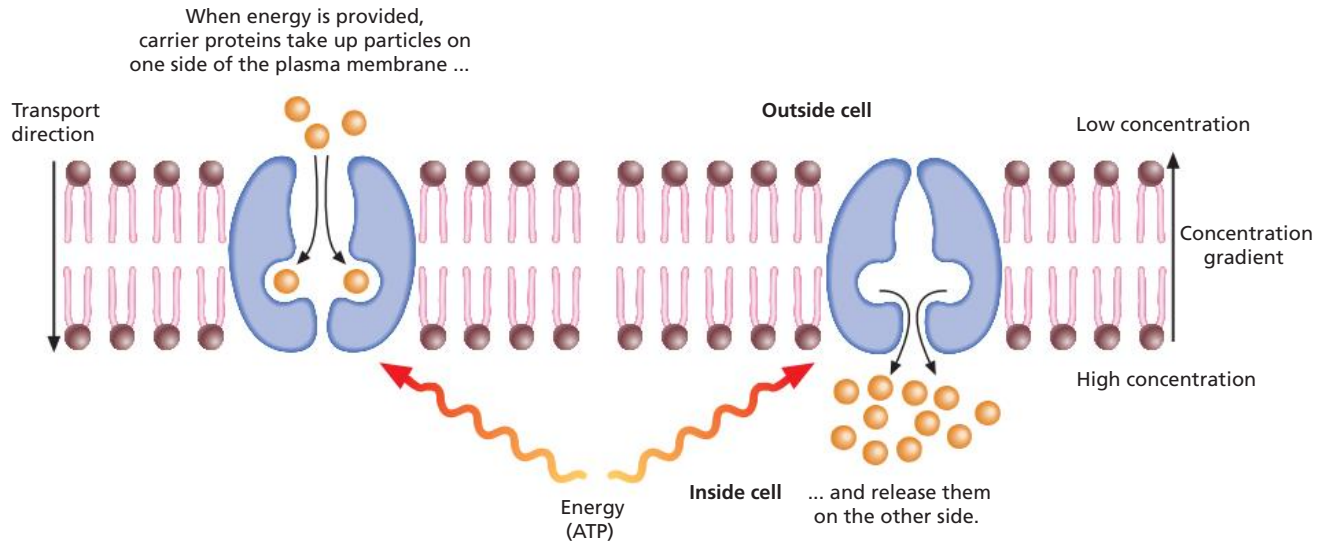


Table 1.2 Summary of types of movement of substances through plasma membranes

Type of movement	Movement through plasma membrane	Example
Simple diffusion	Through phospholipid molecules	Water, ethanol
Facilitated diffusion	Through channel proteins and carrier proteins	Ions, glucose
Active transport	Through carrier proteins	Ions

Membrane diversity

The internal environment of all cell types is not exactly the same. Different cell types require and produce different compounds. It is the selective permeability of the plasma membrane that allows the specific requirements of the cell to be met. Each type of cell contains a specific set of transport proteins that determine which ions or molecules can cross the membrane. There is a remarkable diversity between membranes. This diversity is due primarily to the different types and functions of the proteins present in each membrane.

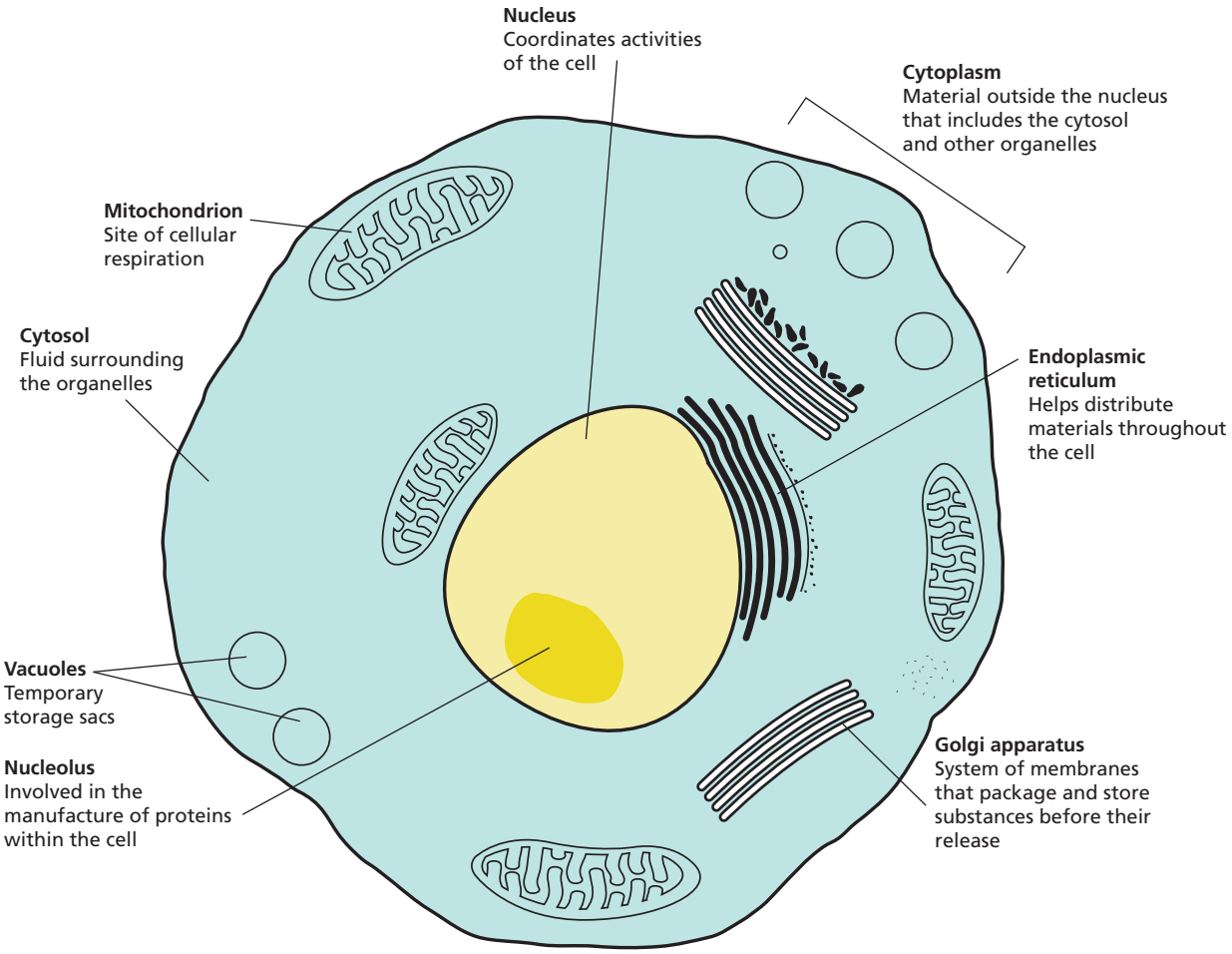
Not only do different cell types have different internal environments; within a cell, organelles often have a different internal environment compared to the surrounding cytosol. Eukaryotic cells contain several types of organelles, including mitochondria, chloroplasts, the endoplasmic reticulum, the Golgi apparatus and lysosomes. Each of these organelles performs a specific function critical to the cell's survival.

Cellular respiration and photosynthesis are studied in depth in Chapter 3.

Mitochondria are small, cigar-shaped organelles that are found in the cytosol of eukaryotic cells. Each mitochondrion consists of an outer smooth membrane and a highly folded inner membrane. The folds in the inner membrane, called **cristae**, protrude into the inner space of the mitochondrion. This membrane arrangement is important in the process of cellular respiration. Likewise, the outer and inner membrane of chloroplasts are arranged to facilitate the process of photosynthesis.

Organelles are separated from the rest of the cellular space by a membrane. The membranes that surround eukaryotic organelles are based on lipid bilayers that are similar (but not identical) to the cell's outer plasma membrane.

Like the plasma membrane, organelle membranes function to keep the inside 'in' and the outside 'out'. This partitioning permits different kinds of biochemical reactions to take place in different organelles. Because the lipid bilayer of organelle membranes is



▼ **Figure 1.16**
Eukaryotic cells, such as this animal cell, localise specific cell functions in membrane-bound organelles. They have different internal environments. The differences in their membrane proteins maintain these differences.

impermeable to most hydrophilic molecules, the membrane of each organelle must contain specific membrane transport proteins that are responsible for the import and export of necessary molecules unique to that organelle. The diversity of these proteins allows specific substances to move into and out of the organelle, thus maintaining the difference in their internal environment. Transport proteins in the lysosome membrane, for example, allow amino acids generated inside the lysosome to cross into the cytosol, where they can be used for the synthesis of new proteins. In plants, membrane proteins called **aquaporins** that facilitate the transport of water are found in the vacuole membrane.

Together, the total area of a cell's internal membranes (surrounding organelles) far exceeds that of its plasma membrane. In terms of its area and mass, the plasma membrane is only a minor membrane in most eukaryotic cells.

RECALL

- Transport proteins facilitate the movement of substances across membranes.
- Energy is not directly required for movement of substances through channel proteins and carrier proteins. Energy is required for movement of substances through ATP-powered pumps.
- The specific set of transport proteins determines which ions or molecules can cross a membrane.

RECAP 1.4

1. Create a table naming the three types of membrane transport proteins. In the table, describe their structure, the type of movement, and if energy is required for movement.
2. In what way can the permeability of a plasma membrane be likened to a sieve? In what way is this analogy insufficient to explain membrane permeability?
3. Explain how specific membrane transport proteins maintain the diversity of the internal environments of different cell organelles.

EXPERIMENT 1.1

SELECTIVE PERMEABILITY AND TEMPERATURE

Aim

To investigate the effect of temperature on the plasma membrane

Materials

- whole fresh beetroot
- mounted needle
- hot plate
- thermometer
- six small test tubes
- 10 mL measuring cylinder
- two 250 mL beakers
- fruit knife
- cork borer
- stopwatch

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Hot plates can burn.	Take care around hot plates. Place hot plates back from the edge of benches when not in use.

Procedure

1. Use a cork borer to cut cylinders of tissue from the beetroot.
2. Using a knife, cut the beetroot cylinders into discs 3 mm thick. You will need 36 discs.
3. Wash the discs in a beaker of water for 5 minutes, changing the water every minute or so.
4. Label the six test tubes as 30, 40, 50, 60, 70 and 80, and using a measuring cylinder place 6 mL of cold tap water in each.
5. Prepare a water bath by half filling a 250 mL beaker and heating it on a hot plate to 30°C, using a thermometer to test the temperature.
6. When the beetroot discs have been washed, thread six onto a mounted needle, leaving a 1 mm space between the discs. Plunge the discs into the hot water for exactly 1 minute.
7. Push discs off the needle and drop them into the tube labelled 30.
8. Increase the heat of the beaker to 40°C, repeating the procedure with another six discs of beetroot.
9. Keep repeating the procedure, raising the temperature by 10°C each time, until all of the discs have been used.
10. After leaving the discs in the test tubes for at least 20 minutes, shake the tubes and record the colours of the liquid, using a scale from 1–5, with 1 being a very pale shade of pink and 5 being a dark shade of red.

Results

Copy and complete the table to display your results.

Temperature (°C)	30	40	50	60	70	80
Colour						

Analysis of results

1. Describe the pattern of your results.

Discussion

1. What evidence do you have that the living membrane is selectively permeable?
2. Explain why it was necessary to wash the discs thoroughly before heating.
3. Suggest how high temperatures affect membranes.

Importing and exporting across membranes

At times, very large particles or even whole cells have to be moved into a cell across its plasma membrane. In other circumstances, relatively large molecules have to be exported from a cell. The large size of these particles makes their movement through the membrane by diffusion or active transport impossible.

Cells make very small containers or sacs (called vesicles) within them from the plasma membrane itself. These vesicles transport such things as solids or liquids across the membrane, outwards in the case of exocytosis and inwards in the case of endocytosis.

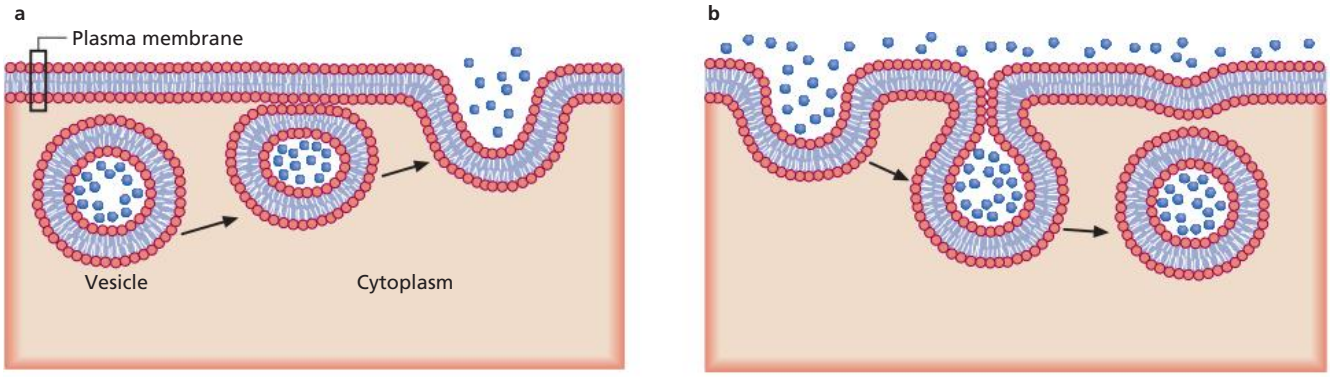


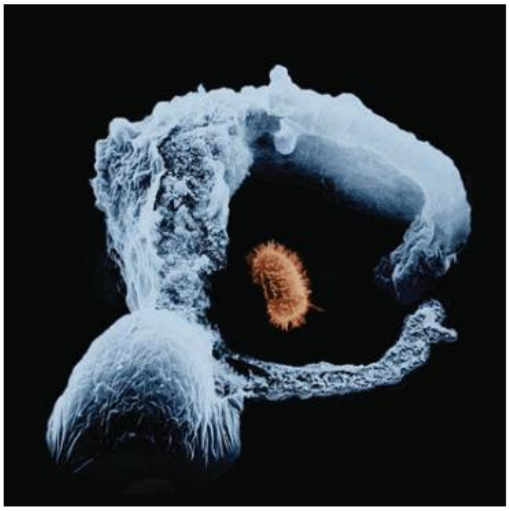
Figure 1.17 ▲
The process of (**a**) exocytosis and (**b**) endocytosis

During exocytosis, a small membrane-bound vesicle moves through the cytoplasm to the plasma membrane. The vesicle fuses with the plasma membrane and then releases its contents to the exterior of the cell (Figure 1.17a). During endocytosis, the plasma membrane is drawn inwards and engulfs particles and liquid droplets. It encloses the material within it to form an endocytotic vesicle, which then stores or transports the material within the cytoplasm (Figure 1.17b).

These cases of **bulk transport** are active processes, requiring energy to move vesicles around the cytoplasm and to change the shape of the cell.

Figure 1.18 shows a unicellular *Amoeba* feeding on a smaller organism, illustrating the process of endocytosis. The *Amoeba* changes shape by sending out projections that surround the prey. Membrane fusion occurs when the plasma membrane of the projections meet, resulting in the formation of a vesicle. Two types of endocytosis are named according to the type of material consumed. The process that engulfs solids, like an *Amoeba* feeding, is called **phagocytosis**, and the process that takes in liquid is **pinocytosis**.

Figure 1.18 ▼
A scanning electron micrograph of an *Amoeba* surrounding its prey (*Tetrahymena*) for ingestion

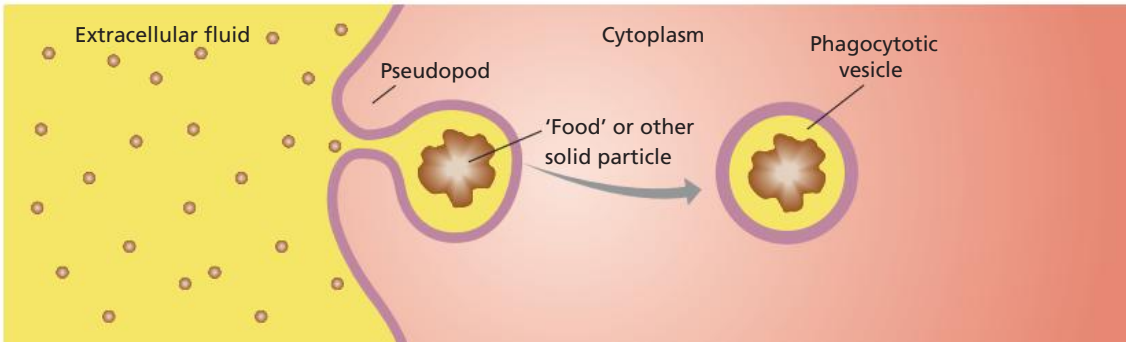


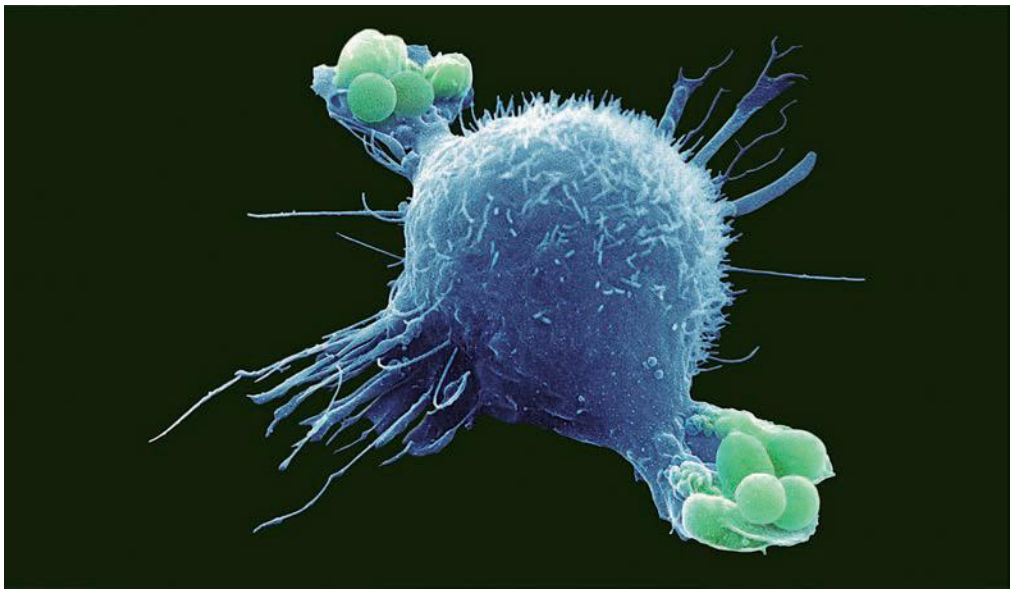
amanaimages/Visuals Unlimited

Human macrophages (a type of white blood cell) are called phagocytes because, in defending the body against disease, they engulf bacteria by phagocytosis (Figure 1.20). Macrophages use recognition proteins in the plasma membrane of the cells they encounter to discriminate between invading bacteria and body cells, demonstrating that phagocytosis is a selective process.

Pinocytosis occurs when the plasma membrane engulfs extracellular fluid in much the same way as phagocytosis. Fat droplets found in the small intestine after a meal move into cells by means of pinocytosis.

Figure 1.19 ▶
The process of phagocytosis





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◀ Figure 1.20
A macrophage engulfing cells by phagocytosis

ACTIVITY 1.1

MODELLING ENDOCYTOSIS AND EXOCYTOSIS

Aim

To enhance your understanding of movements of plasma membranes during endocytosis and exocytosis

You will need

- small object like a coin or eraser
- two pieces of string, one 1 m long and the other 30 cm long

What to do

1. Form the outline of a cell using both pieces of string end to end: do not join the pieces of string.
2. Place the coin near the outer side of the short piece.
3. Use the shorter piece of string to form an endocytotic vesicle around the coin.
4. Move the vesicle into the cytoplasm, closing the plasma membrane behind it.
5. To simulate exocytosis, carry out these directions in reverse.

What did you discover?

1. What happened to the amount of plasma membrane when the model cell carried out endocytosis?
2. Predict what action the cell must take to regulate the amount of plasma membrane when it carries out endocytosis.

RECALL

- Exocytosis and endocytosis move very large particles across a plasma membrane.
- In exocytosis a cytoplasmic vesicle fuses with the plasma membrane, releasing the vesicle contents outside the cell.
- In endocytosis a small patch of the plasma membrane sinks inward and seals back on itself, forming a vesicle inside the cytoplasm.

RECAP 1.5

1 Outline one similarity and one difference between endocytosis and exocytosis.

2 Distinguish between endocytosis, phagocytosis and pinocytosis.

Organelles in action

The plasma membrane is an essential component of cells, holding the contents of cells together. It does not function in isolation and its activities are directly linked with those of other organelles. Within the cell, various organelles work in collaboration to move substances from one part of the cell to another and prepare other substances for export from the cell. Through observations and experiments such as labelling protein cell products with fluorescent dyes or tracking radioactively labelled atoms, specialised cell organelles that facilitate transport have been identified. To understand the role played by various cell compartments we will follow the journey of protein molecules in the **secretory pathway**.

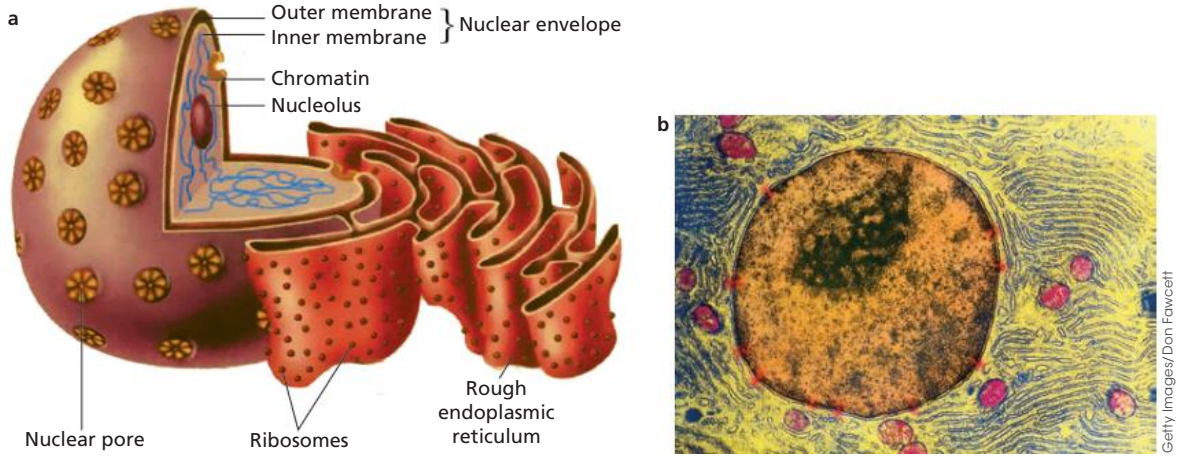
Proteins are the workhorses of the cells. They are important in structures and in moving molecules across the plasma membrane. Eukaryotic cells have elaborate mechanisms to assemble, package and transport proteins within the cell.

The nucleus controls activities in the cell

The **nucleus** is one of the most prominent structures in the eukaryotic cell. It is the information centre of the cell and it controls all activities of the cell because it controls the production of proteins. The code that directs all activities of the cell is found in the nucleic acid, DNA, in the **chromosomes**.

The nucleus is contained within a double membrane called the **nuclear envelope**. The outer membrane is continuous with the endoplasmic reticulum (ER), another membranous organelle that helps distribute materials throughout cells. The nuclear envelope contains numerous openings, called **nuclear pores**. These pores are channels through which water-soluble molecules can move between the nucleus and the cytoplasm.

Figure 1.21 ▼
(**a**) The features of the nucleus (**b**) Transmission electron micrograph of the nucleus of a pancreas acinar cell



McGraw-Hill Botany Visual Resources Library, adapted

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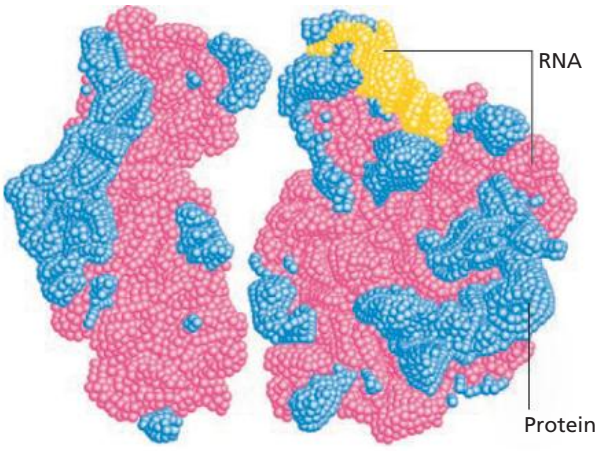
Structures known as **nucleoli** are present in the nucleus. These are responsible for the synthesis of ribosomal RNA (rRNA) and the assembly of **ribosomes** from rRNA and proteins. **Messenger RNA (mRNA)** molecules carry the coded instructions for assembling proteins, which is catalysed by ribosomes.

Ribosomes synthesise proteins

Ribosomes are extremely small organelles that exist free-floating in the cytosol of cells or attached to membranes of the endoplasmic reticulum. They are abundant in cells, reflecting the work that they do in producing a constant supply of different kinds of proteins.



▲ **Figure 1.22** Ribosomes are composed of strands of RNA and proteins. The two subunits of the ribosome lock around the messenger RNA (mRNA) molecule.

Ribosomes consist of two subunits that are composed of the nucleic acid RNA and various proteins (Figure 1.22). In eukaryote cells, **ribosomal RNA (rRNA)** molecules are synthesised in the nucleolus. They pass through the nuclear pores into the cytosol. There they begin the process of synthesising **polypeptides** from **amino acids** according to the instructions given by mRNA copied from DNA within the nucleus.

Cells with high rates of protein synthesis have prominent nucleoli and many ribosomes. For example, the liver is the industrial centre of the body so it is not surprising that each liver cell has a few million ribosomes.

Endoplasmic reticulum

How do proteins produced in ribosomes move to other parts of the cell and towards the membrane for export? The **endoplasmic reticulum (ER)** is an interconnecting system of thin membrane sheets dividing the cytoplasm into compartments and channels. The membrane of the ER is able to pinch off into small vesicles and deliver proteins to all parts within the cell. The ER is therefore an **intracellular** transport system.

▼ **Figure 1.23** Rough ER studded with ribosomes

Most of the ER in cells is studded with ribosomes and thus is known as **rough endoplasmic reticulum (rough ER)**. Proteins produced by ribosomes attached to the rough ER can move directly into the ER lumen (internal space) and move about the cell in vesicles that bud off from the ER. Proteins produced in the rough ER can also be exported, transported to the extracellular side of the plasma membrane, or secreted into other cells. Such proteins include enzymes and hormones. Therefore, the ER is also an **intercellular** transport system, helping to move proteins from one cell to another. Only proteins that are destined to remain embedded in an organelle membrane or the plasma membrane, or be exported from the cell, are produced by ribosomes on the rough ER. Cytoplasmic proteins are synthesised by free ribosomes in the cytosol.

In certain parts of some cells, the ER has no ribosomes attached to it and is known as **smooth endoplasmic reticulum (smooth ER)**. The amount and function of this smooth ER depends on the type of cell it is located in. Its main role is to transport proteins, synthesise lipids and assist in the manufacture of plasma membranes. In liver cells it also detoxifies drugs, and in adrenal cortical cells it produces steroid hormones. Some carbohydrates are produced on smooth ER. It is also a place for storage of calcium ions, which are necessary for muscle contraction and interactions between some membrane proteins.

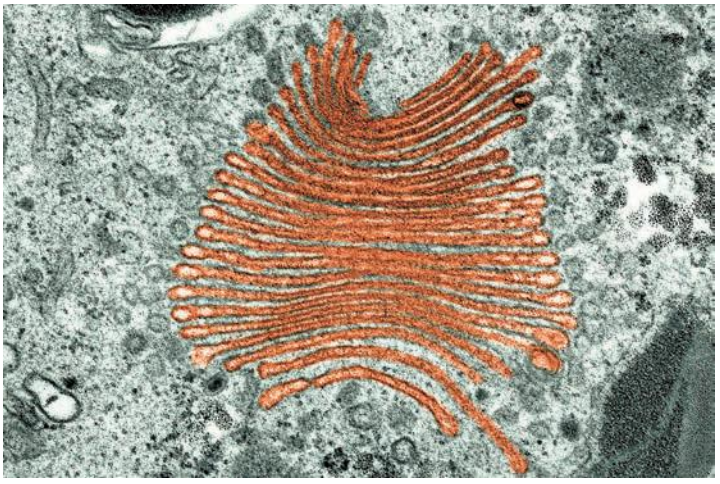


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Figure 1.24 ▲
Electron micrograph of the Golgi apparatus (magnification ×80000)

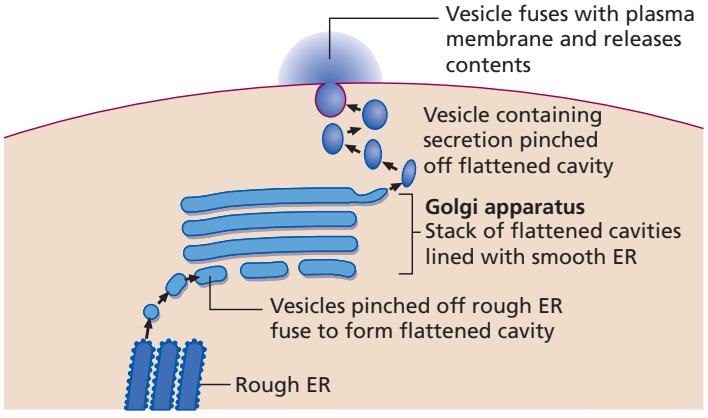

Figure 1.25 ▲
The secretory pathway

Golgi apparatus

Consider a grass-eating animal such as a kangaroo. The cells in grass have a tough cell wall. In order to be able to digest and absorb the nutrients from inside the grass cell, the cell wall must be broken down by enzymes. Cells in the digestive glands of the kangaroo produce such enzymes. Being protein, the digestive enzyme is produced initially by the ribosomes on the rough ER. It moves through the channels within the ER where it is secreted within the cytoplasm of the cell. From there it moves into the **Golgi apparatus** (also known as Golgi body), where different enzymes put the final touches to it and it is packaged and stored before being secreted from the cell to move into the intestines of the kangaroo. This is where it can begin its work of digesting the cellulose in the cell wall of the grass.

The Golgi apparatus consists of a system of membranes within the cytoplasm. Parts of the Golgi apparatus membrane are able to pinch off into small vesicles. These vesicles can move to the plasma membrane, where they join to the membrane and discharge their contents to the outside of the cell.

Golgi apparatus are more numerous in cells that have a secretory function, such as pancreatic cells, which produce digestive enzymes and the hormone insulin.

Movement out of the cell

Exocytic vesicles are associated with transporting large molecules and particles across the plasma membrane and out of the cell. During exocytosis, a small membrane-bound vesicle moves through the cytoplasm to the plasma membrane, where it fuses with the membrane and releases its contents to the exterior of the cell (Figure 1.26).

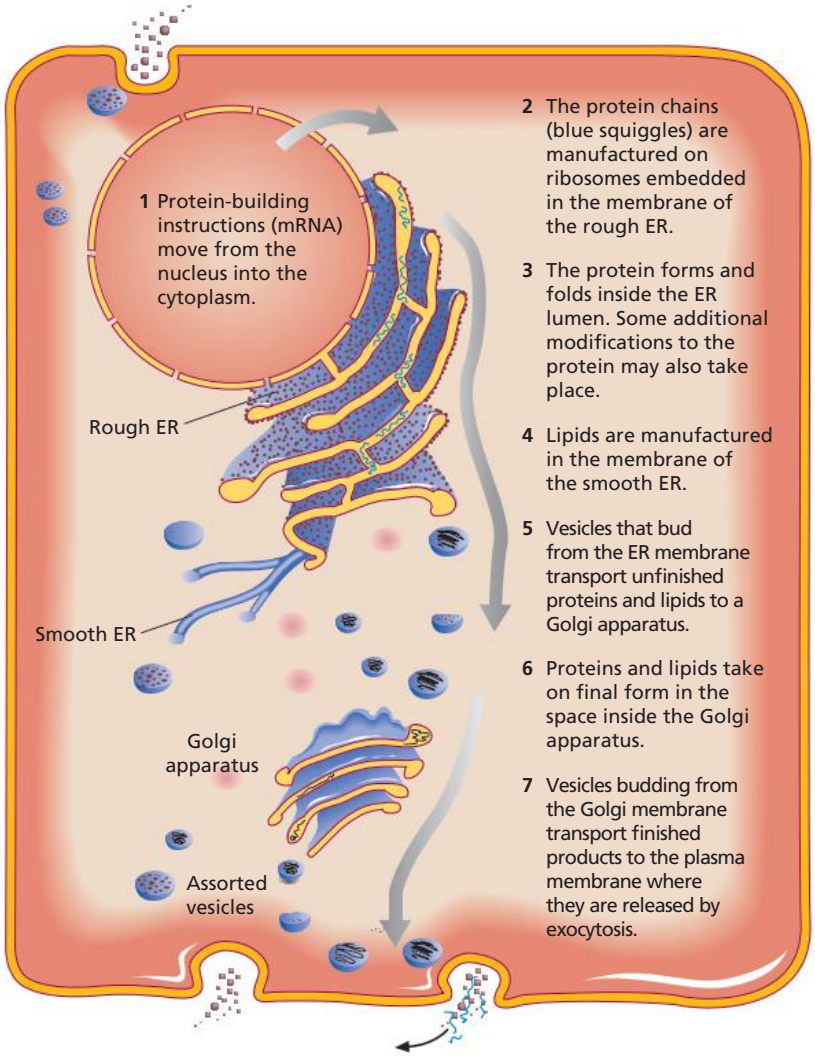

Figure 1.26
Secretory pathways; includes details of the production, transport and secretion of proteins in cells

RECALL

- In the secretory pathway, cellular products move through a cellular transport system, are packaged and are then released from the cell through the plasma membrane.
- Organelles involved in the secretory pathway include the nucleus, ribosomes, endoplasmic reticulum, Golgi apparatus and the plasma membrane.

RECAP 1.6

1. Explain the role of the nucleus in the secretory pathway.
2. Describe the structure and function of ribosomes.
3. Describe what the endoplasmic reticulum and Golgi apparatus have in common and describe how they differ functionally.
4. Distinguish between rough ER and smooth ER.

Biological knowledge and society: An issue in the fish oil industry

Children whose diets lack vitamin D and who do not get enough sunshine develop a bone disease called rickets. Chemists in the 1920s developed methods of extracting vitamins from cod liver oil and the production of synthetic vitamin D tablets began. These methods could be patented and the tablets could be mass produced, generating large profits for companies.

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Figure 1.27 ▲
(**a**) A spoonful of cod liver oil provided essential nutrients for developing children in the 1930s. (**b**) Fish oil tablets are now one of the biggest markets in the dietary supplement industry.

Discovering the role of omega-3 essential fatty acids in the diet

In 1929 George and Mildred Burr fed rats a special diet of purified protein and sugar. The diet was devoid of fats and oils and their rats became sick. However, when they introduced omega-3 fatty acids to the diet, the rats recovered. They discovered fatty acids were essential for rat diets. Fatty acids were later discovered to also be essential for infants, when babies being fed skim milk developed a deficiency disease. Essential fatty acids are now added to infant formula. Omega-3 fatty acids are required for healthy cell membranes, particularly membranes of neurons developing in the brain. They are long lipids that have a kink in them; their incorporation into plasma membranes increases membrane fluidity, which enhances membrane integrity and facilitates the movement of substances across the membrane (Figure 1.7). They are also required for synthesis of a number of hormones involved in the immune response. There are three types of omega-3 fatty acid: linolenic acid (ALA) found in plant oils, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in marine oils. DHA is essential for infants.

Discovering health benefits of omega-3 fatty acids

In the 1970s Bang and Dyerberg travelled to Greenland to conduct a study of Inuit people who had a low incidence of heart disease. They tested the blood of 170 Inuit and found a high concentration of omega-3 fatty acids. These fatty acids were not in high concentrations in the blood of a control population of Inuit that had moved to Denmark and changed their diet. They proposed that eating oily fish correlated with protection against heart disease. Since this time, omega-3 fatty acids have been found to be associated with a number of health benefits in the areas of mental health, rheumatoid arthritis, autism, cancer, stroke and Alzheimer's disease. Many of these correlations arise from the results of case studies rather than randomised controlled clinical trials. However, on the basis of such associations, omega-3 fatty acids, especially DHA, came to be included in dietary recommendations for adults. In response, companies began producing omega-3 dietary supplements and fish farms concentrated on producing fish with high omega-3 fatty acid content.

Producing fish oil dietary supplements

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Getty Images/Visuals Unlimited, Inc./Patrick Endres

Figure 1.28 ▲
(**a**) Foraging fish swim in schools, providing protection in numbers from predators such as dolphins. (**b**) Purse seine fishing takes advantage of this behaviour.

Omega-3 fatty acids originate in algae and pass along the food chain to foraging fish and then to predator fish like salmon, tuna and shark. While these predatory fish have higher omega-3 levels in their fat, they also accumulate higher levels of harmful mercury. The most common source of fish for producing dietary supplements are foraging fish such as herring, mackerel, anchovies and menhaden, which feed on plankton and algae and have lower mercury levels. These fish forage in schools, making it easy to catch vast numbers in nets. Planes spot schools of fish and alert fishing ships to move into the area. A wall of netting is cast around the school. A pump is then placed into the net and fish are vacuumed into the hull. Predator fish feeding on the school or young predator fish that sometimes travel with the school also end up in the hulls of these fishing boats.

Demand increases and overfishing emerges

Fish oil is a multibillion dollar business. It is used in feed for fish farming, to make dietary supplements and to improve the shelf life of many food products. While supplements are still the smallest part of the fish oil market, sales topped $1 billion in the US in 2011 and over $700 million in the UK, ranking them one of the most popular dietary supplements taken in these countries. This increases the strain on ocean ecosystems. Overfishing of foragers can result in predators starving, including fish, birds and marine mammals that rely on foraging fish. In many highly fished areas, dead zones have emerged as foraging fish no longer filter out enough of the algae.

New studies raise questions about the health benefits of fish oil supplements

Not only is overfishing an issue but new studies are unveiling concerns relating to the health benefits of omega-3 oils (Table 1.3).

Table 1.3 Outlines of recent studies and concerns of lobby groups questioning the efficacy and safety of fish oil supplements

Authors and year or recently formed lobby group	Study conducted or concern raised	What was revealed
Kwak *et al.* (2012)	Meta-analysis of approximately 1007 studies of the effect of omega-3 supplements on preventing cardiovascular events. Of the 1007 studies, identified valid data drawn from 14 randomised blind (placebo-controlled) trials, providing cumulative data from 20 484 patients with a history of heart disease.	Omega-3 fatty acid supplements did not reduce the overall risk of cardiovascular events or stroke. Further clinical studies required over a longer time period to determine if supplements reduce risk.
Sydenham, Dangour and Lim (2012)	Systematic review of three randomised placebo-controlled trials. Pre and post cognitive data for 3536 cognitively healthy older participants who either received omega-3 supplements or placebo sunflower oil for 6 or 24 months.	Direct evidence of the effect of omega-3 on incident dementia was not found. Trials showed no benefit of omega-3 supplement on cognitive function in cognitively healthy older people. Western diets may have enough omega-3 so the effect of supplements may not be noticed.
Benson Chiles and Chris Manthey, co-founders of FishOilSafety.com, are environmentalists who work to promote sustainable fishery	Mounted a court case against fish oil supplement companies regarding labelling of fish oil products that have been found to contain varying levels of the pollutants polychlorinated biphenyl (PCB) and dioxins. Both are recognised as cancer-causing agents and are known to interfere with reproductive success.	While studies confirm there are varying amounts of pollutants in fish oil supplements, it is generally accepted that the levels in farmed fish fed on fish oil supplements are higher. This varies depending upon the feed given to farmed fish. Wild caught fish are considered to have the lowest levels of PCBs.
Brasky *et al.* (2013)	A randomised placebo-controlled trial with 35 500 participants to test whether selenium and vitamin E reduce risk of prostate cancer. Within this study a case–cohort side study was carried out to examine association between blood plasma fatty acids and prostate cancer risk among participants. The cohort consisted of 834 men diagnosed with prostate cancer and a comparison group of 1393 men selected randomly from the 35 500 participants in the original study.	Reported an increased prostate cancer risk among men with high blood concentrations of omega-3 fatty acids. Suggested that these fatty acids could be involved in prostate tumour development. In response a number of scientists have cautioned against the validity of these findings as the study was not a randomised controlled study for omega-3. A clinical trial would be required to test this correlation for causation.

Over to you

1. What are the issues?
2. Identify the relevant biology that relates to these issues.
3. 'A little learning is a dangerous thing.' Write a 500 word argumentative article for a general audience on this theme, discussing the interrelationship between the scientific knowledge of omega-3 fatty acids in the diet (including the reliability of the science) and how this has impacted upon society and the environment.

References

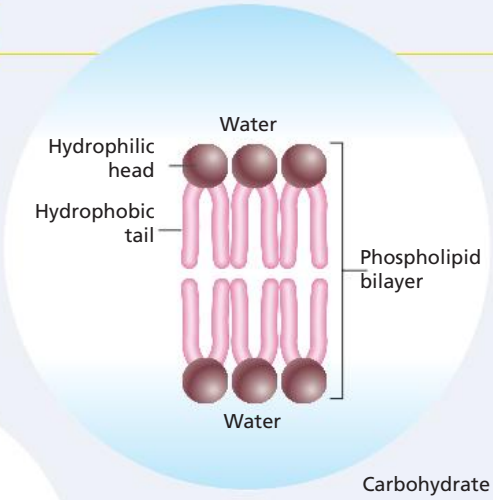
Brasky, T., Darke, A., Song, X., Tangen, C., Goodman, P., Thompson, I., Meyskens, F., Goodman, G., Minasian, L., Parnes, H., Klein, E., and Kristal, A. (2013). Plasma phospholipid fatty acids and prostate cancer risk in the SELECT trial. *Journal of the National Cancer Institute.* 105(15): 1132–1141.
Kwak, S., Myung, S., Lee, Y., and Seo, H. (2012). Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Internal Medicine.* 172(9): 686–694.
Sydenham, E., Dangour, A., and Lim, W. (2012) Omega 3 fatty acid for the prevention of cognitive decline and dementia. Cochrane Database of Systematic Reviews. 6: 1469–1493, retrieved 31 June, 2015 from http://researchonline.lshtm.ac.uk/21084/1/CD005379.pdf.

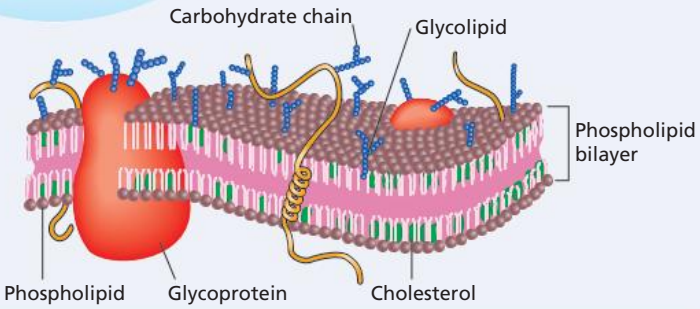
CONCEPT SUMMARY

How do cells maintain life?

Phospholipid bilayer

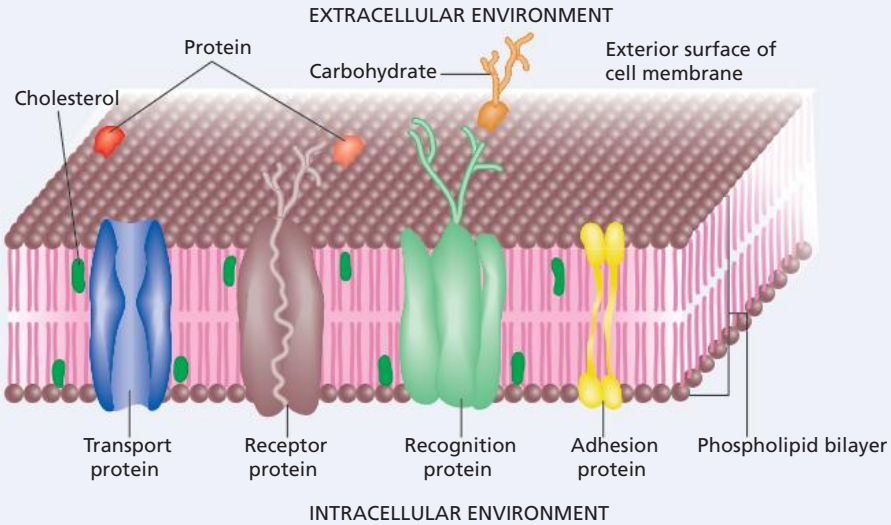
- This flexible structure allows the cell to grow, expand, contract, break and reassemble
- The two-dimensional fluid is made up of a double layer of phospholipid molecules.





The fluid mosaic model

- The phospholipid head is dissolvable in water, whereas the hydrophobic tails are forced to face inwards towards each other.
- Individual phospholipid molecules are highly mobile within the membrane.
- Specialised protein molecules are embedded in the bilayer: transmembrane proteins, receptors/ligands, glycoproteins, proteins for recognition, adhesion and transport.
- Cholesterol (animals) and phytosterol (plants) increase membrane flexibility.



Polarity: like dissolves like

Hydrophilic solutions: Polar solvent + polar solute $\longrightarrow$ solution

Hydrophobic solutions: Non-polar solvent + non-polar solute $\longrightarrow$ solution

Movement of substances through membranes

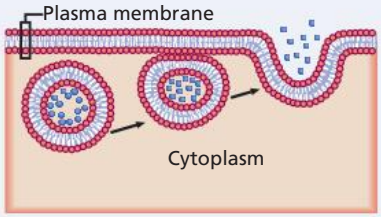
The plasma membrane is differentially permeable. Size, charge and polarity affect permeability.

Transport across membranes

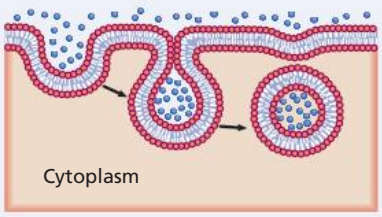
- Simple diffusion: Occurs through phospholipid molecules, such as water and ethanol
- Facilitated diffusion: Occurs through channel proteins and carrier proteins, such as ions and glucose
- Active transport: Occurs through carrier proteins, such as ions; requires ATP

Bulk transport

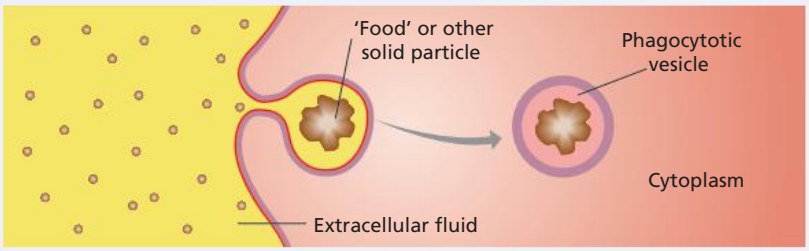
- Exocytosis
- Endocytosis – includes phagocytosis (solids) and pinocytosis (liquids)



Exocytosis

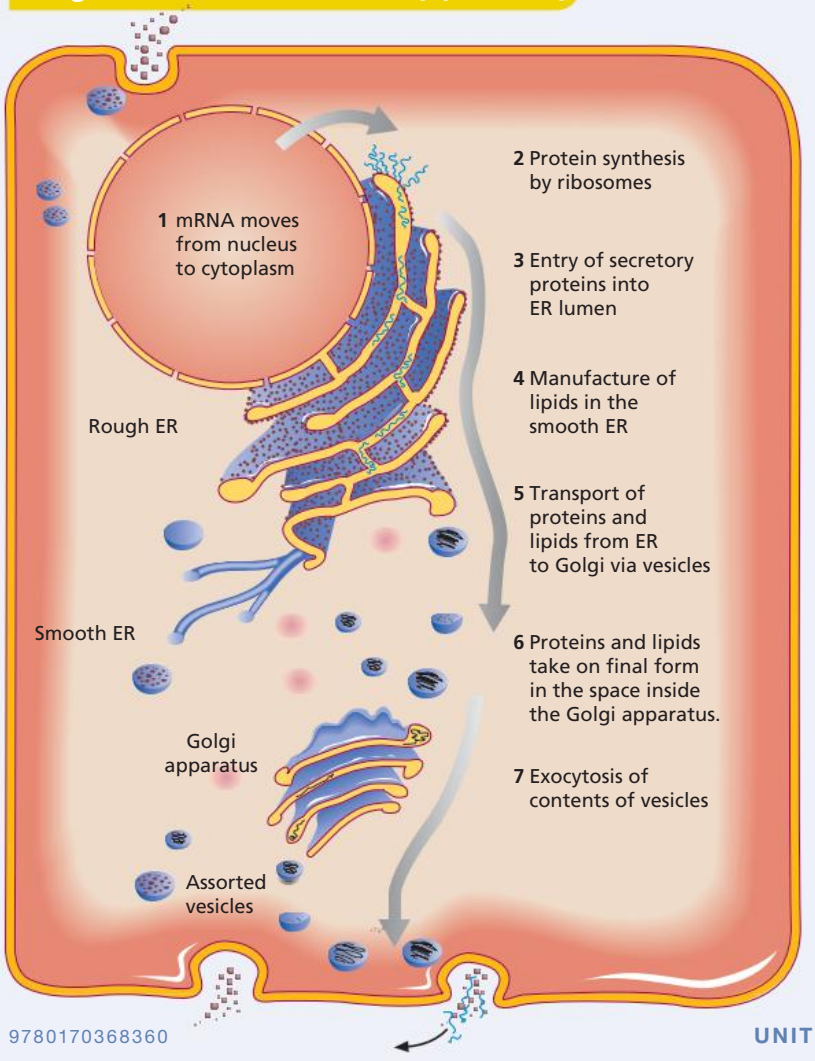


Endocytosis



Phagocytosis

Organelles in the secretory pathway



- The nucleus controls activities in the cell. It consists of chromosomes, nuclear envelope, nuclear pores and nucleoli.
- Ribosomes (free-floating or embedded in ER membrane) synthesise proteins (polypeptide chains) from amino acids.
- The endoplasmic reticulum is an intracellular and intercellular transport system.
  - Rough ER contains ribosomes embedded in its membrane. These produce polypeptides that are destined to be transmembrane or secreted proteins.
  - Smooth ER is the site of production of steroid hormones and lipids, detoxification of toxins, production of some carbohydrates and storage of calcium ions.
- The Golgi apparatus is a system of membranes that are able to pinch off into small vesicles, harbouring machinery to modify proteins and lipids in the secretory pathway.
- Cellular products are released from the secretory pathway by exocytosis.

CHAPTER GLOSSARY

active transport the process whereby cells actively transport substances across a membrane against a concentration gradient (from a low concentration to higher concentration of the substance); consumes energy

adenosine triphosphate (ATP) a high-energy compound composed of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

adhesion proteins proteins on the surface of cells that are involved in binding with other cells or to an extracellular matrix in a process called cell adhesion

amino acid a nitrogen-containing compound that is the building block of proteins

aquaporin a type of membrane protein that facilitates the transport of water

ATP-powered pump a type of membrane protein that uses the energy from ATP to move molecules across the membrane against a concentration gradient

bulk transport the transport of large quantities of materials into or out of the cytoplasm all at the one time

carrier protein a protein within a membrane that assists other molecules to cross the membrane in facilitated and active transport

channel protein a protein that forms a channel within a membrane to allow the passage of substances across the membrane

cholesterol a type of lipid found in animal cell membranes that stabilises membrane fluidity

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

cristae the folding of the inner membrane into the matrix of a mitochondrion, thus increasing the total surface area of the inner membrane

cytoplasm the material outside the nucleus that includes the cytosol and other organelles

cytoskeleton a network of filaments within a eukaryotic cell that provides structural support, anchorage, shape, motility and the capacity to move and arrange organelles within the cell

cytosol the fluid part of the cytoplasm surrounding the organelles

differentially permeable describes a membrane that allows some substances but not others to pass across it

diffusion the passive movement of molecules from a high to a low concentration of that substance

endocytosis the movement of solids or liquids into a cell from the environment via vesicle formation

endoplasmic reticulum (ER) an organelle in eukaryotic cells consisting of an interconnecting system of thin membrane sheets

exocytosis the movement of solids or liquids from a cell to the environment via vesicle fusion with the plasma membrane

extracellular fluid all fluid outside the cells in multicellular organisms

facilitated diffusion a form of diffusion that requires a substance to be attached to a specific carrier molecule to move across a membrane

fluid mosaic model a model that explains the structure and function of the plasma membrane

glycoprotein a protein with an attached carbohydrate group

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

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

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

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

intercellular occurring between cells

intracellular occurring within a cell or cells

ion atom or molecule with an overall positive or negative charge

ligand a molecule that binds to a receptor

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

non-self a molecule that is not recognised by the immune system as being part of the organism itself

nuclear envelope the double membrane that surrounds the nucleus in eukaryotic cells and separates DNA from the cytosol

nuclear pore an opening in the nuclear envelope

nucleolus a granular structure within the nucleus where ribosomal RNA is transcribed and ribosome subunits are assembled

nucleus a membrane-bound compartment in eukaryotic cells that contains the chromosomal DNA

phagocytosis the bulk transport of solids into a cell inside a vesicle

phospholipid a lipid molecule that has a hydrophilic phosphate group 'head' and hydrophobic lipid 'tail'

phospholipid bilayer the two layers of phospholipids that form a plasma membrane

phytosterol a type of lipid found in plant cell membranes

pinocytosis the bulk transport of liquids into a cell inside a vesicle

plasma membrane the insoluble boundary of the living cell that maintains the contents of the cell and regulates the movement of substances into and out of the cell. All cells have a plasma membrane

polarity a term that refers to a molecule having distinct regions of opposite charge

polypeptide a linear polymer built from amino acid monomers

receptor a molecule on the surface or interior of the cell that binds specifically to a substance (ligand) to detect or receive a stimulus

recognition protein a protein that acts as a marker on membranes

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

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

rough endoplasmic reticulum (rough ER) ER with ribosomes attached

secretory pathway the movement of proteins produced by ribosomes attached to the ER, through a series of compartments and vesicles, via the Golgi apparatus, to secretory vesicles for export from the cell by exocytosis

selectively permeable see **differentially permeable**

smooth endoplasmic reticulum (smooth ER) ER with no ribosomes attached

transmembrane protein a protein with one or more regions that span the membrane

transport protein a protein that carries molecules across membranes

vesicle a small, membrane-bound sac in the cytoplasm that transports, stores or digests substances

CHAPTER REVIEW QUESTIONS

Remembering

1 Name the molecules responsible for each of the following features of the plasma membrane:
   - **a** ability to change shape
   - **b** flexibility
   - **c** ability to reseal a puncture

2 Explain why a plasma membrane is described as a 'lipid bilayer'.

3 Name the four types of membrane proteins and construct a table that summarises their functions.

4 Define 'hydrophobic' and 'hydrophilic'.

5 Name two methods of membrane transport that do not require protein channels or carriers.

Understanding

6 **a** Describe the part of the plasma membrane described as fluid. Which part is described as mosaic?
   - **b** Explain why these terms are used.

7 Plasma membranes are selectively permeable. What does this mean?

8 Explain why certain white blood cells are known as phagocytes.

9 Explain the role of the Golgi apparatus in the transport of materials out of the cell. Describe the kinds of materials it packages.

Applying

10 Write a few sentences to explain the process by which carbon dioxide might move from the external environment into a cell.

11 By means of an annotated diagram, relate the structure of the plasma membrane to its function.

12 Predict the function of a cell that contains more rough ER than smooth ER.

13 Explain the concept of 'like dissolves in like' and the significance of this to molecules in cells.

14 The effect of drinking a glass of wine can be felt fairly quickly.
   - **a** Predict whether alcohol (ethanol) is water- or lipid-soluble. Suggest how it moves through the plasma membrane.
   - **b** Caffeine is a polar molecule. How would the speed of its effects compare with that of ethanol?

15 Radioactively labelled amino acids were supplied to a pancreatic cell that produces digestive enzymes to be released into the digestive system.

- **a** In which organelles of the cell would the amino acids subsequently be detected? List them in order.
- **b** In what form would they appear in these organelles? You might like to present your answer as a diagram.

Analysing

16 When viewed with an electron microscope, small sac-like vesicles are seen to form inside a cell by budding from the plasma membrane, then detach from the plasma membrane.

- **a** Name these vesicles.
- **b** Describe the function of these structures.
- **c** Name the process described.
- **d** An observer described the process as pinocytosis. What would you say to argue for or against this observation?

17 Explain why a hydrophobic substance does not readily dissolve in water.

18 Compare and contrast the structure and role of membrane transport proteins.

19 Discuss reasons why different types of cell organelles have different types of transport proteins in their membranes.

20 A culture of living cells (A) is placed in a solution with a high concentration of sodium ions. A second culture of cells (B) is exposed to temperatures high enough to denature the proteins in their membranes and then placed in the concentrated sodium ion solution.

- **a** Predict the relative concentration of sodium ions in the internal cellular environments of both cultures.
- **b** Account for your prediction.

Evaluating

21 A student made the comment that 'the formation of vesicles by endocytosis should reduce the size of the plasma membrane'. Apply your knowledge to critically examine this comment.

22 'Transport proteins act like gates facilitating movement across the membrane.' Evaluate this statement, referring to the three types of membrane transport proteins in your explanation.

Creating

23 Water and paint thinner do not mix. Using these two substances, design an experiment that would determine whether another substance called X is polar or non-polar.

24 Size and polarity determine if a molecule can or cannot pass directly through a plasma membrane. Create a poster to demonstrate your understanding of this concept.

CHAPTER 2

FROM BIOLOGICAL INFORMATION TO BIOLOGICAL FUNCTION

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

KEY KNOWLEDGE

Nucleic acids and proteins

- nucleic acids as information molecules that encode instructions for the synthesis of proteins in cells
- protein functional diversity and the nature of the proteome
- the functional importance of the four hierarchal levels of protein structure
- the synthesis of a polypeptide chain from amino acid monomers by condensation polymerisation
- the structure of DNA and the three forms of RNA including similarities and differences in their subunits, and their synthesis by condensation polymerisation
- the genetic code as a degenerate triplet code and the steps in gene expression including transcription, RNA processing in eukaryotic cells and translation.

Gene structure and regulation

- the functional distinction between structural genes and regulatory genes
- the structure of genes in eukaryotic cells including stop and start instructions, promoter regions, exons and introns
- the use of the *lac* operon as a simple prokaryotic model that illustrates the switching off and on of genes by proteins (transcriptional factors) expressed by regulatory genes.

Biological knowledge and society

- techniques that apply DNA knowledge (specifically gene cloning, genetic screening and DNA profiling) including social and ethical implications and issues

KEY SCIENCE SKILLS

Analyse and evaluate data, methods and scientific models

- explain how models are used to organise and understand observed phenomena and concepts related to biology, identifying limitations of the models

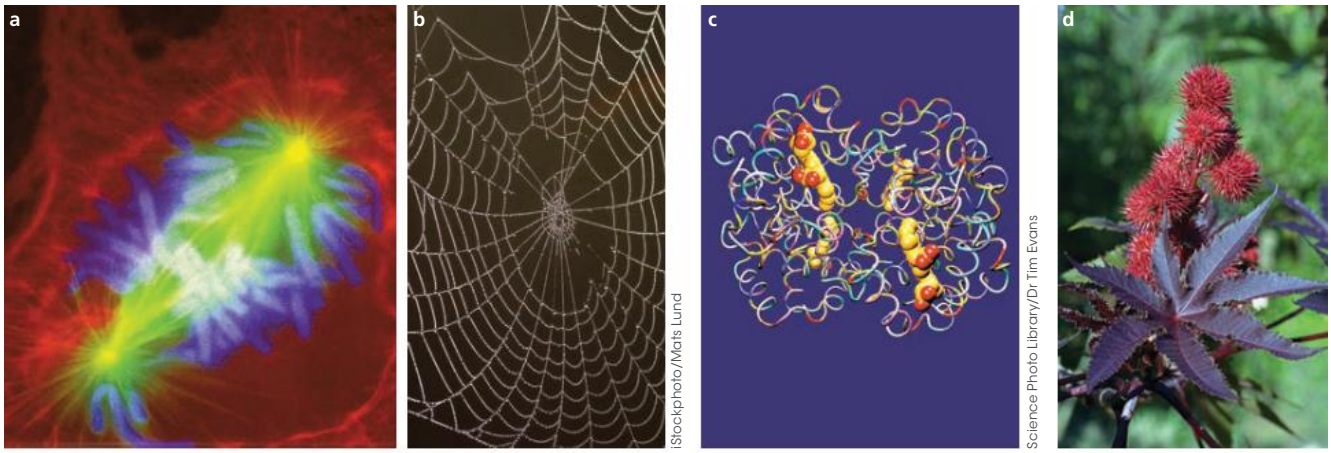
Proteins

Virtually everything a cell is or does depends on the **proteins** it contains. What does your hair have in common with the feathers of birds, the rattle of a rattlesnake and the spines of an echidna? They are all composed of a strong fibrous protein known as keratin. Keratin is just one of a vast variety of proteins produced by the activities of cells.

Proteins are large complex molecules that are the building blocks for many different structures. Proteins can also be **enzymes**, which control the thousands of chemical reactions that maintain life processes (Table 2.1, Figure 2.1).

Table 2.1 The functional diversity of proteins

Type of protein	Function	Examples
Motility	Allow movement of cells and their organelles	Tubulin forms microtubules to move flagella, cilia, chromosomes and organelles. Actin and myosin work together to move muscles.
Structural	Provide support, strength and protection	Collagen supports body tissues. Fibroin makes a spider web stronger, weight for weight, than steel. Keratin forms nails and hair.
Enzymes	Catalyse biochemical reactions	Catalase removes toxic hydrogen peroxide from cells by breaking it down into water and oxygen. All organisms have DNA polymerase, an enzyme that duplicates genetic information (DNA).
Transport	Carry molecules from one location to another or across cell membranes	Haemoglobin carries oxygen to body cells. Porin forms a hydrophilic pore in the outer membrane of mitochondria for the passage of molecules.
Hormones	Signalling between different cell types; stimulation or inhibition	Insulin travels in the blood and binds to cell receptors to trigger the uptake of glucose. Follicle-stimulating hormone (FSH) stimulates the maturation and release of ova (female gametes).
Cell-surface receptors	Receiving signals such as hormones and growth factors, transmission of nerve impulse	Insulin receptors bind insulin to trigger the uptake of glucose by the cell. Rhodopsin in the retina membrane is a light-sensitive receptor that allows us to see dim light.
Neurotransmitters	Signalling between neurons	Endorphins activate nerve receptors to alleviate pain or stress. Enkephalins act as analgesics (pain relievers) and sedatives affecting mood and motivation.
Immunoglobulins	Recognition of foreign substances (antigens)	Antibodies cause foreign material to clump and be ingested by large white cells (macrophages).
Poisons or toxins	Chemicals for defence and to aid in the capture of food	The castor oil plant produces ricin, a deadly toxin. Snake venom contains many proteins that can paralyse and digest prey.



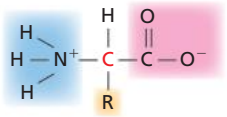
▲ **Figure 2.1** Proteins have a diverse range of functions. (**a**) Spindle fibres attach to chromosomes in cell division. (**b**) A spider web is composed of fibroin. (**c**) Haemoglobin carries oxygen to the cells in the body. (**d**) The castor oil plant produces the deadly toxin ricin.

This diversity of proteins can be explained by the way their building blocks, the 20 amino acids, are sequenced in various combinations. It is rather like arranging 20 kinds of beads in unique ways to make different necklaces of different lengths. The necklace chains can then be arranged variously in loops and folds to give each its characteristic features.

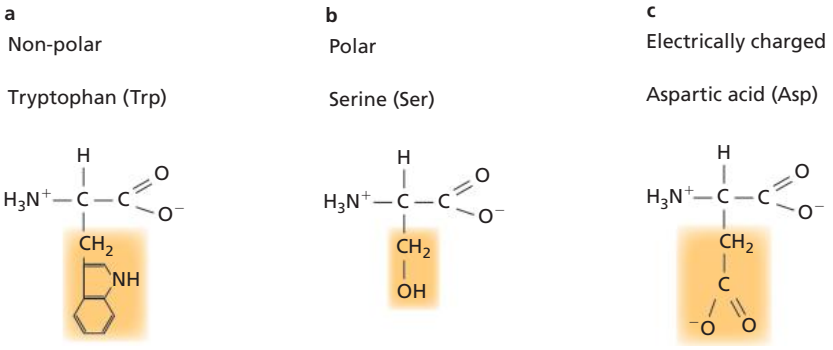
Despite the diversity of proteins they all have the same basic structure: up to thousands of amino acids bond to form linear polymers known as **polypeptides** that are folded, twisted or coiled. Plants synthesise their own amino acids, but animals depend on obtaining almost half of them from their diet. Well over 100 kinds of amino acids can be found in cells but only 20 are used to make up proteins.

Amino acids

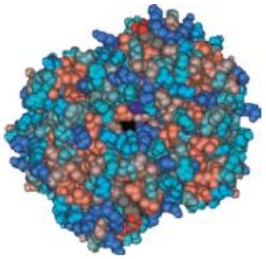
Amino acids are small molecules that have the same basic structure – a central carbon atom to which are attached a hydrogen atom, an amine group (NH_2), a carboxylic acid group (COOH) and what is called an R group. The amine and acid groups react with water to become charged NH_3^+ and COO^- groups respectively (Figure 2.2). There are only 20 different amino acids found in the proteins of living organisms. It is the difference in the R group that distinguishes one amino acid from another and gives each amino acid its particular chemical properties (Figure 2.3).



▲ **Figure 2.2** Structural formula for an amino acid, at about pH 7. Each amino acid differs according to the structure and properties of the R group.



◀ **Figure 2.3** The structural formulas of three of the 20 amino acids, grouped according to their properties: (**a**) non-polar; (**b**) polar; and (**c**) electrically charged



Jacinta Duncan

Figure 2.4 ▲
This model of haemoglobin shows hydrophobic amino acids (brown) associating in the centre of the molecule and the hydrophilic (blue) amino acids tending to interact with the surrounding aqueous environment.

Some R groups give the protein molecule polar regions and other R groups make regions of the protein non-polar. Non-polar **hydrophobic** regions are generally tucked away within the protein molecule, away from the water molecules in the aqueous environment (Figure 2.4). Polar and charged amino acids are **hydrophilic**. They tend to be on the surface of protein molecules because of their affinity for the polar water molecules in their environment (Figure 2.4).

RECALL

- Proteins are a class of biological molecules with a diverse range of functions central to cell structure, organisation and operation.
- Proteins comprise linear polypeptide chains made up of amino acids that have been joined together.
- There are 20 different amino acids that can be grouped according to their properties.

RECAP 2.1

1 List at least five types of proteins, state their functions, and give an example of each.
2 How does an amino acid get its name?
3 Distinguish between hydrophilic and hydrophobic amino acids. Suggest where each is likely to be found in a folded protein and explain why.

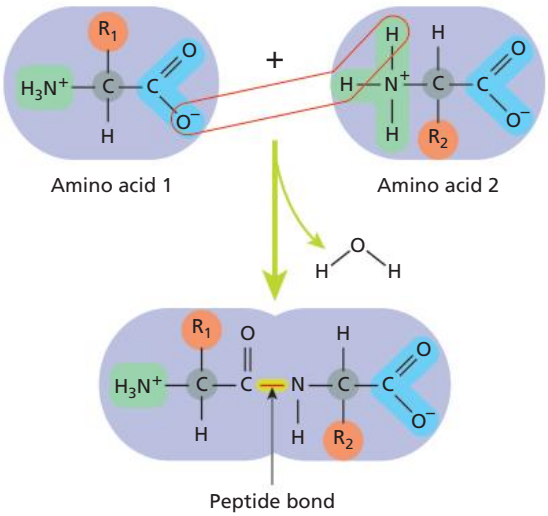


Figure 2.5 ▲
A condensation polymerisation reaction forms the peptide bond between two amino acids. Water is released during the reaction.

From amino acids to proteins

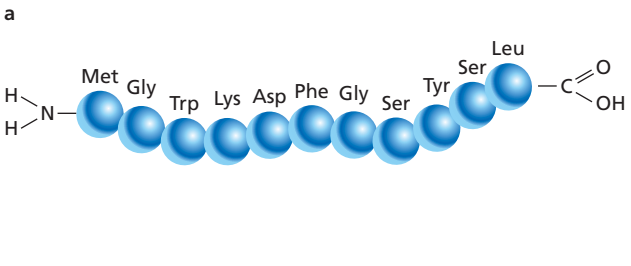
The process of synthesising protein molecules can be explained by the four different levels that give rise to the final structure.

Primary structure

DNA determines the **primary structure**, or sequence of amino acids in the polypeptide. Amino acids bond together in the process of **condensation polymerisation**. Each bond between two adjacent amino acids is called a **peptide bond** (Figure 2.5).

Secondary structure

Once the polypeptide chain is formed, various parts undergo coiling and folding due to hydrogen bonding between the peptide bonds of neighbouring amino acids. These coiled and folded portions form the **secondary structure** of the protein. Tight coils are known as **α-helices** and flattened folding forms as **β-pleated sheets** (Figures 2.6 and 2.7). Other parts of the polypeptide chain do not fold into defined arrangements and are called **random loops**. The β-pleated sheets and random loops often form the basis of the active site in enzymes, being less rigid than α-helices.



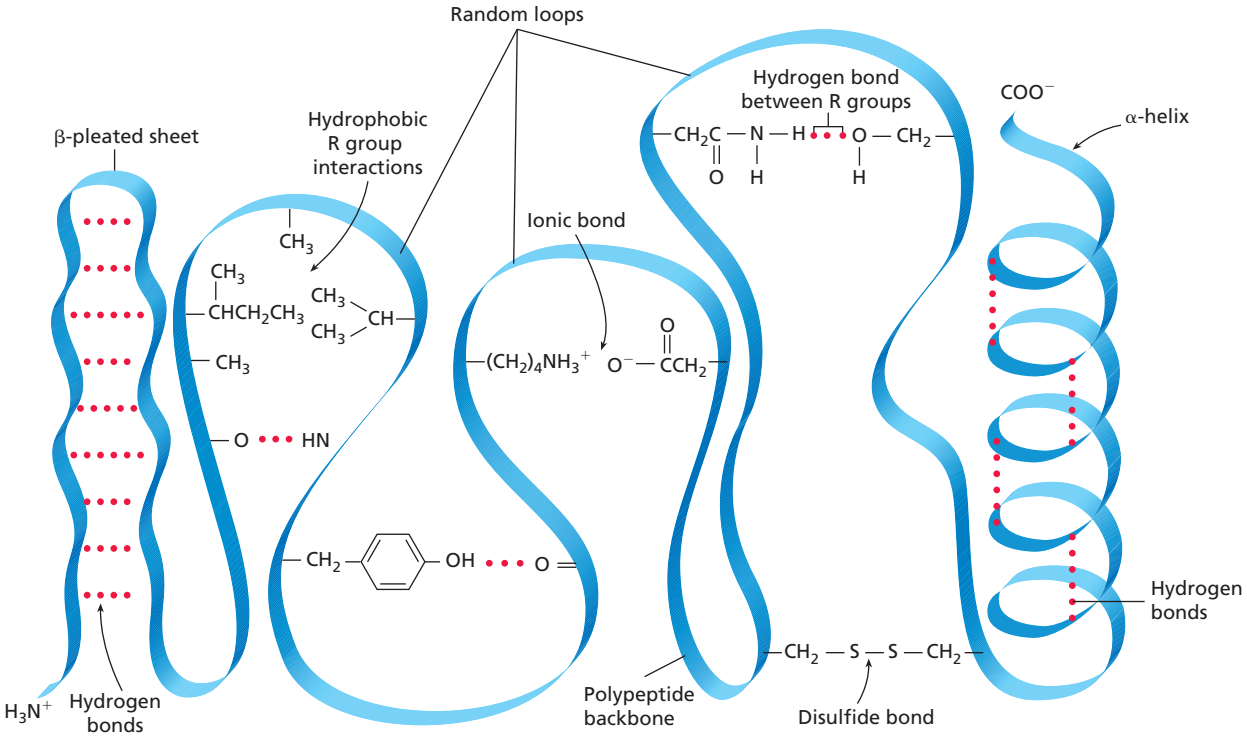
◀ **Figure 2.6**
(**a**) The order of amino acids in the polypeptide is called the primary structure. (**b**) Coiling (α-helices) and folding (β-pleated sheets) result in the secondary structure of a protein. Coils and sheets are connected by random loops.

Tertiary structure

Hydrophilic R groups attract other hydrophilic R groups, and hydrophobic R groups attract other hydrophobic R groups according to the chemical principle 'like attracts like' (Figure 2.7). These interactions between the R groups of the amino acids cause the polypeptide chains to become folded, coiled or twisted into the protein's functional shape or **conformation**, described as the **tertiary structure** of the protein. The interactions result in hydrogen bonds, ionic bonds or hydrophobic contact between various R groups, or **disulfide bridges** between cysteine amino acids. Disulfide bridges are strong covalent bonds that form connections within the polypeptide that are resistant to unfolding. Protein molecules with the same sequence of amino acids generally fold into the same shape. A change to just one amino acid can alter the shape of the protein molecule and it may not function properly.

It is the tertiary structure that determines the function of the protein – its **biological functionality**. Some proteins form long, closely packed fibres that are insoluble in water and result in structural components of cells. Most proteins form spherical or globular molecules that are soluble in water and perform a variety of functional tasks.

▼ **Figure 2.7**
Secondary structures, such as α-helices and β-pleated sheets, form by hydrogen bonding within localised regions of the polypeptide. The specific function of a protein is determined by its tertiary structure. The tertiary structure arises from interactions between R groups of amino acids in the polypeptide chain.



Quaternary structure

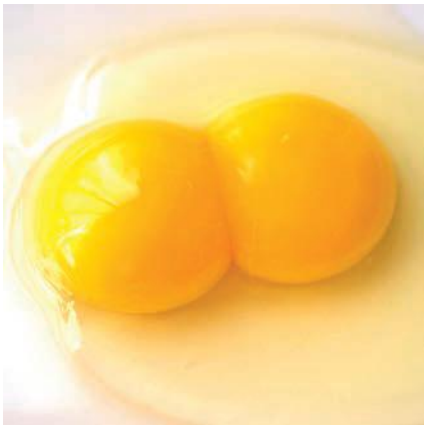
Many large, complex protein molecules consist of two or more polypeptide chains. Haemoglobin, for example, which carries oxygen in the blood, consists of four polypeptide chains. The **quaternary structure** is formed when two or more polypeptides associate into the mature protein. A variety of hydrogen bonds, ionic bonds and covalent bonds hold the polypeptide chains together and give the overall shape to the molecule. Each polypeptide is described as a **subunit** of the protein.

Changing the nature of proteins

The function of a protein depends on its shape. Apart from misreading the DNA code so that polypeptides are unable to fold correctly, proteins may lose their functional shape if they are exposed to high temperatures, strong salty solutions, or very acidic or alkaline conditions. These conditions can **denature** or change the shape of the protein molecules (Figure 2.8). If the change is small the protein molecule can return to its original shape, but it cannot if the change is large.

Observing the three-dimensional structure of proteins allows us to view the active sites of enzymes and the binding sites of receptor proteins so that we can determine molecules that will 'fit' into these sites. This can lead to the development of highly specific drugs that will either stimulate the activity of the target or block its activity.

Figure 2.8 ▶
Raw and cooked egg. The colourless protein of the 'white' of the egg albumen is changed by heat.



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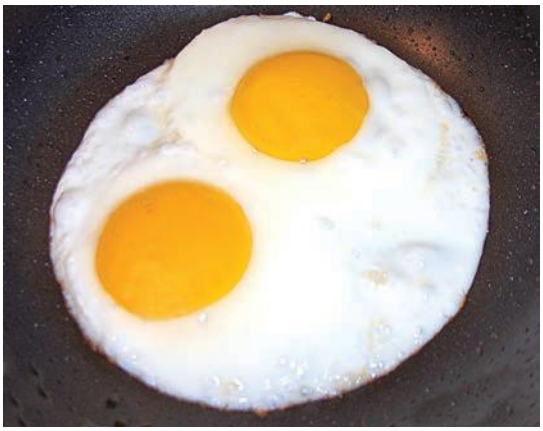

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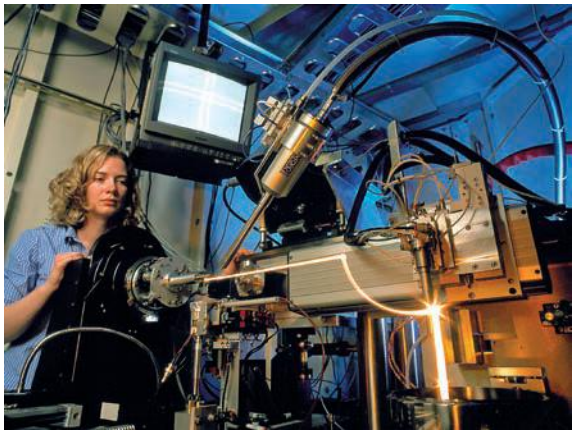

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Figure 2.9 ▲
Researcher with an X-ray crystallography machine used to study the structure of proteins

Determining protein structure

Structural biologists use X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy to find out the structure of a protein that they are interested in. The techniques they use are time-consuming and expensive (Figure 2.9), but they are effective in determining the spatial arrangement of the atoms that make up the protein molecules.

Specialised computer programs analyse the data the biologists collect to determine the most likely configuration of the amino acids of the protein. The research team then submits the structure to a journal and to the Protein Data Bank, a worldwide electronic library for processing and distributing data about the three-dimensional structures of biological macromolecules. This is an example of bioinformatics. Scientists and the public can access this information and view the three-dimensional structure using a variety of computer visualisation programs, such as Cn3D, which is free to download from the National Center for Biotechnology Information (NCBI) website.

The proteome

The whole set of proteins produced by a cell, a tissue or an organism is called its **proteome**. It is estimated that there are over 100 000 different proteins in the human proteome. **Proteomics** is the study of proteomes. Proteomics is a dynamic field of research that is concerned with investigating the collection of proteins and their modifications in a particular cell type or tissue. **Functional proteomics** refers particularly to the study of what proteins do in different cells or tissues. It can be concerned with how the many proteins interact with each other in a specific tissue type, or how the suite of proteins in a tissue changes during disease. The combined expertise of computational biologists, mathematicians and molecular biologists has resulted in the development of powerful tools, techniques and databases for studying proteins.

RECALL

- Proteins fold into shapes that are defined by their amino acid sequence, and they exhibit four levels of structure in the course of folding into their proper shape.
- A protein's function is dependent upon its shape.
- Heating proteins often causes them to unfold irreversibly.
- A proteome is the complete set of proteins in a cell, organ or tissue.

RECAP 2.2

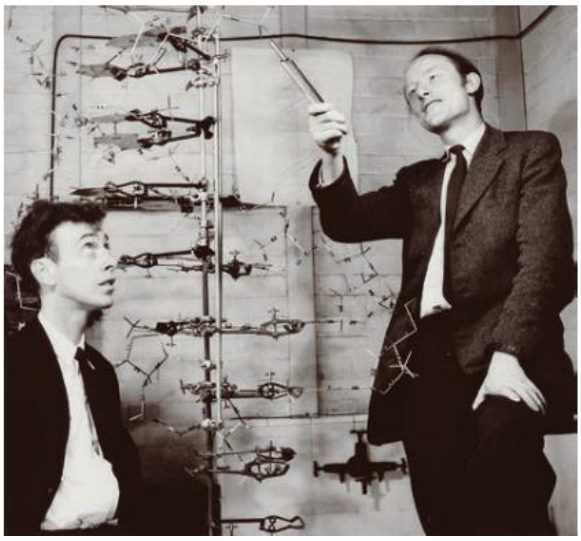
1. Describe how amino acids are linked together.
2. Describe the four levels of protein structure.
3. What three types of folds are associated with a polypeptide's secondary structure?
4. Explain what is meant by the term 'denature' and relate the term to a protein's function.
5. Explain the benefits of proteomics.

Nucleic acids

In 1869, Friedrich Miescher, a German doctor, extracted a white substance from the nuclei of white blood cells that he collected from pus on soiled surgical bandages. He called the substance 'nuclein'. By 1900 the basic chemistry of this mysterious white substance had been worked out. It was a long molecule made up of three distinct chemical parts. Today this molecule is called **nucleic acid**.

Commonly recognised by the letters **DNA (deoxyribonucleic acid)** and **RNA (ribonucleic acid)**, nucleic acids are large, linear polymers that form when monomers bond together. A molecule of DNA is composed of two long polymer strands wound around each other to form the familiar double helix. The claim for the discovery of the structure of DNA has been contentious. Many years of work and investigation by scientists contributed to the body of knowledge that eventually led James Watson and Francis

▼ **Figure 2.10**
Watson and Crick with their DNA model



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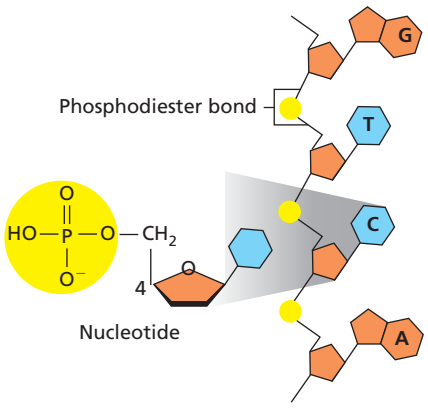


Figure 2.11 ▲
DNA is made up of nucleotides that link together through phosphodiester bonds to form a strand of DNA.

Crick to report the famous model of the double helix in 1953. Watson and Crick used X-ray images of the DNA molecule made by Rosalind Franklin to determine its structure (Figure 2.10).

Nucleic acids store information in a chemical code that directs the machinery of the cell to produce proteins. Nucleic acids are every organism's genetic material; they are the means by which the story of life extends through time and across all life forms.

Structure of nucleic acids

Like proteins, nucleic acids are polymers built of repeating units, or monomers. **Nucleotides** are the monomers that make up nucleic acid molecules. DNA and RNA differ in the structure of their nucleotides. These differences influence the structure and the functions of the nucleic acid polymers.

Structure of DNA

A nucleotide has three distinct chemical components:

1 a five-carbon sugar (in DNA this is deoxyribose)
2 a negatively charged phosphate group, which gives DNA an overall negative charge
3 an organic nitrogen-containing compound called a base (Figure 2.11).

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

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

The carbons of the sugar in a nucleotide are numbered from 1′ to 5′. The nitrogenous base is attached to carbon 1′ of the sugar. The phosphate group is attached to carbon 5′ of the sugar. Consequently, the nitrogenous base projects to the side of the sugar ring and the phosphate group sits above it (Figure 2.11).

The nucleotides of a nucleic acid strand are linked by condensation polymerisation reactions. The phosphate group at carbon 5′ of one nucleotide bonds with the hydroxyl group at carbon 3′ of the next nucleotide in the strand. In the process, a **phosphodiester bond** is formed and a molecule of water is released. Regardless of the number of nucleotides in a strand, the two ends of the strand always differ. A phosphate group projects from one end and is called the **5′ end** of the strand. A hydroxyl group projects from the other end and is called the **3′ end** of the strand.

Nucleic acids are invariably synthesised towards the 3′ end of the strand in a 5′ to 3′ direction. In other words, nucleotides are progressively added to the exposed hydroxyl group at the 3′ end.

The nitrogen bases of a DNA strand project outwards and link with the nitrogen bases of the second strand (Figure 2.12). Hydrogen bonds between the adjoining pairs of nitrogenous bases hold the double helix together, much like the rungs of a twisted ladder. The nitrogen bases do not bond randomly. Rather, A bonds with T and C bonds with G, following strict **complementary base-pairing** rules.

The two strands of the DNA molecule are aligned in an **antiparallel** arrangement. That is, the 5′ end of one strand comes together with the 3′ end of the complementary strand.

Structure of RNA

Like DNA, RNA is built of nucleotides that are linked together by condensation polymerisation reactions. There are, however, basic differences in the structure of

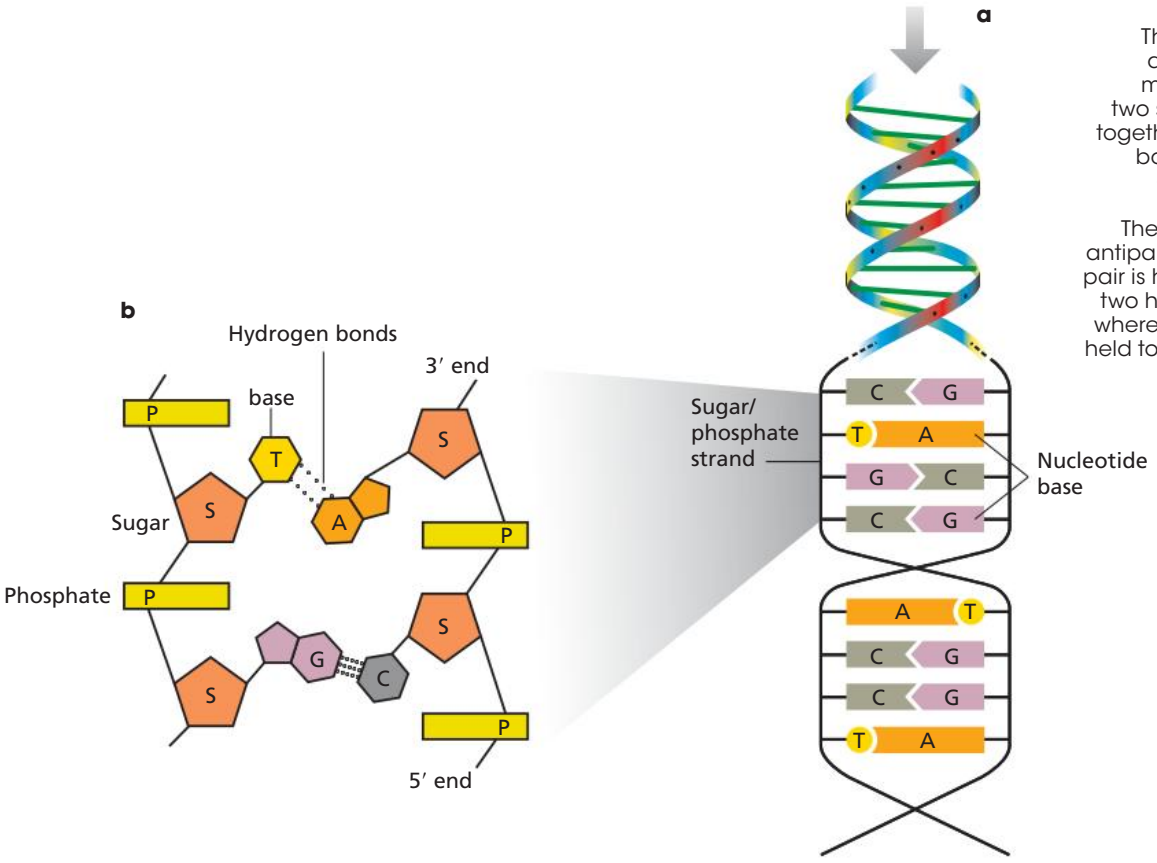


Figure 2.12
The DNA helix is a double-stranded molecule. (**a**) The two strands are held together by hydrogen bonding between complementary nitrogen bases. The two strands are antiparallel. (**b**) An A–T pair is held together by two hydrogen bonds, whereas a G–C pair is held together by three.

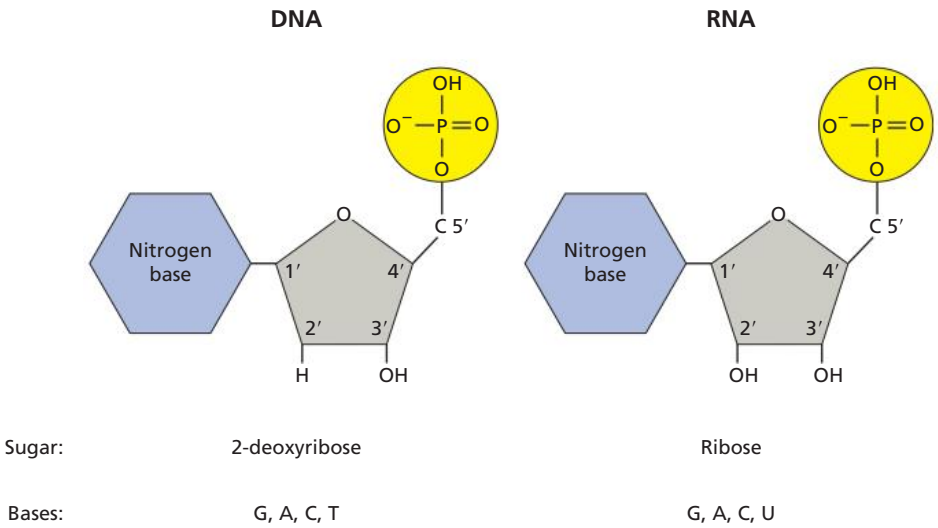


Figure 2.13
Differences between the nucleotides of DNA and RNA

the nucleotides of each (Figure 2.13). The deoxyribose sugar of the DNA and the ribose sugar of RNA differ in that deoxyribose has one less oxygen atom at carbon 2′. The nitrogenous base thymine (T) in DNA is replaced by the base uracil (U) in RNA. The bases T and U are very similar, except that T has four additional atoms (a CH_3 group).

Apart from differences in nucleotide structure, RNA tends to be single-stranded and has a variety of folding patterns. DNA is double-stranded and folds into a double helix. RNA molecules also are much shorter than DNA molecules. The majority of RNA

molecules range from a few dozen up to a few thousand nucleotides. DNA molecules in **chromosomes** range from tens of thousands up to hundreds of millions of **base pairs** in length.

RECALL

- Nucleic acids comprise linear strands made up of nucleotides that have been joined together.
- DNA, the ultimate information molecule, is double-stranded, with the nucleotides on each strand linking together through complementary base pairing.

RECAP 2.3

1. Describe the three key features of a nucleotide and how they are arranged.
2. How are nucleotides linked together?
3. How are the two strands of DNA held together?
4. If DNA structure is described as 'antiparallel', what does this mean?
5. What charge does DNA have, and why?

The functions of nucleic acids

The seemingly trivial differences between DNA and RNA nucleotides dictate major differences in the structure and function of DNA and RNA. Double-stranded DNA is a persistent, long-lived molecule. It is transmitted between cells during cell division and between generations of organisms through sexual reproduction. It is the molecule ultimately responsible for inheritance.

RNA tends to be single-stranded. It is a relatively short-lived molecule that is made and degraded rapidly by cells. It is also a versatile molecule with a variety of functions that facilitate and regulate protein production.

The function of DNA

In prokaryotic cells, the DNA is organised into a single, circular chromosome. Some DNA is also found in the form of small circular pieces of DNA called **plasmids**. The chromosome and plasmids are exposed in the cytoplasm.

The DNA of eukaryotic cells is mainly organised into linear chromosomes contained within the nucleus. Proteins called **histones** are bound to the DNA in eukaryotic chromosomes. Like winding cotton around many reels, histones help pack the large DNA threads into the confined space of the nucleus. Some DNA is also found in the form of circular chromosomes in the mitochondria and chloroplasts.

The complete sequence of DNA in a single set of an organism's chromosomes is called its **genome**. Certain sequences of nucleotides in the DNA are codes for making polypeptides. The sequence of nucleotides in a segment of DNA ultimately determines the sequence of amino acids in the polypeptide. In turn, the sequence of amino acids determines which protein is formed.

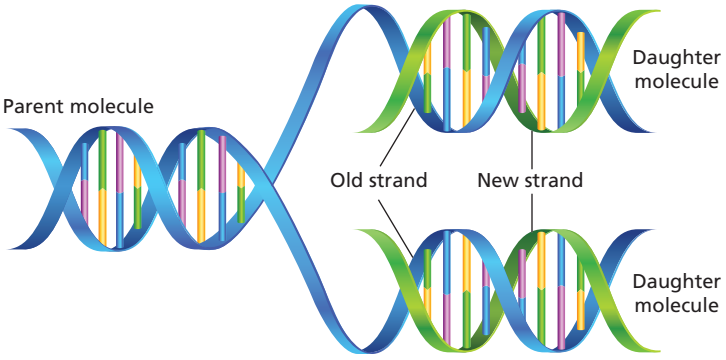
It sounds simple, but when we consider that each cell of our body carries well over a metre in length of DNA, twisted and coiled into 46 chromosomes that have more than three billion base pairs (bp), it is not surprising that there is such a diversity of proteins. However, not all the DNA codes for polypeptides. The segments that code for polypeptides are called **genes**. Genes account for only about 1% of the human genome.

See Nelson Biology VCE Units 1 & 2, *Chapter 9 for more on DNA replication.*

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

See Chapter 8 for more on how alleles come about.

DNA consists of two complementary strands held together by hydrogen bonds. If the nucleotide sequence is known for one strand, it is straightforward to infer the sequence on the other strand because of the base-pairing rules. When DNA is copied, the original strands are separated first. Each single strand then serves as a **template** for the production of a new complementary strand (Figure 2.14). This is described as **semi-conservative replication**. The new strand is built towards its 3′ end. The sequence of nucleotides in the new strand is determined by complementary base pairing with the nucleotides of the template strand.



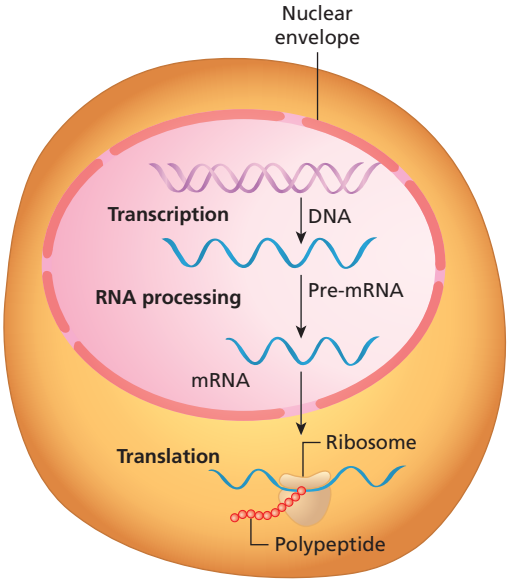
◀ Figure 2.14 Model for semi-conservative replication of DNA. Each daughter molecule contains one strand from the parent DNA molecule and one freshly synthesised strand.

Owing to its unique ability to replicate with high fidelity, DNA passes on this information from one generation of cells to the next and from one generation of organisms to the next. It is the master code that determines the very nature of cells and therefore of life forms.

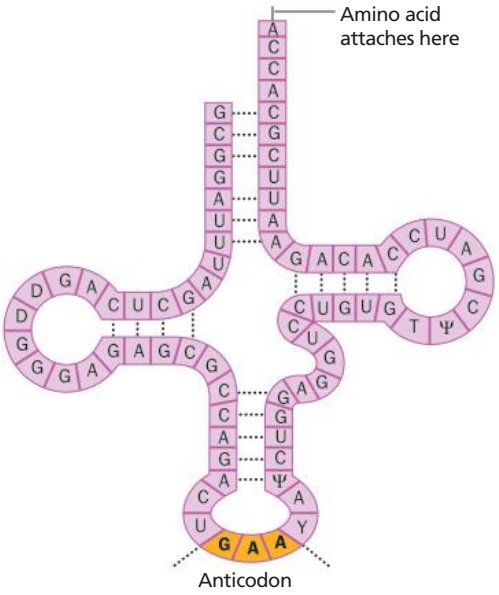
The many roles of RNA

RNA has many functions in producing proteins. It takes the information in the DNA strand and creates the proteins necessary for life. This is called **gene expression** – the gene is 'expressed' by production of its coded protein.

DNA acts as a template and the information in the genes is copied into RNA. This copying process is called **transcription** (Figure 2.15). The RNA produced (called **pre-mRNA**) is subsequently processed into the mature **messenger RNA (mRNA)**. Single-stranded mRNA molecules, or 'transcripts', convey the information encoded in single genes and are therefore much shorter than chromosomal DNA. They leave the nucleus readily. Messenger RNA gets its name from its ability to convey the instructions needed for protein synthesis from the DNA in the nucleus to the cytoplasm where the proteins are made.



◀ Figure 2.15 Gene expression relies on the processes of transcription and translation. During transcription, DNA is copied into pre-mRNA that is processed into mature RNA for export from the nucleus. During translation, the ribosome converts the nucleotide sequence in the mRNA into the amino acid sequence of a polypeptide.



adapted from H. Curtis & N. S. Barnes, Biology, Worth Publishing, New York, 1989

Figure 2.16 Structure of tRNA. The diagram shows the molecule as flat and shaped like a clover leaf. Some nucleotides occupy the same positions in all tRNAs, whereas others vary according to the particular tRNA. The symbols D and ψ represent unusual nucleotides that are characteristic of tRNAs; Y can represent either C or U. Although T is not found in other forms of RNA, it does occur in tRNA. Base pairing only occurs in certain regions. The three bases shown at the bottom of the representation constitute the mRNA binding site, or anticodon. The amino acid binding site is shown at the top of the molecule.

Ribosomes in the cytoplasm serve as sites for polypeptide synthesis. The ribosome docks with the mRNA and uses the nucleotide sequence of the mRNA to guide polypeptide synthesis. This process is called **translation** (Figure 2.15). The ribosomes are composed of **ribosomal RNA (rRNA)** strands complexed together with a large number of proteins. The rRNA therefore acts as structural molecules for formation of the ribosome.

As mRNA is reeled through the ribosome, amino acids are linked together to build the polypeptide. The amino acids are brought in by **transfer RNA (tRNA)** molecules. These exist as free-floating molecules within the cytoplasm and, unlike mRNA, tRNA molecules are folded into loops with a distinctive clover shape (Figure 2.16). Each type of amino acid is carried by a different tRNA molecule. Three nucleotides in the central loop of the tRNA, called the **anticodon**, bind to mRNA following base-pairing rules. The stem of the tRNA is attached to the amino acid. The sequence of nucleotides in the mRNA directs which tRNA should bind next. This determines which amino acid is next in the polypeptide sequence. When a tRNA molecule binds with the mRNA in the ribosome, the accompanying amino acid is positioned for 'transfer' to the growing polypeptide. In this way, tRNA molecules act as adaptor molecules which help to turn a nucleotide sequence into an amino acid sequence.

RECALL

- Genes are the segments of DNA that code for polypeptides.
- RNA is normally single-stranded.
- Different types of RNA perform different functions in the process of expressing a gene as a polypeptide.

RECAP 2.4

1 Identify three fundamental differences between the structures of DNA and RNA.
2 Define two key processes in gene expression.
3 List three major types of RNA and describe their functions.

Transmitting information through a triplet code

The nucleic acids function primarily as information molecules. DNA determines the sequence of the 20 different types of amino acids put together in the polypeptide. But how does a four-letter DNA code specify for each of 20 different amino acids in polypeptides? The challenge is overcome by interpreting the nucleotides in groups of three (triplets). If

one of four nucleotides can occur in each position of the triplet, then $4 \times 4 \times 4 = 64$ possible combinations can be specified, more than enough for the 20 amino acids.

As we have seen, the instructions encoded in the genes in the DNA are relayed through a series of processes that involve RNA in the production of specific proteins. What are the events that convey that information from gene to protein?

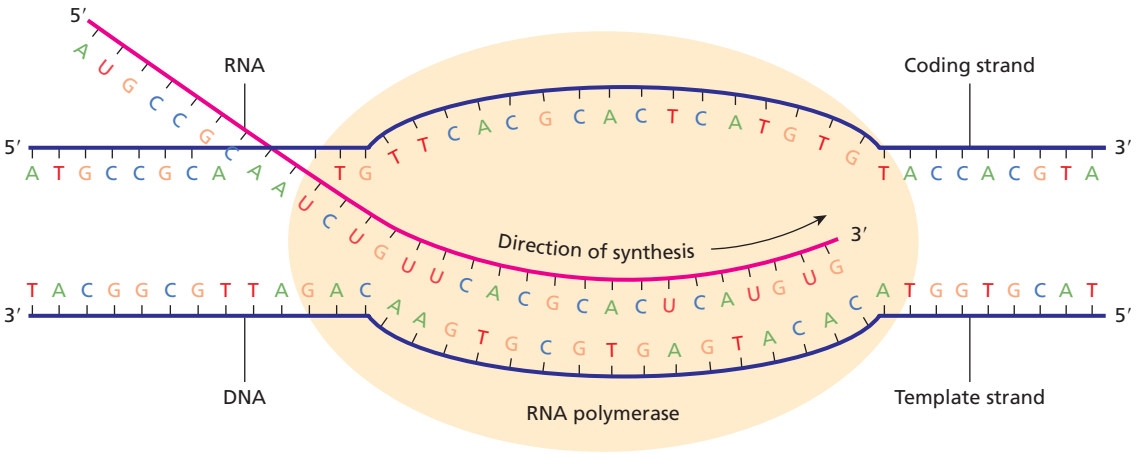
Transcription

The DNA in the region of the gene first unwinds, then unzips, exposing the nucleotide bases of both strands. One strand of the DNA has the sequence that codes for the polypeptide. This is called the **coding strand**. The other strand of DNA is used to direct the synthesis of mRNA and is called the **template strand**.

At the site in the DNA where a gene occurs, the nucleotide sequence defines the gene's structure. A particular sequence of non-coding DNA, called the **promoter**, signals the start of the gene. The promoter is often rich in T and A bases and this region is sometimes referred to as the TATA box. Proteins position the enzyme **RNA polymerase** onto the DNA to bind with the promoter. As RNA polymerase proceeds along the DNA, it progressively builds a strand of RNA that is complementary to the template strand.

Although both DNA strands are exposed during transcription, the antiparallel structure of DNA ensures that only the correct strand is used as the template. Nucleic acid strands are invariably built towards the 3′ direction, in the opposite direction to their template. This means only the template strand (running 3′ to 5′) can be **transcribed**, or copied, into RNA (running 5′ to 3′). The nucleotide sequence of the RNA is identical to the coding strand of DNA (running 5′ to 3′), except that T is replaced by U (Figure 2.17).

▼ Figure 2.17
During transcription, pre-mRNA is synthesised in the 5′ to 3′ direction from the template strand of DNA.



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

Processing pre-mRNA

Following transcription, some segments of the pre-mRNA are removed. Most eukaryotic genes have regions of base sequences that are not translated into the amino acids of polypeptides. These regions, which 'interrupt' the coding sequences, are called **introns**. Introns are interspersed among regions of DNA called **exons**, which contain the actual information for polypeptide formation. Both the exons and introns are transcribed into pre-mRNA, but introns are removed and exons re-joined by RNA splicing before the mRNA leaves the nucleus (Figure 2.18).

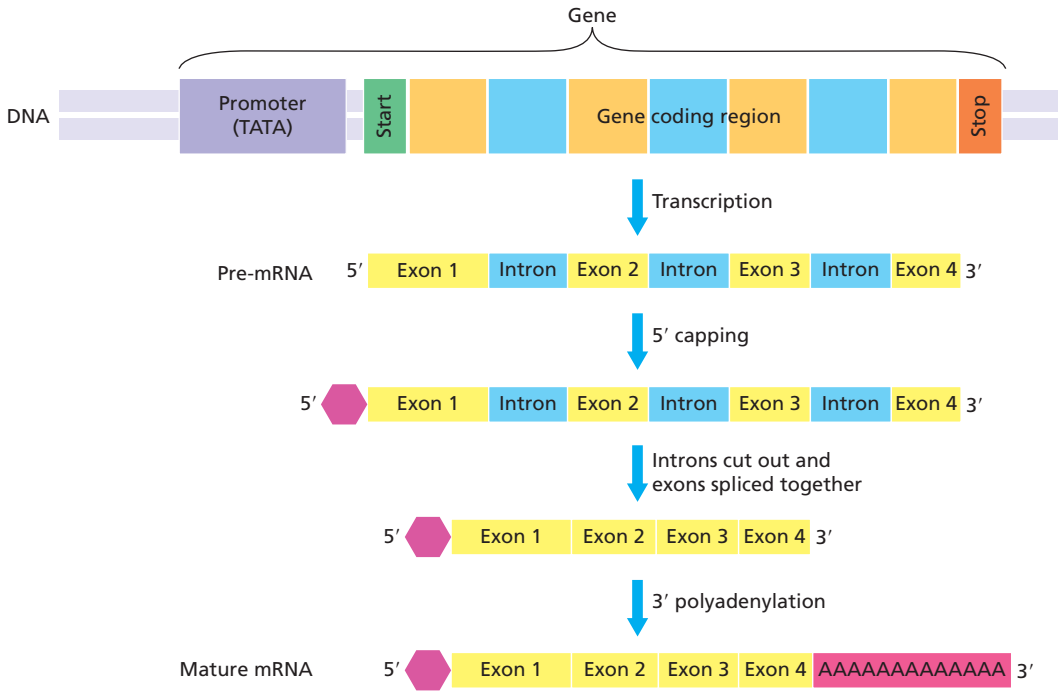


Figure 2.18 ▶
Transcription and processing to generate mature mRNA from pre-mRNA in the nucleus of a cell.

As it is being transcribed, the pre-mRNA is modified by the addition of a methylated cap at the 5′ end (Figure 2.18). Before it leaves the nucleus, the pre-mRNA is capped at the 3′ end by **polyadenylation** (Figure 2.18). That is, a **poly-A tail** consisting of about 100–200 adenine nucleotides is added at the 3′ end. The addition of these structures protects the RNA from degradation. When the methylated cap and poly-A tail are later removed, the RNA is rapidly digested and the nucleotides are recycled for further RNA synthesis.

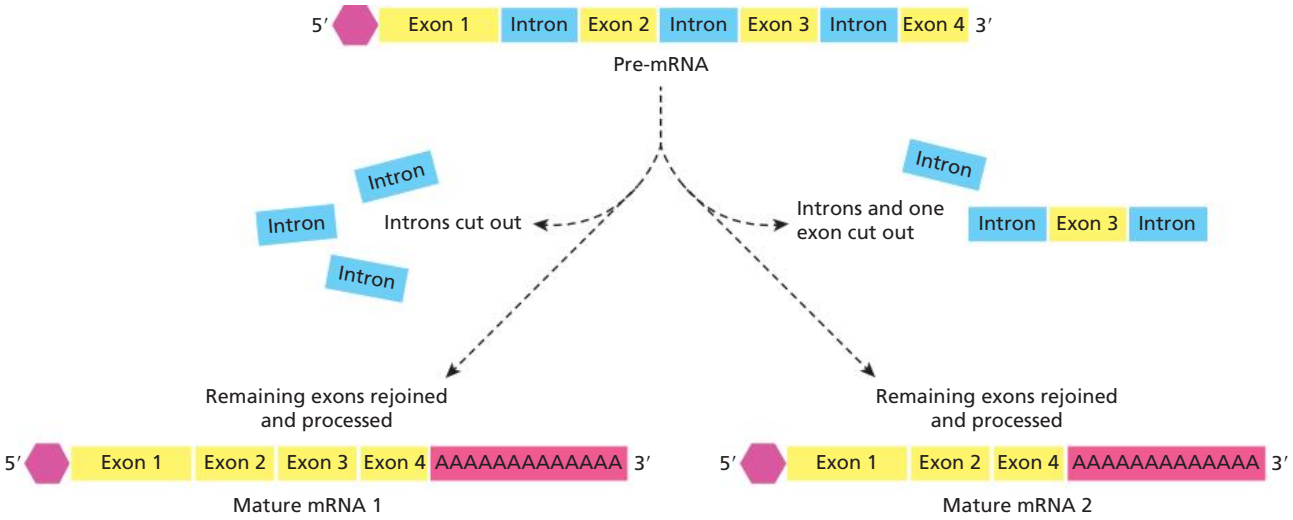
After processing, the mature mRNA is ready to leave the nucleus and move into the cytoplasm, where translation of the nuclear code into polypeptides is carried out by ribosomes. The average mRNA strand is about 1000–2000 bases long, including the methylated cap and 100–200 adenine bases in the poly-A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

Alternative splicing

Before the human genome was sequenced, it was predicted that it would contain over 100 000 genes, based on the estimated number of proteins in the human body. Surprisingly, after sequencing, it appeared the number of genes was between 20 000 and 25 000. What could explain this puzzle?

It turns out that a single gene can code for several different polypeptides. During RNA processing, different exons may be removed along with the introns to produce mRNA molecules of different length and sequence (Figure 2.19). This process is referred to as **alternative splicing**. The polypeptides translated from the alternative mRNA molecules are of different sizes, have different sequences, and have their own unique functions.

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



▲ **Figure 2.19** Alternative splicing allows the production of different mRNA molecules, and therefore different polypeptides, from the same gene.

RECALL

- During transcription, RNA is copied from template DNA.
- The pre-mRNA is processed by cutting, splicing and capping to become mature mRNA before it leaves the nucleus.
- The pre-mRNA can be alternatively spliced to generate different mRNA transcripts.

RECAP 2.5

1. What is the promoter of a gene?
2. Explain why only one of the DNA strands can serve as the template strand during transcription.
3. Define exons and introns, and explain how they relate to the processing of pre-mRNA.
4. Describe how alternative splicing generates different mRNA transcripts.
5. What are the 5′ and 3′ caps, and what do they achieve for the mature mRNA?

The genetic code

The nucleotide sequence of the gene is copied and processed into a functional strand of mRNA. The mRNA now serves as a linear sequence of instructions for making the primary structure of a polypeptide. The information stored in the mRNA is grouped into units of three nucleotides called **codons**. Each codon specifies a particular amino acid. The order of the codons in the mRNA specifies the order of the amino acids in the polypeptide. One codon, AUG, which codes for the amino acid called methionine, also specifies the start of polypeptide synthesis. Three codons do not code for any amino acids but direct polypeptide synthesis to stop. These fundamental rules are the foundation of the **genetic code** (Figure 2.20). With very few exceptions, the genetic code is universal to all organisms.

The genetic code shows the relationship between the codons in mRNA and the amino acids that are translated. As there are 64 codons and just 20 amino acids, many amino acids are encoded by multiple codons. Amino acids such as serine or arginine are specified by up to six codons (Figure 2.20). These observations show that there is a level of redundancy built into the genetic code. The genetic code can therefore be described as **degenerate** because most amino acids can be encoded by two or more codons.

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

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

Figure 2.20 ▲
The genetic code. The mRNA codons correspond to the 20 amino acids made by translation on the ribosomes. Three codons act as stop codons, and under certain conditions the codon AUG initiates protein synthesis.

Translation

When mRNA moves into the cytoplasm, it binds with a ribosome, where it causes amino acids to assemble in a particular order. In other words, the codons in the mRNA are translated into the amino acid sequence of a polypeptide. Polypeptide synthesis also requires the activity of tRNA molecules. The ribosome serves as the machinery for protein synthesis while tRNA provides the raw materials.

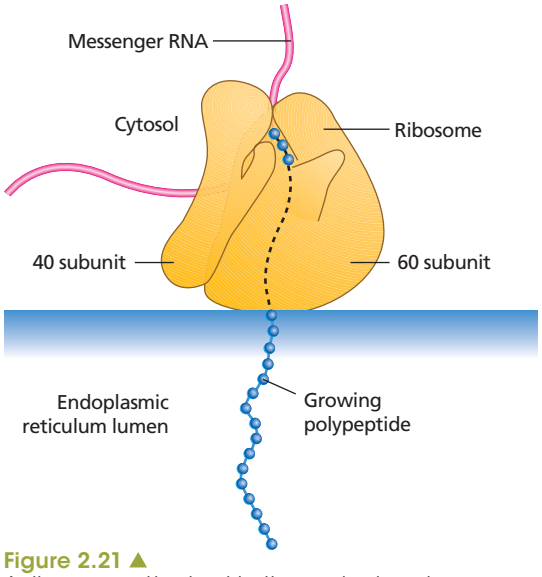


Figure 2.21 ▲
A ribosome attached to the endoplasmic reticulum is synthesising a polypeptide. The polypeptide is transferred to the lumen (inside) of the endoplasmic reticulum.

The ribosome

Ribosomes usually exist in the cytoplasm in two subunits, a smaller one called the 40 S subunit and a larger one called the 60 S subunit (S is a unit of size). Both contain numerous protein molecules together with rRNA, and both subunits are assembled in the nucleolus. The subunits move from the nucleus into the cytoplasm, where they combine to form the functional units of translation.

In eukaryotic cells, ribosomes are found free throughout the cytosol. Ribosomes are also bound to rough endoplasmic reticulum (Figure 2.21). Generally, proteins that are destined for the cytosol are made on free ribosomes.

Proteins that are made on bound ribosomes are transferred through the membrane and enter the lumen (interior) of the rough ER (Figure 2.21). They are then secreted from the cell or relayed to other cell compartments, such as lysosomes.

Ribosomes are also found in prokaryotes and in mitochondria and chloroplasts. These ribosomes are smaller

than the ribosomes found in the cytoplasm of eukaryotic cells, although they also consist of two subunits and are involved in protein synthesis.

Ribosomes are able to form chains, called **polyribosomes** or polysomes, all binding to a single mRNA strand (Figure 2.22). The advantage of polyribosomes is that they allow a large number of polypeptides to be made from a single mRNA strand in a comparatively short time. The rate of polypeptide synthesis is greatly increased. In bacterial cells, polypeptide synthesis happens even more rapidly. This is because prokaryotes lack a nucleus and protein synthesis can begin even before mRNA synthesis is complete.

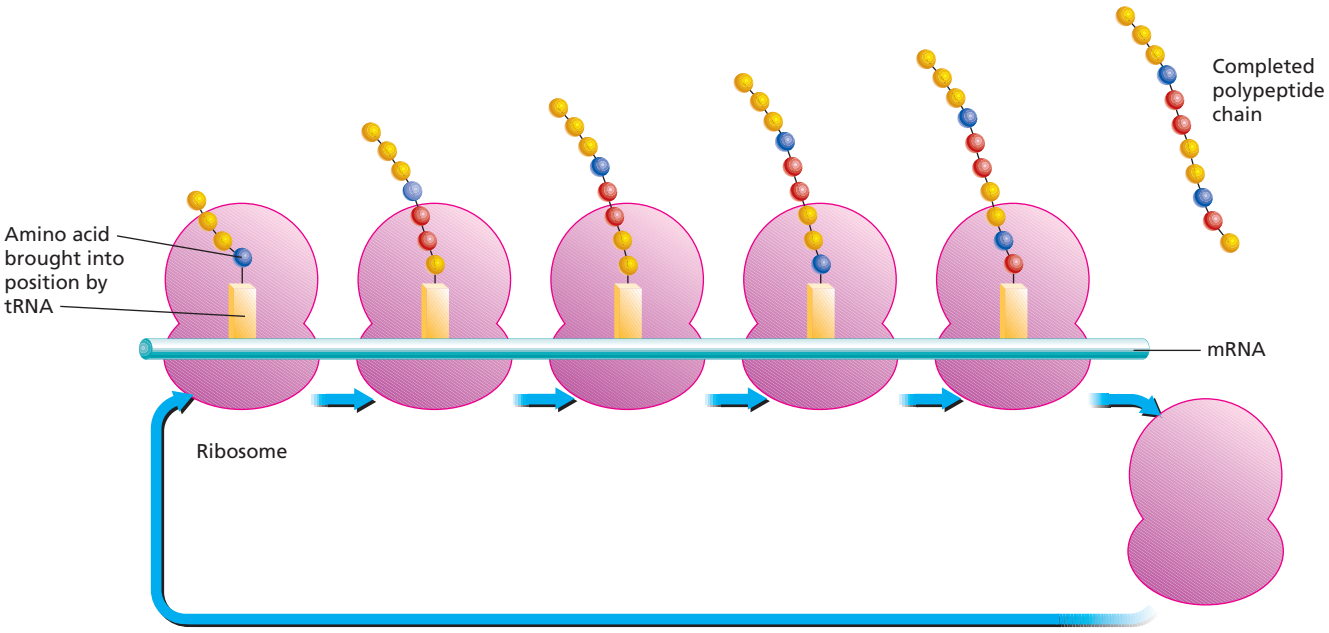


Figure 2.22 ▲
How a polyribosome works. A series of ribosomes moves along an mRNA molecule. Each ribosome synthesises a polypeptide molecule as it moves along the mRNA. When the ribosome reaches the end of the mRNA strand, it releases its polypeptide chain and can return to the beginning.

Codon–anticodon interaction

The tRNA molecules bring the amino acids into position on the ribosome for incorporation into the polypeptide. The order of codons in the mRNA must be 'read' by the incoming tRNA molecules so the amino acids are in the correct order for polypeptide synthesis. This is determined by direct interaction between tRNA and mRNA.

The anticodon of each tRNA is complementary to a particular codon of the mRNA. For example, if the mRNA codon is GCU, the anticodon of the tRNA must be CGA. The particular amino acid carried by each tRNA is determined by the sequence of the anticodon. According to the genetic code (see Figure 2.20), the GCU codon specifies the amino acid alanine. This means the tRNA has brought an alanine into position for addition to the growing polypeptide chain. A tRNA molecule with the anticodon CGA will only be attached to an alanine amino acid.

Likewise, a tRNA with anticodon UGA will only be attached to the amino acid threonine. This tRNA binds to the mRNA codon ACU. In essence, the ACU codon in mRNA specifies a threonine amino acid in the polypeptide (see Figure 2.20).

Translation in action

A small ribosome subunit loaded with an initiator Met-tRNA (one that can start the process) recognises an mRNA strand as it leaves the nucleus and travels to the cytoplasm. The ribosome subunit bonds to the methylated cap on the mRNA and

moves along it 'scanning' for an AUG start. Once found, a large ribosomal subunit joins with the small one. The ribosome then passes along the mRNA strand and, as it passes the next triplet of bases (codon) in the mRNA, a **charged tRNA**, carrying the appropriate amino acid, moves into position on the ribosome. The three bases in the mRNA codon bond to the complementary three bases (anticodon) in the tRNA molecule.

Once aligned, adjacent amino acids are linked by peptide bonds, initiating formation of the polypeptide chain. As the amino acids join up, the polypeptide chain peels off from the tRNA molecules. When its job is complete, the **uncharged tRNA** detaches from the mRNA and returns to the pool of tRNAs in the cytoplasm. The process is shown in Figure 2.23.

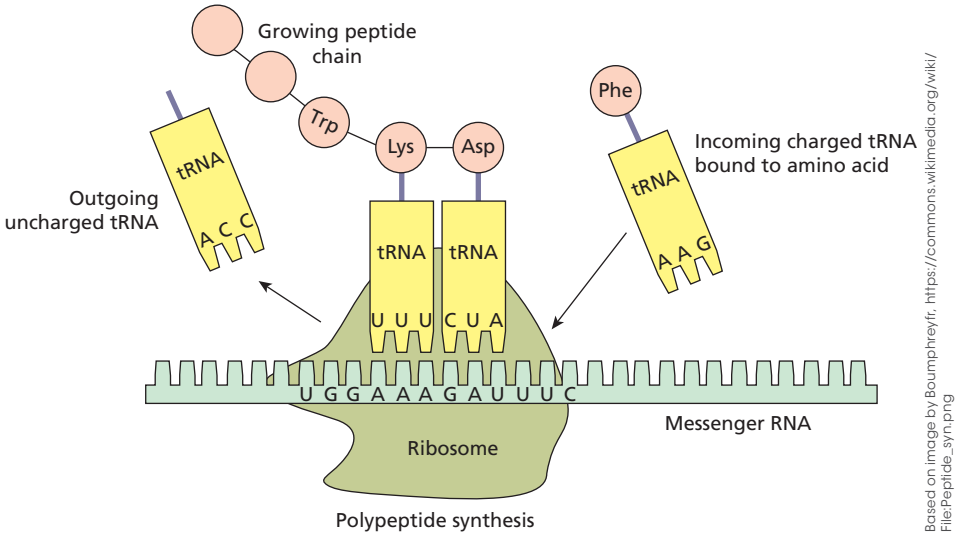


Figure 2.23 ▶
Through the action of tRNA, the mRNA dictates the order in which amino acids link up to form the polypeptide chain. The anticodons at the ends of tRNA molecules complement the codons in the mRNA.

The ribosome then moves on to the next codon of the mRNA strand, another charged tRNA molecule with a complementary anticodon sequence joins the codon and another amino acid is drawn into position, and so on. As the ribosome moves along the mRNA strand, more and more amino acids are joined by peptide bonds to the growing polypeptide chain. In this way, the amino acids are linked in an order corresponding to the sequence of codons in the mRNA. As this is determined by the sequence of base triplets in the original DNA, it follows that the base sequence in the DNA determines the order in which the amino acids link up.

Meanwhile other ribosomes are carrying on the same process, moving along the mRNA strand simultaneously, each synthesising a polypeptide chain as it goes. On reaching a stop codon the ribosome releases the mRNA strand and the newly synthesised polypeptide chain. A protein molecule is made up of one or more polypeptide chains joined together to make a three-dimensional structure.

RECALL

- Information in the DNA is coded into groups of three nucleotides, or triplets, and each triplet corresponds to a specific amino acid.
- In the cytoplasm, the mRNA is translated into polypeptide by ribosomes in the cytosol or ribosomes bound to the endoplasmic reticulum.
- Translation is accomplished with the assistance of tRNA molecules that bring amino acids into position for incorporation into the polypeptide.

RECAP 2.6

1 Explain what is meant by a 'degenerate' genetic code.
2 Explain the relationship between a codon and an anticodon.
3 Explain how charged and uncharged tRNA molecules relate to the process of translation.
4 A particular mature mRNA contains 102 nucleotides, excluding the 5′ and 3′ caps.
   a How many codons does this mRNA contain?
   b How many amino acids will be translated from the mRNA?

Gene regulation

The information in the DNA also prescribes which combinations of proteins are to be made in particular cells or tissues, and under what circumstances particular proteins are to be made. The process of turning gene expression on or off is referred to as **gene regulation**. Genes are regulated during cell differentiation, development, or in response to physiological or environmental cues. There are a number of mechanisms cells employ to regulate gene expression. As more genomes are sequenced and studied, more may be discovered. For our purposes, it is instructive to consider the functional difference between two types of genes.

Regulatory genes are those involved in controlling the expression of one or more other genes. The products of these genes may be functional pieces of RNA or proteins. The proteins may be enzymes, signalling molecules, receptor molecules, or DNA-binding proteins. The key feature of the regulatory gene is that its product alters the expression of other genes.

Structural genes are any genes that are not regulatory genes. Structural genes are, however, regulated by regulatory genes.

In order to appreciate the interaction between regulatory and structural genes, we shall explore the example of the *lac* operon in the bacterium *Escherichia coli*.

An example of gene regulation

The bacterium *E. coli* inhabits the mammalian intestine, living on sugars and other nutrients. During infancy, mammals feed on milk from their mothers. This milk contains the sugar lactose. If lactose is present, *E. coli* produces proteins to import it into the cell and break it down. If lactose is absent, these proteins are not produced as it would be a waste of the bacterium's resources to do so. If lactose is supplied to the *E. coli* that has been starved of lactose, the bacteria will start synthesising the necessary proteins.

E. coli uses three proteins in this process: β-galactosidase, β-galactosidase permease, and β-galactosidase transacetylase. One of the proteins facilitates lactose transport into the cell. The other two proteins aid in digesting and processing lactose. The coding regions for the three corresponding genes appear next to each other on the *E. coli* genome. They are under the control of one promoter and are transcribed as a single mRNA strand. As these three structural genes are expressed as a single unit they are described as an **operon**. This is the *lac* operon (Figure 2.24).

Elsewhere in the *E. coli* genome is a regulatory gene that codes for a transcription factor. A **transcription factor** is any protein that binds to DNA to control the rate of transcription from a gene. Transcription factors can serve to initiate or enhance transcription, or they may act to prevent it. The transcription factor for the *lac* operon is a **repressor protein** (Figure 2.24a). When lactose is absent, the repressor protein binds to a region of non-coding DNA, called an **operator** (Figure 2.24c). The binding sites of the operator are situated around the promoter in the *lac* operon. Binding of the repressor protein blocks RNA polymerase from attaching to the promoter region. The three genes of the *lac* operon cannot be transcribed and so gene expression is switched off.

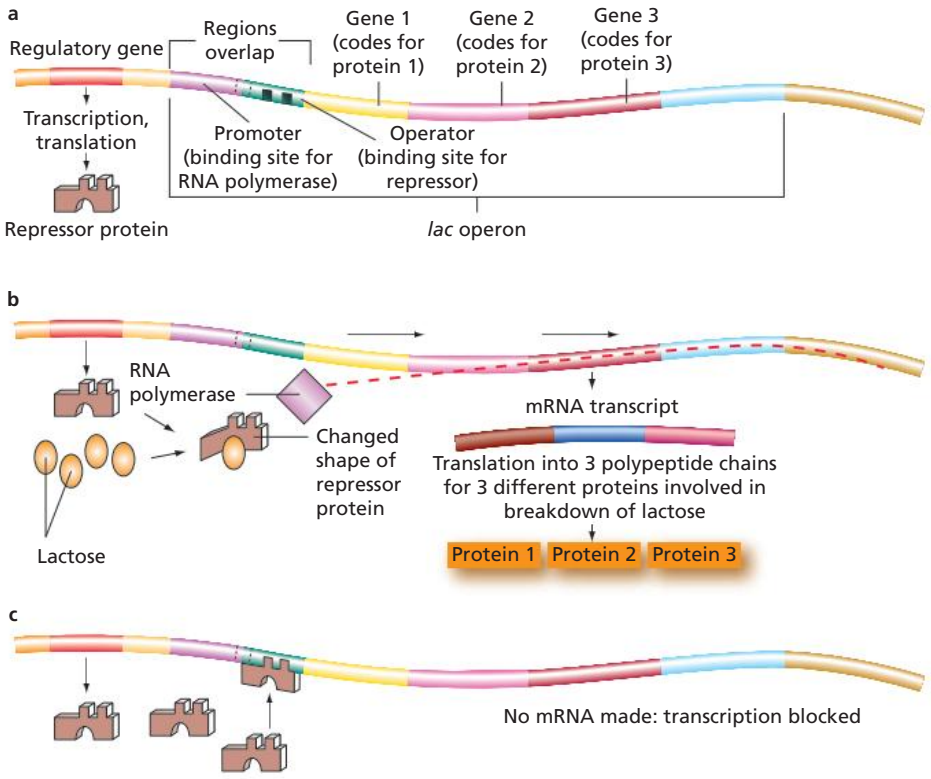


Figure 2.24 ▶
(**a**) The *lac* operon consists of an operator (the binding site for the repressor protein), a promoter (binding site for RNA polymerase) and three structural genes that code for proteins involved in lactose breakdown. A repressor protein regulates the three genes by binding to the operator and inhibiting transcription. (**b**) When lactose is present, it binds the repressor, altering its shape so that it cannot bind to the operator. RNA polymerase can transcribe the three genes into a single mRNA transcript. (**c**) When lactose is absent, the repressor protein binds the operator, covering part of the promoter. This means that RNA polymerase cannot bind to the promoter and transcription of the genes is blocked.

When lactose is introduced to the bacterium's environment, some enters the *E. coli* cell. The lactose functions as an **inducer**, a kind of signalling molecule, that activates the *lac* operon. To achieve this, lactose binds to the repressor, altering its shape so that it cannot bind to the operator. The promoter is now exposed for RNA polymerase to bind, and the three structural genes are transcribed into mRNA. Gene expression is switched on (Figure 2.24b).

The binding of the lactose and the repressor is reversible. For as long as lactose concentrations are sufficiently high, lactose binds the repressor and keeps the *lac* operon activated. When lactose levels drop, the repressor binding declines. Without lactose, the repressor protein returns to its original shape and attaches to the operator in the *lac* operon. RNA polymerase can no longer bind to the promoter and transcription of the structural genes stops.

RECALL

- Gene regulation is the process of switching on or switching off gene expression. The *lac* operon is an example of gene regulation.
- A regulatory gene codes for a repressor protein that blocks RNA polymerase from transcribing the *lac* operon.
- Lactose acts as an inducer that interacts with the repressor to switch on expression of the *lac* operon genes.

RECAP 2.7

1 What is the difference between a regulatory gene and a structural gene?
2 What is meant by an 'operon'?
3 How does the *lac* repressor block transcription?
4 How does lactose switch on gene expression at the *lac* operon?
5 What benefit is there to the cell by regulating gene expression of the *lac* operon?

ACTIVITY 2.1

TRANSMITTING THE CODE

The nucleus of eukaryotic cells contains DNA, the molecule that encodes for all the proteins produced by the cell. The sites of synthesis of proteins, the ribosomes, are found in the cytoplasm, outside the boundary of the nucleus. DNA is unable to leave the nucleus, so in order to produce a protein a message must be sent from the nuclear DNA to the ribosome.

To do this, two processes take place:

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

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

Aim

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

You will need

Each pair of students requires:

- paper
- scissors
- coloured pencils

What to do

The sequence of nucleotides below belongs to the *lol* gene from a fungus.

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

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

1 Draw the label 'DNA' and the sequence of nucleotides for each strand on two separate strips of paper. Ensure the base pairs align when the two strips are brought together.

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

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

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

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

Figure 2.20 shows the mRNA codon sequences for each of the 20 amino acids needed to produce all the proteins required by cells.

4 Manoeuvre the mRNA transcript to the cytoplasm of your model cell. Place a fourth strip alongside it and label it 'polypeptide'. Use the genetic code table to translate the mRNA codons into amino acids.

5 Complete Table 2.2 to show the sequences.

Table 2.2 Transcription and translation of the *lol* gene

mRNA sequence						
mRNA codons						
tRNA anticodons						
amino acid sequence						

What did you discover?

1 a Identify from the model which DNA strand was the coding strand, and which was the template strand for transcription.
   b Explain how you decided which strand to use as the template strand.
   c Describe the relationship of the nucleotide sequence in the mRNA with the nucleotide sequences of the coding and template strands.
2 Explain how you decided which end of the mRNA to start translating from.
3 What is the first amino acid in the polypeptide sequence? Is it possible for any amino acid to appear first in any polypeptide? Explain.
4 How many nucleotides were in the mRNA sequence? How many amino acids were made? What relationship is there between the number of nucleotides in the mRNA transcript and the number of amino acids in the translated polypeptide?
5 Has this model of transcription and translation made the processes easier to understand?
6 Identify any improvements that could be made to this model to make the processes easier to understand.

RECALL

- The direction of RNA synthesis and the antiparallel structure of DNA determine which are the coding and which are the template strands.
- The nucleotide sequence of a gene is transmitted from DNA to mRNA to tRNA through complementary base pairing.
- The sequence of the mRNA is identical to that of the coding DNA strand except that T in DNA is replaced by U in mRNA.

RECAP 2.8

1 Which is the growing end of the RNA during transcription?
2 How does the antiparallel structure of DNA determine the template strand?
3 Explain the relationship in the sequence between the triplets in the coding strand of DNA, the mRNA codons, and the tRNA anticodons.
4 Do all codons code for an amino acid? Explain.

Biological knowledge and society: The speed gene


Getty Images/Heinz Kluetmeier/Sports Illustrated

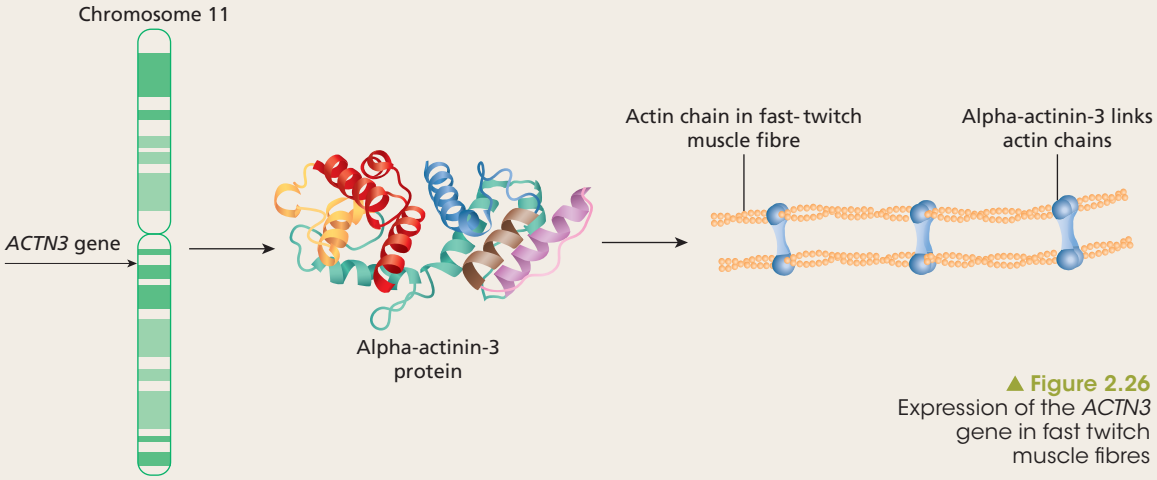

amanaimages/Tim Williams/ActionPlus

Figure 2.25 ▲
Studies reveal genetic differences between elite sprint (left) and marathon (right) runners.

A gene located on chromosome 11 called the *ACTN3* gene has generated controversy. It has been dubbed the 'speed gene' as it is correlated with elite athletic ability in sprint events. A controversy has arisen around genetic screening for the speed gene and concerns of discrimination and the potential for 'designer athletes' and gene doping.

The genetics

The protein product of the *ACTN3* gene is called alpha-actinin-3. It is expressed in fast twitch muscle fibres where it connects actin protein chains to coordinate fast, repetitive and powerful muscle contractions (Figure 2.26). These fast twitch muscle fibres are powered by glucose and use anaerobic glycolysis pathways to provide energy in the form of ATP. (See Chapter 3 for more information.)

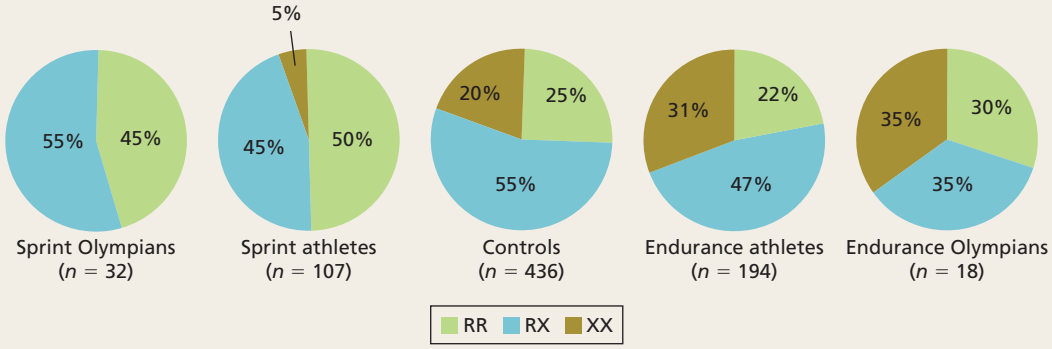


▲ **Figure 2.26** Expression of the *ACTN3* gene in fast twitch muscle fibres

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

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

A genetic test can be carried out to determine the *ACTN3* allele status of individuals (you will learn about these tests in Chapter 11). In 2003 Professor North collaborated on a study with the Australian Institute of Sport (AIS). The study involved determining the *ACTN3* allele status for: 436 people in the general population as a control; 32 sprint Olympians; 107 sprint athletes; 194 endurance athletes; and 18 endurance Olympians. The results are shown in Figure 2.27.



◀ **Figure 2.27** The *ACTN3* allele status of Olympian sprint and endurance athletes, non-Olympian sprint and endurance athletes, and the general population (controls) (adapted from a paper by Berman and North, 2010).

'A Gene for Speed: The Emerging Role of α-Actinin-3 in Muscle Metabolism', Yemima Berman, Kathryn N. North, *Physiology*, 1 August 2010 Vol. 25 no. 4, 250-259 DOI: 10.1152/physiol.00008.2010, © 2010 the American Physiological Society, adapted, percentages shown are approximates

Alpha-actinin-3 influence on athletic performance

To study the effect of alpha-actinin-3, scientists generated a strain of *ACTN3* knockout (KO) mice that could not produce the protein. They found the KO mice produced higher amounts of a protein called alpha-actinin-2. This closely related protein is produced to replace the missing alpha-actinin-3. Table 2.3 shows results of further tests comparing the KO strain to controls.

Table 2.3 Overview of results comparing knockout mice that cannot produce alpha-actinin-3 with control mice that do produce this protein (summarised from a paper by Berman and North, 2010).

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

Identifying youth with high athletic potential is big business around the world, with many countries, including Australia, investing in programs to identify and train athletes. The Australian Institute of Sport provides programs to adolescents based on physical and psychological tests.

Many parents are also willing to invest in their children's sporting future. Similar results to those seen in Figure 2.27 have been replicated in a number of independent studies around the world, so they are considered reliable. Companies such as 23andMe and Atlas Sports Genetics are offering genetic tests to consumers. Atlas First recommend parents test children aged 0 to 8 years of age in order to provide early information on genetic predisposition for success in speed/power or endurance events. Critics are concerned that these companies are misrepresenting the science. Professor North cautions that the *ACTN3* gene in isolation is a poor predictor. The athlete phenotype is complex, with hundreds of genes involved and environment playing an important role.

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

Over to you

1 What are the issues?

2 Identify the relevant biology that relates to these issues.

3 How can the results of the North (2003) study shown in Figure 2.27 be explained by the results of the Berman and North (2010) study shown in Table 2.3?

4 Referring to the results in Table 2.3, discuss the altered muscle performance in KO mice and then compose a theory as to why an absence of alpha-actinin-3 leads to increased endurance performance and decreased sprint and power performance.

5 List the social and ethical implications and issues that could arise from the use of genetic information in athlete selection.

References

Berman, Y., and North, K. (2010). A gene for speed: The emerging role of alpha-actinin-3 in muscle metabolism. *Physiology*. 25(4): 250–259.
National Health and Medical Research Council (2013). Use of genetic information in sport. Retrieved 19 October 2015 from https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/g003_genetic_sequencing_in_sport_150622.pdf

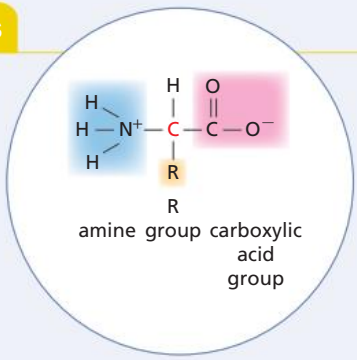
CONCEPT SUMMARY

Proteins

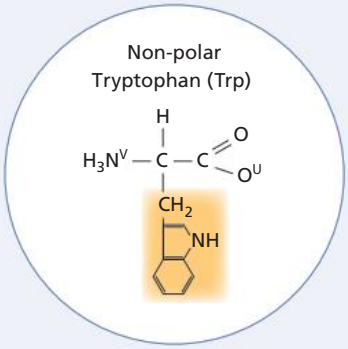
Types of protein

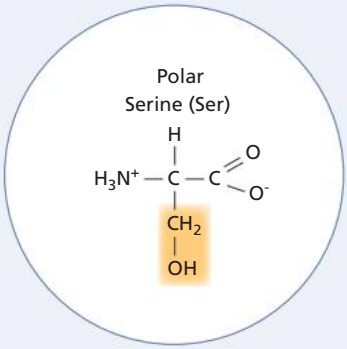
- Motility
- Structure
- Enzymes
- Transport
- Hormones
- Cell-surface receptors
- Neurotransmitters
- Immunoglobulins
- Poisons or toxins

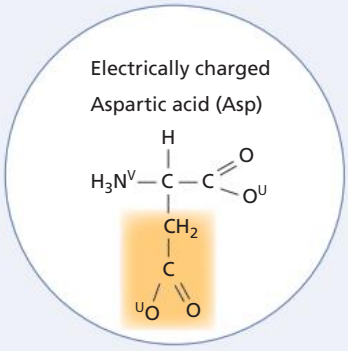
Amino acids



Types of amino acids







- Only 20 different amino acids are found in the proteins of living organisms; the difference in the R group is what distinguishes one amino acid from another and gives each its particular chemical properties.
- The R group can be uncharged and non-polar, making it hydrophobic.
- R group can be charged or polar, making it hydrophilic.

Structure of proteins

- Primary structure: a sequence of amino acids in the polypeptide chain
- Secondary structure: hydrogen bonding within the polypeptide chain forms α-helices and β-pleated sheets
- Tertiary structure: R groups of amino acids interact to result in hydrogen bonds, ionic bonds or disulfide bridges between adjacent cysteine amino acids, giving the protein its functional conformation
- Quaternary structure: hydrogen bonds, ionic bonds and covalent bonds of different polypeptide chains associate to form the mature protein

» Heat or solutions of high pH or different pH to their native environment can cause proteins to denature.

» A proteome is the complete set of proteins in a cell, organ, or tissue.

Genetic code

The genetic code is transferred from the nucleotide sequence of DNA, to the codon sequence of mRNA, via the anticodon sequence of tRNA, to the amino acid sequence of the protein that is produced during transcription. One codon can signal a transcriptional start site, and three codons act as transcription stop sites.

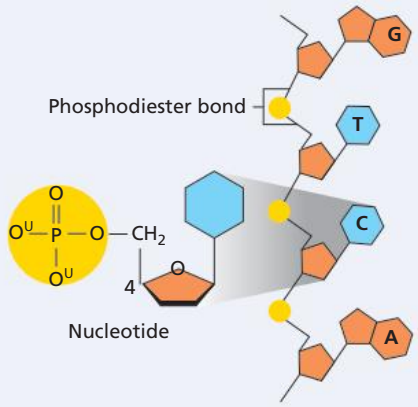
Gene regulation

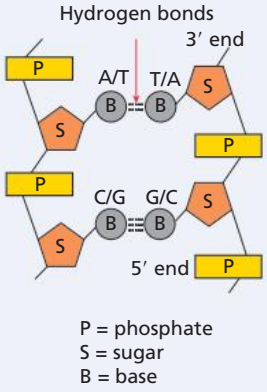
- Involves switching gene expression on/off, up/down
- Important during cell differentiation, development, or in response to physiological or environmental cues.
- Regulatory gene products control gene expression. They include RNA, enzymes, signalling molecules, receptor molecules and DNA-binding proteins such as transcription factors.
- Structural genes: any genes that are not regulatory genes

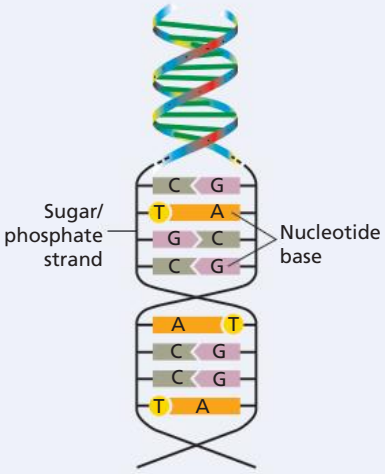
9780170368360

Nucleic acids

- Polymers of nucleotides joined together by phosphodiester bonds (between the 5′ phosphate group and the 3′ hydroxyl group) that form the backbone of the molecule
- DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) differ in the structure of their nucleotides







- The two strands of the DNA molecule are aligned in an antiparallel arrangement with opposing strands binding via hydrogen bonding according to the complementary base-pairing rule.

Differences between DNA and RNA

- The deoxyribose sugar of DNA has one less oxygen atom at carbon 2′ than the ribose sugar of RNA.
- The nitrogenous base thymine (T) in DNA is replaced by the base uracil (U) in RNA.
- RNA tends to be single-stranded with a variety of folding patterns. DNA is double-stranded and folds into a double helix.
- RNA molecules are much shorter than DNA molecules – a few thousand nucleotides compared with hundreds of millions of base pairs.
- Double-stranded DNA is a persistent, long-lived molecule, responsible for inheritance.
- RNA is a short-lived molecule with a variety of functions that facilitate and regulate protein production (mRNA, rRNA, tRNA).
- DNA is packaged in chromosomes, containing the double helix wound around histones to form nucleosomes.
- DNA contains genes, which can exist as different sequence variants called alleles.
- The genome is the complete set of DNA in a cell.

Transcription

DNA is the template molecule. It is copied into pre-messenger RNA (pre-mRNA), then processed into mature mRNA that can leave the nucleus. Key terms:

- Coding strand and template strand
- Promoter
- RNA polymerase
- Exons and introns, splicing and alternative splicing
- 5′ methylated cap and 3′ poly-A tail

Translation

mRNA is loaded into a ribosome and moves through it, serving as a template of codons that are used to build the polypeptide strand. Transfer RNA (tRNA) has anticodons and each anticodon is associated with a particular amino acid. Some ribosomes are bound to the surface of the rough ER. Cytoplasmic ribosomes can form polysomes along the mRNA strand.

CHAPTER GLOSSARY

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

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

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

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

amino acid a nitrogen-containing compound that is the building block of proteins

anticodon the three nucleotides in tRNA that bind to mRNA following base-pairing rules

antiparallel parallel but orientated in opposite directions

base pair a pair of complementary nitrogen bases linked by hydrogen bonding

biological functionality the function of a protein

charged tRNA tRNA when linked to its appropriate amino acid

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

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

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

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T and C pairs with G

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

conformation the proper or functional shape of a protein

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

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

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

disulfide bridge a strong bond formed between two sulfur atoms within a protein

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

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

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

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

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

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

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

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

histone a protein that binds and packages DNA in eukaryotic chromosomes

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

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

inducer a signalling molecule that switches on expression of a gene

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

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

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

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

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

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

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

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

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

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

polyadenylation the process of adding a chain of adenine nucleotides to the 3′ end of an mRNA strand

polypeptide a linear polymer built from amino acid monomers

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

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

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

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

protein a polymer built from amino acid monomers; may comprise a single such polymer or multiple polymers bonded together into a functional molecule

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

proteomics the study of proteomes

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

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

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

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

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

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

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

RNA polymerase the enzyme that catalyses the synthesis of RNA

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

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

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

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

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

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

transcribe copy DNA into RNA

transcription the process by which DNA is copied into RNA

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

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

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

uncharged tRNA tRNA without an attached amino acid

CHAPTER REVIEW QUESTIONS

Remembering

1. Why is nitrogen (N) considered to be an essential element for all living things?
2. List two ways that different tRNAs are the same, and two ways they are different.
3. Outline the main steps in protein synthesis.

Understanding

4. All amino acids contain the same two functional groups. How then do the 20 amino acids differ from one another?
5. Polymers result when bonds between monomers are formed with the removal of water. Suggest a way that the bonds between monomers could be broken. Justify your answer.
6. Explain how some proteins are located in the cytosol, whereas others are secreted by the cell.
7. One strand of DNA consists of the base sequence ATGCGTACTCAATAG.
   - a Write:
     - i the sequence of bases for the complementary DNA strand
     - ii the sequence of bases in an RNA copy of the original DNA strand.
   - b Translate the RNA into amino acids.

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

Applying

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

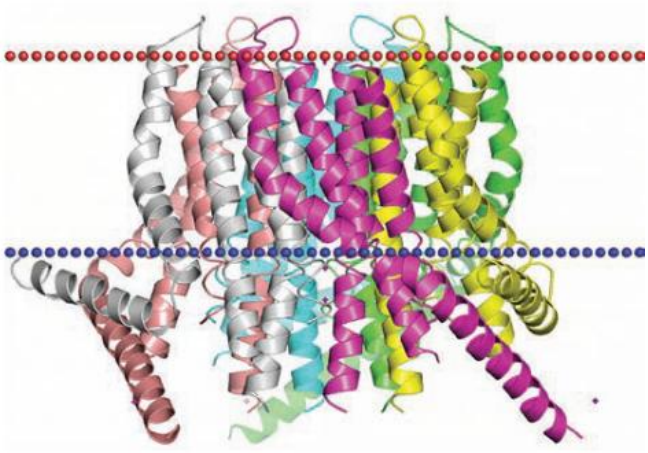
10 Antibodies, which are proteins of the immune system, contain many disulfide bridges. Suggest why this feature of antibodies might suit them during a fever.

11 Histones are composed predominantly of positively charged amino acids.
   a Would these proteins be soluble or insoluble in water? Explain why.
   b Explain why histone proteins are suited to packaging DNA into chromosomes.

12 The *Ara* operon of *E. coli* contains three genes that import and digest the five-carbon sugar arabinose. The *Ara* operon is under the control of a protein called AraC. Gene expression from the operon is normally switched off. If arabinose is present, gene expression from the operon is switched on. Use your understanding of the *lac* operon to describe the mechanisms regulating gene expression at the *Ara* operon. Use annotated diagrams to show what is happening in the absence and presence of arabinose.

Analysing

13 Figure 2.28 shows a calcium channel protein inserted in a membrane.
   a What sort of technique might have been used to determine the structure of the protein?
   b What type of secondary structure dominates the protein?
   c What properties might the amino acids on the face of the protein embedded in the membrane have?
   d What properties might the amino acids have where they line the inside of the channel?
   e Each polypeptide chain in the protein is coloured differently. How many polypeptides make up the mature protein, and what level of protein structure is this?



Andrei L. Lomize, OPM Database

Figure 2.28 ▲
Calcium channel protein 1 embedded in the membrane of the endoplasmic reticulum. The red and blue circles mark the boundary of the membrane. The protein transports calcium ions from the cytosolic side of the membrane (red circles) to the inside of the endoplasmic reticulum (blue circles).

Evaluating

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

Creating

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

CHAPTER 3

STRUCTURE AND REGULATION OF BIOCHEMICAL PATHWAYS

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

KEY KNOWLEDGE

Structure and regulation of biochemical pathways

- the role of enzymes as protein catalysts in biochemical pathways
- the mode of action of enzymes including reversible and irreversible inhibition of their action due to chemical competitors at the active site, and by factors including temperature, concentration and pH
- the cycling of coenzymes (ATP, NADH, and NADPH) as loaded and unloaded forms to move energy, protons and electrons between reactions in the cell.

Photosynthesis

- the purpose of photosynthesis
- chloroplasts as the site of photosynthesis, an overview of their structure and evidence of their bacterial origins
- inputs and outputs of the light dependent and light independent (Calvin cycle) stages of photosynthesis in C3 plants (details of the biochemical pathway mechanisms are not required)
- factors that affect the rate of photosynthesis, including light, temperature and carbon dioxide concentration.

Cellular respiration

- the purpose of cellular respiration
- the location of, and the inputs and outputs of, glycolysis including ATP yield (details of the biochemical pathway mechanisms are not required)
- mitochondria as the site of aerobic cellular respiration, an overview of their structure and evidence of their bacterial origins
- the main inputs and outputs of the Krebs (citric acid) cycle and electron transport chain including ATP yield (details of the biochemical pathway mechanisms are not required)
- the location of anaerobic cellular respiration, its inputs and the difference in outputs between animals and yeasts including ATP yield
- factors that affect the rate of cellular respiration, including temperature, glucose availability and oxygen concentration.

Biological knowledge and society

- techniques that apply DNA knowledge (specifically gene cloning, genetic screening and DNA profiling) including social and ethical implications and issues
- the distinction between genetically modified and transgenic organisms, their use in agriculture to increase crop productivity and to provide resistance to insect predation and/or disease, and the biological, social and ethical implications that are raised by their use

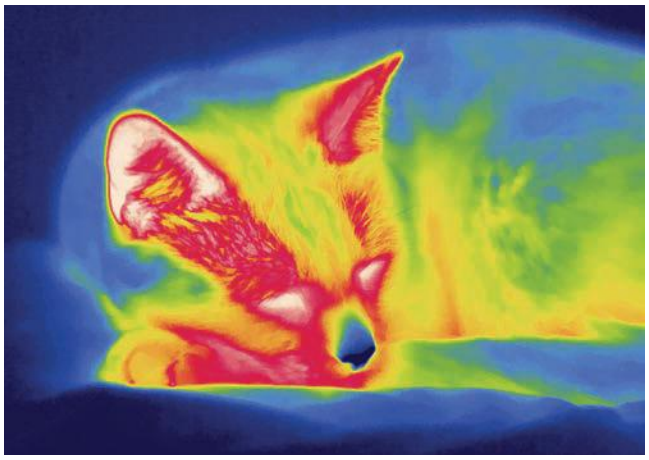
KEY SCIENCE SKILLS

Comply with safety and ethical guidelines

- apply relevant occupational health and safety guidelines while undertaking practical investigations, including following relevant bioethical guidelines when handling live materials

Conduct investigations to collect and record data

- systematically generate, collect, record and summarise both qualitative and quantitative data



amanaimages/Tyrone Turner/National Geographic Creative

Figure 3.1 ▲
Thermal imaging reveals the heat emitted from objects. Living organisms with a high metabolism tend to give off more heat, which is why mammals, such as cats, glow more brightly than plants.

If we viewed the world using infrared vision, we would see only the thermal radiation that is emitted from objects and it would look a very different place. Living organisms would stand out brightly from inanimate objects as they give off more heat (Figure 3.1). This heat is generated by the activity of cells as they break down and build molecules at a furious pace.

Cellular metabolism

The sum of the thousands of chemical reactions that occur constantly in each living cell is known as **cellular metabolism**. The rate of cellular metabolism varies among organisms and thus the amount of heat energy given off varies. The rate of cellular metabolism also affects the life span of an organism. For instance, the antechinus, a small mammal, has a very short life span due, in part, to having a very high rate of metabolism, while trees, which have a slower rate of metabolism, can live for a very long time. Next time you are stirring sugar into a drink, spare a thought for the factories that produced it – not the refinery, but the sugar cane plant. It may seem that plants are not particularly busy with their sedentary lifestyle, but appearances can be deceptive. A journey into the cells of any plant reveals a world full of chemical activity.

Chlorophyll molecules in plant leaves capture solar energy from the Sun and use this energy to split water molecules and excite electrons. Energy harnessed from these electrons is trapped in chemical bonds when carbon dioxide molecules and hydrogen atoms from water are joined to form glucose. This process, called photosynthesis, occurs in plants and some single-celled protists. Chemical reactions in which atoms and molecules are joined together to make more complex molecules are called **anabolic reactions**. Energy is required to form new chemical bonds, and reactions that require an input of energy are called **endergonic reactions**.

If you hold a match to a peanut in the presence of oxygen, the peanut will catch fire and give off heat as the bonds in the stored complex carbohydrates and oils are broken down. In a more controlled fashion, cells process nutrient molecules to provide energy to maintain cellular processes. The energy stored in the bonds of glucose molecules is used to provide the cell with useable energy in a reaction called cellular respiration. This reaction can occur either in the presence of oxygen (**aerobic cellular respiration**) or in the absence of oxygen (**anaerobic**). Reactions that break down complex molecules into simpler molecules are called **catabolic reactions**, and reactions that release energy are said to be **exergonic reactions**. Cells use the available energy released from catabolic reactions to fuel anabolic reactions (Figure 3.2).

The metabolic reactions that occur in cells do not take place haphazardly; all are controlled and regulated to maintain cell functions and to meet the energy needs of the cell. To achieve this, chemical reactions in cells occur in a series of regulated steps collectively called **biochemical pathways**. These reactions must occur at a rate that allows the cell to function. Each step in the pathway is controlled by an enzyme, which is a protein that speeds up the rate of chemical reactions without undergoing any change itself. Enzymes often require the assistance of other molecules to ensure that reactions are maintained and that the energy requirements can be met.

The main metabolic pathways that transfer energy through living systems relate particularly to photosynthesis and cellular respiration, reactions that transform energy to keep organisms alive.

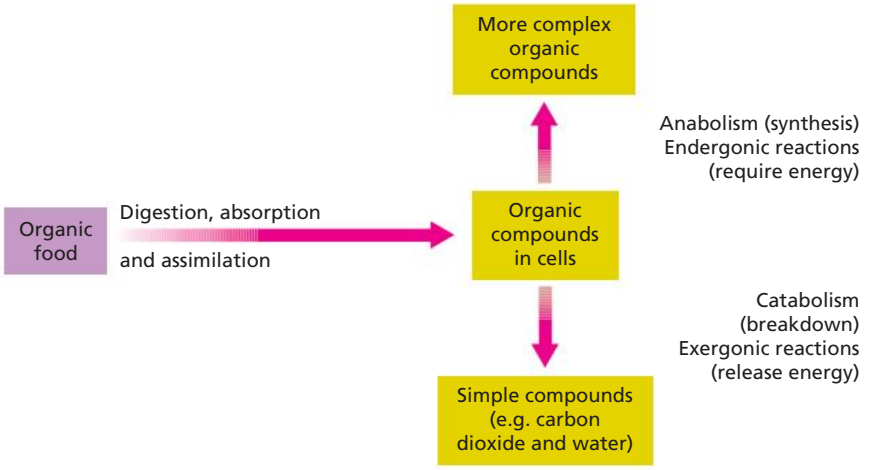


Figure 3.2
The relationship between catabolic and anabolic reactions in a typical animal cell. The ingested organic compounds may be either built up into more complex compounds or broken down into simpler compounds.

RECALL

- Cellular metabolism refers to all the chemical reactions that occur in each living cell.
- Biochemical reactions can build molecules (anabolic) or break them down (catabolic).
- Biochemical reactions can occur in the presence of oxygen (aerobic) or in its absence (anaerobic).
- Enzymes control most steps of a biochemical pathway.

RECAP 3.1

1 Distinguish between anabolic and catabolic reactions and give an example of each.
2 Define the term 'metabolism'.
3 'Endergonic reactions and exergonic reactions are interdependent.' Explain this statement.
4 Why do chemical reactions in cells proceed in a series of steps called a biochemical pathway?

Biochemical pathways

Biochemical pathways can be seen as systems. The system has inputs, which are processed, and outputs. Metabolism is the sum total of all the biochemical pathways and systems that occur in all living cells. Different cells have different requirements and rates at which they work. For example, heart muscle cells have a relatively high rate of metabolism to keep the heart beating. The oxygen that is transported in blood is used for cellular respiration in all cells, including cardiac muscles that contract together to form a heartbeat.

The energy released from catabolic reactions such as cellular respiration is shuttled into anabolic reactions, particularly those that recharge cellular energy stores. To understand how this works we need to understand the role of biological reactions in making and breaking molecules.

A biochemical process or reaction occurs when the chemical bonds of inputs or reactants are broken and the atoms recombine to form a new substance or substances – the output or products. In endergonic reactions, a total net amount of energy is absorbed and locked up in the bonds of the products, which have more stored energy than the

reactants. Endergonic reactions, therefore, use up energy. In exergonic reactions, a total net amount of energy is released from the bonds of the reactants and the products have less energy than the reactants (Figure 3.3). That is, exergonic reactions release energy.

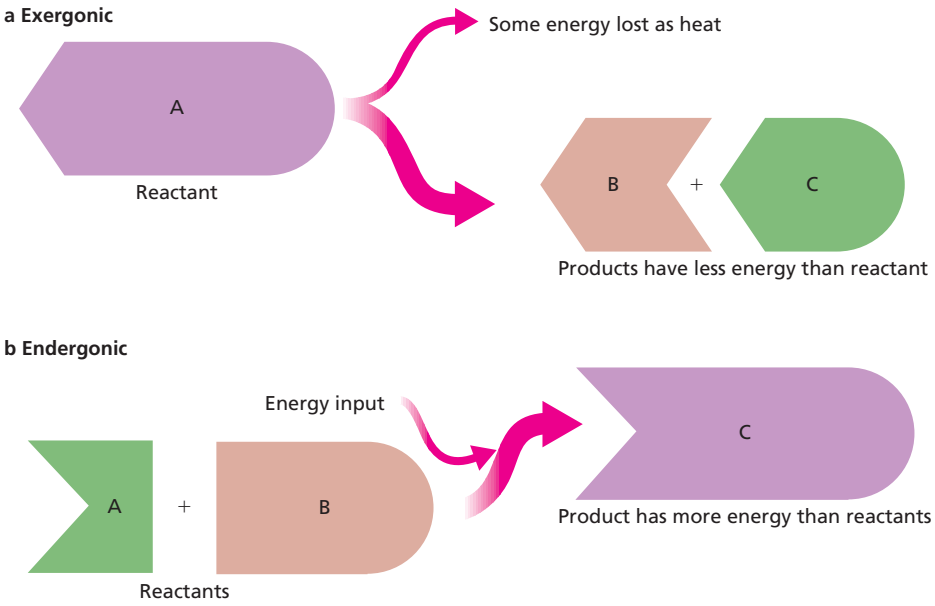


Figure 3.3 ▶
The reactants of (**a**) exergonic reactions have more energy than the products, but those of (**b**) endergonic reactions have less energy than the products.

$$A + B \rightarrow C + D$$
reactants products

Atoms and molecules in a cell do not stay still; they are constantly in motion and colliding. For reactants A and B above, a certain amount of energy is required for a reaction to occur to give rise to the products C and D. The amount of energy needed to strain and break the reactants' bonds is called the **activation energy**. When enough activation energy is available to break the chemical bonds of the reactants, new bonds form between the atoms, thus generating one or more products.

For cells to continue functioning, enough energy must be provided to maintain the process of generating products from reactants, and they must control the rate of energy released so they do not burn up like the peanut mentioned earlier. Chemical reactions are reversible under certain conditions, and it is important that products are removed from a cell so that they do not build up and thus slow down vital metabolic reactions. Thus, biochemical reactions go through a series of steps in which the product of one step becomes the reactant for the next step (see Figure 3.4). In this way, a product from one reaction is continually removed by being the reactant for the next reaction.

Cells have a number of ways of removing the final product from solution so that a biochemical pathway keeps operating in the right direction. In a plant cell, the final product of photosynthesis is glucose. Glucose, a soluble substance, is converted into

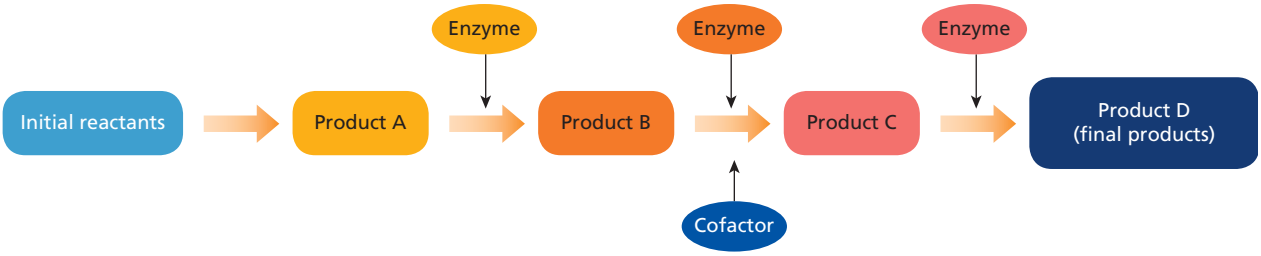


Figure 3.4 ▼
A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.

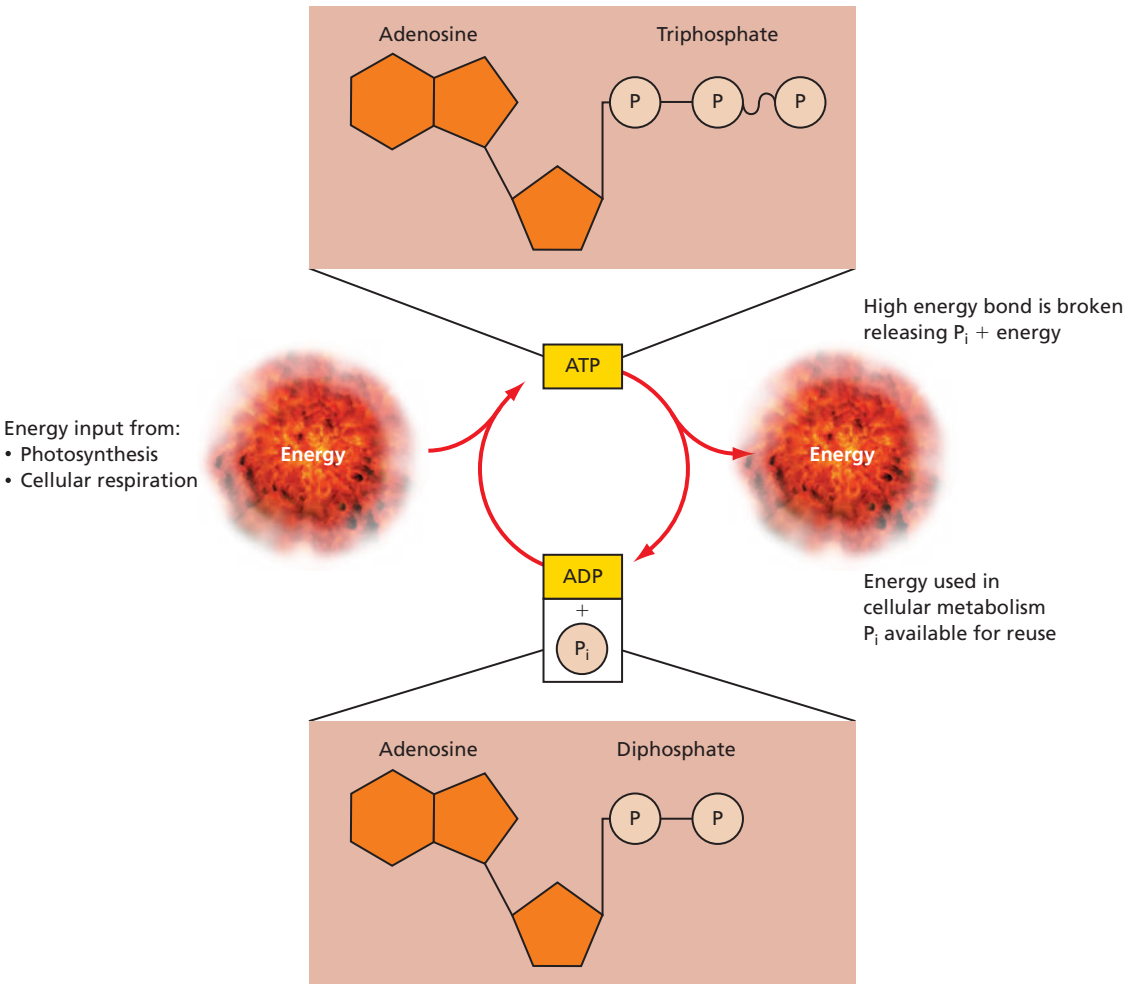
the insoluble polysaccharide starch, which is stored by the plant. Thus, the plant is able to continue to produce and store glucose. In cellular respiration the products diffuse from cells and are expelled into the atmosphere by a number of means. Each step in the biochemical pathways of photosynthesis and cellular respiration is controlled and regulated by enzymes, with energy being supplied or released.

The energy shuttle

Cells capture the chemical energy released from exergonic reactions to fuel endergonic reactions. The two reactions happen simultaneously in cells. In this process some energy is lost as heat, which escapes from cells into the atmosphere. As these reactions do not always occur in the same place within the cell, energy has to be transferred between reactions. This transfer is achieved by a molecule called **adenosine triphosphate (ATP)**. ATP is the universal primary source of free energy for all living organisms, from bacteria to humans. ATP contains adenosine attached to a sugar group (ribose), which is bound to a chain of three phosphate groups.

ATP is a renewable energy source. When a cell requires energy to drive an endergonic reaction, the high-energy chemical bonds attaching the last phosphate group to ATP are broken, thus releasing stored energy. This energy is now available to fuel a cellular reaction. The remaining molecule now has only two phosphate groups and is called **adenosine diphosphate (ADP)**. Free energy obtained from an exergonic reaction can be used to add a phosphate group to ADP, converting it to ATP. The ATP–ADP cycle is the cell's way of shuttling energy between reactions (Figure 3.5).

▼ **Figure 3.5**
The ATP–ADP cycle is the cell's way of renewing its supply of immediate energy.



The addition of a phosphate group to an organic molecule of any sort is called **phosphorylation**. This changes the conformation of a molecule, regulating its activity and usually causing the molecule to become more reactive. The ATP–ADP system provides an efficient linking or coupling of energy-yielding processes to energy-requiring processes within the cell by conserving, transferring and releasing energy.

RECALL

- Biochemical reactions can consume energy (endergonic) or release it (exergonic).
- The activation energy of a reaction is the energy required to break the specific bonds in the reactant molecule(s) that allow new bonds to form and create the product molecule(s).
- ATP is the primary source of available energy for all living things. It is produced by phosphorylation of ADP.

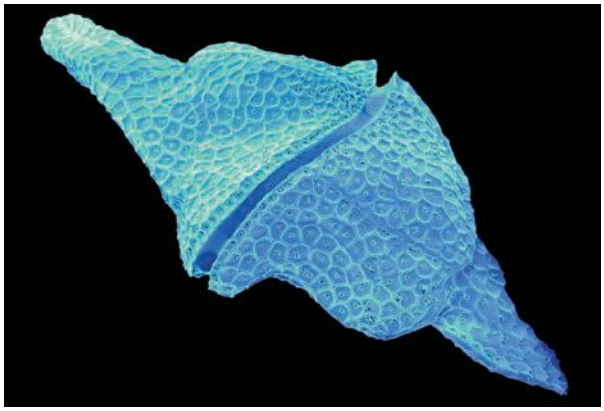
RECAP 3.2

1 What would happen to a biochemical reaction if the final product were not removed?
2 List five features of the ATP–ADP system.
3 Would an endergonic reaction result in the production of ATP or ADP molecules? Explain.

Enzymes control biochemical pathways

The rate of any chemical reaction increases with an increase in temperature. As the temperature rises, all the molecules in a system gain more kinetic energy and therefore move around more. Molecules collide with each other more often when they move around more, and this increases the chance that the specific reactants of a reaction will interact and undergo the reaction. However, this method of speeding up reactions does not work for cells, which operate at a constant temperature. A rapid increase in temperature changes the shape of protein molecules, which means that they can no longer perform their function. When this occurs, proteins are said to be **denatured**. Denaturation of cellular proteins destroys the function of cells, which then die quickly. Since higher temperatures cannot be used to overcome the activation energy needs of reactants in cells without devastating the cell, the need to reach activation energy must be overcome in another fashion. So how do cells achieve this? The cell's answer to this is to use **enzymes**, proteins that act as **organic catalysts**.

Towards the end of the 19th century the German chemist Eduard Buchner was trying to find a way of preventing yeast extracts from going bad. In one trial he added sugar to yeast extract and, rather than preventing change, he found that the sugar was fermented and converted to alcohol. Louis Pasteur had already demonstrated that yeast was responsible for the fermentation of sugar, but Buchner took the research further. He showed that the fluid extracted from living yeast cells was responsible for fermentation, not the yeast cells themselves. To describe the active ingredient in the extract that caused the fermentation he coined the term enzyme, from the Greek word 'zýmē', meaning leavened. 'Enzyme' is now the collective term for the thousands of organic protein molecules extracted from cells and found to act as organic catalysts.



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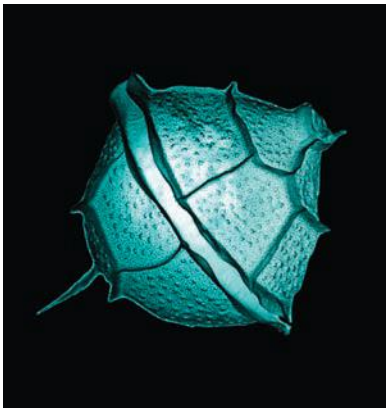

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◀ Figure 3.6
The glow of light in the waves at this beach is a result of the enzyme luciferase, which is expressed by microscopic dinoflagellates living in the sea water. Luciferase converts chemical energy to light energy.

Thus, cells use enzymes to speed up reactions. These biological **catalysts** interact with reactant molecules to increase the rate of reaction. Reactants that are acted upon by enzymes in biochemical reactions are called **substrates**.

As enzymes are not consumed in a reaction they can be recycled. Enzymes are the workhorses of the cell. Without enzymes, the reactions that occur in living organisms would be so slow as to hardly proceed at all, which would be incompatible with the maintenance of life.

Enzymes not only increase the rate of reactions, they also control them. Each step in a biochemical pathway is controlled and regulated by a specific enzyme. Over 1000 different reactions can take place in an individual cell in any given moment. The functional organisation that this demands is achieved by each individual reaction being catalysed by a specific enzyme in a particular place within the cell. Enzyme specificity is at the heart of how enzymes control each step in a biochemical pathway. There are as many enzymes in living organisms as there are types of chemical reactions, and each enzyme is specifically shaped to act on a particular substrate to speed up a reaction.

Naming enzymes

Even though enzymes are manufactured inside cells, their site of function may be either within the cell (intracellular) or outside the cell (extracellular). Intracellular enzymes speed up and control metabolic reactions inside cells. Extracellular enzymes are secreted from the cell and catalyse reactions outside the cell. For example, digestive enzymes are secreted from specialised cells in the lining of the gut and act on food in the gut.

Normally, an enzyme is named by attaching the suffix '-ase' to the name of the substrate on which it acts. Thus, carbohydrases act on carbohydrates, lipases act on lipids, proteases act on proteins, nucleases act on nucleic acids and ATPase acts on ATP, converting it to ADP and a free phosphate molecule, sometimes called inorganic phosphate (P_i). The '-ase' rule, however, does not always apply. For example, pepsin and trypsin, which are enzymes found in the mammalian gut, act on proteins. Both pepsin and trypsin are proteases that were discovered and named before the '-ase' naming system was introduced.

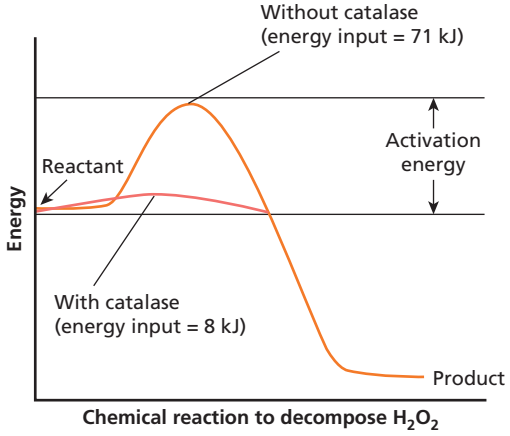


Figure 3.7 ▲
Catalase reduces the activation energy needed to break down hydrogen peroxide. Enzymes are powerful because they reduce the activation energy for chemical reactions.

Enzyme power

Enzymes generally work very rapidly. One of the fastest enzymes is catalase. This enzyme is found in several organs and tissues, including the liver, where its job is to speed up the decomposition of hydrogen peroxide (H_2O_2) into oxygen and water.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide is a toxic by-product of metabolism so it is essential that the cell removes it as fast as possible. Hydrogen peroxide has high activation energy, which means that the energy input required before it will decompose into oxygen and water is high.

The simple addition of some ferric ions (Fe^{3+}) into a solution of hydrogen peroxide increases the rate of decomposition by a factor of 30 000. The ferric ions act as a catalyst and lower the activation energy required for hydrogen peroxide to decompose. In living cells the enzyme catalase does a similar job (Figure 3.7). Catalase is a porphyrin, a catalytic protein containing an iron (Fe) atom. The decomposition of hydrogen peroxide in the presence of catalase can proceed up to 100 million times faster than without it. The action of enzymes in reducing the activation energy of reactions is represented graphically in Figure 3.7.

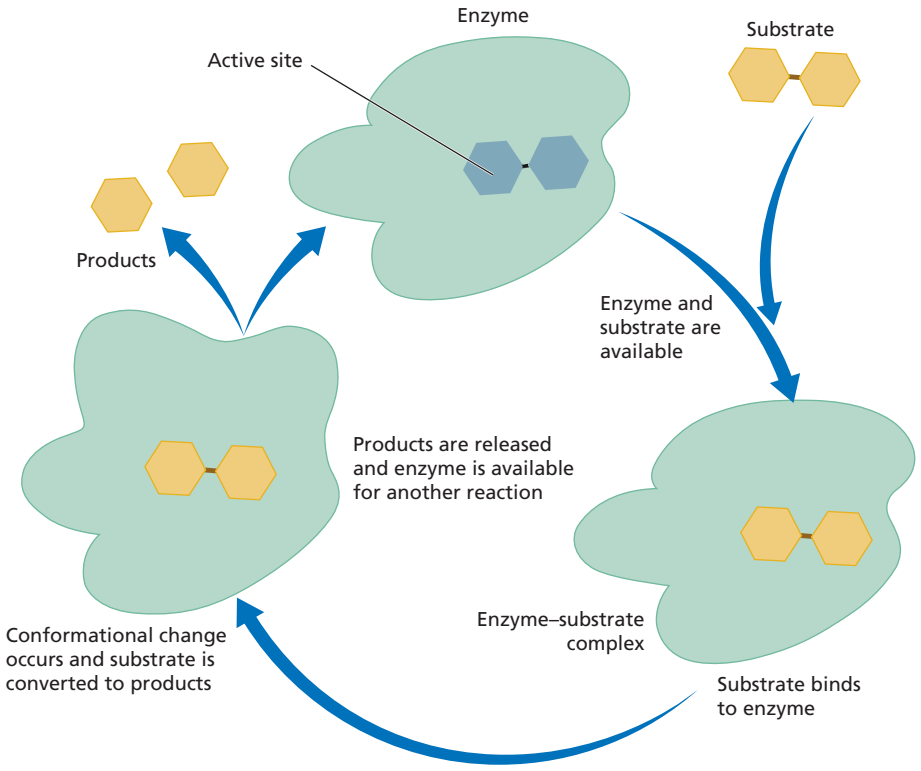
Models for enzyme action

Most enzymes are large globular proteins. In Chapter 2 we looked at the tertiary structure of proteins and how their shape determines their function. The folding of an enzyme into its tertiary structure forms a groove or pocket. This groove can accommodate one or more particular substrate molecules and is called the **active site** (Figure 3.8). An active site is highly specific for a particular substrate, which must be of a compatible shape for binding to occur. This model of enzyme action is known as the **lock-and-key model**.

The bonds that form between an enzyme and its substrate can also modify the shape of the enzyme so that the substrate can be fully accommodated by the enzyme. This interaction is called the **induced-fit model** of enzyme action (Figure 3.9). In this situation, the bonds within the substrate molecule are stretched and bent by molecular interactions with the amino acid groups that line the active site. The active site provides a particular environment, such as an acidic one. As a result of the stresses the active site's environment places on the substrates, the activation energy required to kick-start the reaction is dramatically lowered and new product molecules are formed at a faster rate. As the product molecules are not specific to the active site, they are released and the active site becomes available for another substrate molecule.

Enzymes need help

Some enzymes are inactive until they bind with other molecules or ions that change their conformation. This alters the shape and the charge of the enzyme's active site



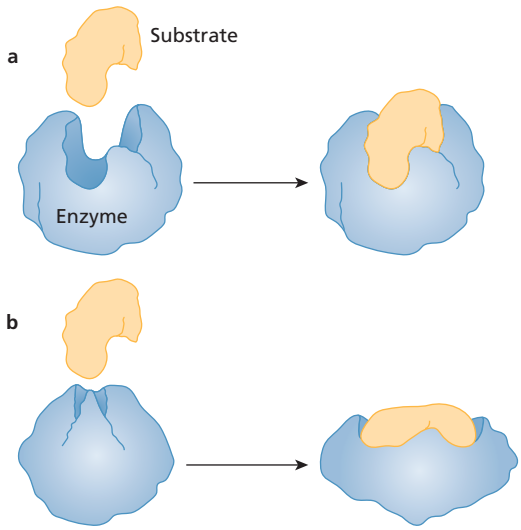
◀ **Figure 3.8**
Enzymes are highly specific molecules. A small part of the enzyme, called the active site, has the right shape, or conformation, to bind with a specific reactant (substrate). The conformational change that results prepares the substrate for reaction.

so that it can capture substrate molecules and catalyse reactions more efficiently. Two classes of substance bind to enzymes or to the substrate to activate the enzyme: **cofactors** and **coenzymes**.

Cofactors are small inorganic substances, such as zinc ions and magnesium ions. Cofactors sometimes bind amino acids to help the enzyme fold into its tertiary structure. For example, two negatively charged amino acids may need to come together for an enzyme to fold into its correct shape. Ordinarily, these two amino acids would repel each other. However, if they are bridged by a positively charged calcium ion, the enzyme folds properly. Sometimes cofactors are also found in the active site and assist the enzyme to carry out the reaction.

Coenzymes are non-protein organic substances that are required for enzyme activity. Coenzymes are relatively small molecules compared to the enzyme. Many are made by organisms from dietary vitamins, and act as carriers of small groups of atoms or ions to and from reactions that are catalysed by enzymes. In the course of participating in metabolic pathways, coenzymes are reversibly **loaded** and **unloaded** with the groups of atoms they carry. The cause of beri-beri, a disease that causes brain damage and affects short-term memory, is a deficiency of the vitamin thiamine, also known as vitamin B1. Thiamine is converted to the coenzyme thiamine pyrophosphate, which aids in the breakdown of glucose to pyruvate during cellular respiration. Beri-beri affects the brain because the brain depends on the metabolism of glucose for energy.

Other examples of coenzymes are NADH and NADPH, which are essential for photosynthesis and cellular respiration. This is discussed later in the chapter. These molecules are able to accept electrons and protons during biochemical reactions and transfer them to another reaction in a different step of the process.



▲ **Figure 3.9**
Lock-and-key model (**a**) versus the induced-fit model (**b**) for enzyme action. In the induced-fit model, the substrate molecule enters the enzyme's active site, causing the enzyme molecule to change its shape so that the two molecules fit together more closely.

RECALL

- Enzymes are biological catalysts that interact with substrate molecules to increase the rate of a reaction.
- Enzymes reduce the activation energy required for a biochemical reaction to proceed.
- Enzymes act on substrate molecules, which fit into the enzyme's active site with a lock-and-key fit, or by induced fit.
- Cofactors and coenzymes, which may be reversibly loaded and unloaded, may be required for full enzyme activation.

RECAP 3.3

1 Define the term 'catalyst'.
2 What is the role of enzymes in a cell?
3 What happens to an enzyme after it has catalysed a reaction?
4 How do enzymes affect the activation energy required by reactants for a reaction to occur?
5 Explain what is meant by the 'induced-fit model' of enzyme action. How is this different from the 'lock-and-key model'?

Factors affecting enzyme activity

Enzymes are involved in the processing of inputs within and outside cells. The intracellular and extracellular environments in which the enzymes work are regulated to ensure that enzymes perform in a manner suited to the cell's needs. Enzymes are sensitive to changes in substrate and product concentrations, temperature, pH, and other substances that may compete with a substrate for an active site.

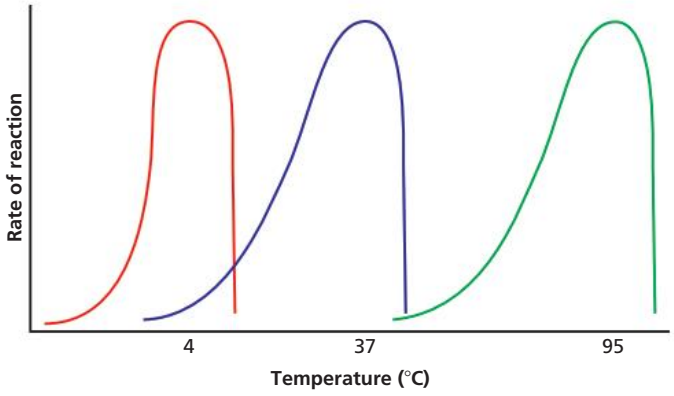
Effect of temperature

The activity of enzymes gradually increases with increasing temperature. This is because molecules become more excited, move around more quickly, and collide more often as they warm up. The increase in collisions increases the opportunity for a substrate to bump into its enzyme and enter its active site. The rate of reaction therefore increases as the temperature rises. As temperature continues to increase, the enzyme activity reaches a peak called the **optimum temperature**. This is the temperature at which the enzyme works at its fastest. But, if the temperature gets too high, the bonds that determine the three-dimensional shape of proteins can break. As a result, the protein loses its functional shape. It becomes denatured and the substrate can no longer fit into the active site. The enzyme's activity decreases.

The optimum temperature differs for different enzymes, and reflects the conditions in which the enzyme is normally found. For example, enzymes operating in the human body work best at temperatures of about 37°C (Figure 3.10), which is the relatively constant core temperature of the body.

The enzymes of **psychrophiles**, micro-organisms that live in near-freezing environments such as the wind-blasted rocks of snow-covered mountain summits, can operate at very low temperatures. This may be because some of the bonds that keep

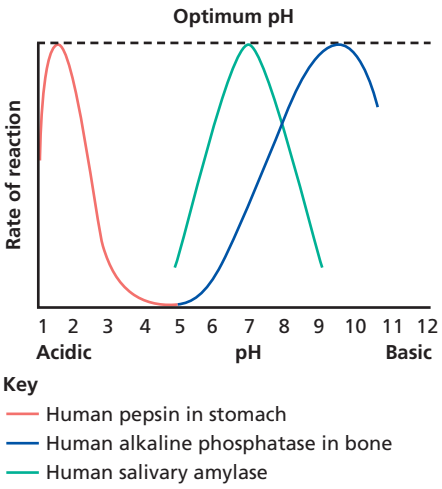
Figure 3.10 ▼
The temperature range for three different enzymes. Activity gradually increases until the optimum temperature for enzyme activity is reached. As temperature continues to increase, enzymes become denatured so the reaction rate decreases.



proteins rigidly folded are absent in psychrophiles. Having a more flexible structure means enzymes require less energy to work. The micro-organism *Pyrodictium* exists in the geothermally heated areas of the sea floor. It is a **thermophile**, and its enzymes operate best at temperatures of about 95–105°C. Enzymes from another thermophile, *Thermus aquaticus*, are regularly used in the laboratory. The enzyme *Taq* polymerase is used in a technique called the polymerase chain reaction, to make millions of copies of DNA, because it operates at the required reaction temperature of 72°C and is not denatured at the elevated temperatures required at times in the reaction. This technique is discussed in Chapter 11.

Effect of changing pH

The pH of the solution surrounding enzymes, whether acidic, basic or neutral, can have a profound effect on the structure and activity of the active site of an enzyme and its interactions with a substrate. Each enzyme has an **optimum pH** at which it works (Figure 3.11). Some enzymes can work in a wide range of pH environments, while others are very sensitive and will only work in a narrow pH range. Most enzymes work most effectively around a neutral pH of 7. The optimum pH of an enzyme is the pH at which it works at its fastest rate. Like optimum temperature, the optimum pH relates to the environment in which it works. Some enzymes work in environments of extreme pH, such as the enzyme pepsin, which operates in the acidic environment of the stomach. It has an optimum pH of 1.5. Amylase, which works in the neutral environment of the mouth, has an optimum pH of 7, and alkaline phosphatase, which is found in the relatively alkaline environment of the bone, has an optimal pH of 9.5.



▲ **Figure 3.11** The pH range for three different enzymes: the enzyme pepsin digests proteins in the acidic juices of the stomach; the enzyme salivary amylase digests carbohydrates in the mouth at a neutral pH; and the enzyme alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone.

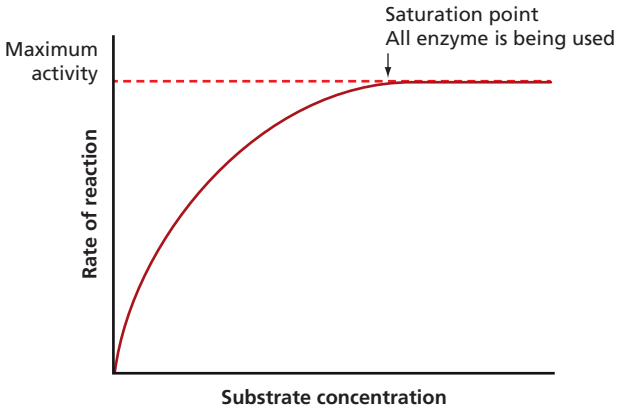
Buffered solutions, such as sodium phosphate, are better for enzyme activity than are non-buffered solutions, such as water. Cellular solutions need to be buffered because proteins influence the pH of a solution by donating hydrogen ions or hydroxyl ions. Changes in pH affect the amino acids making up a protein. As a solution becomes more basic, proteins tend to lose hydrogen ions. In acidic solutions, proteins gain hydrogen ions. If the charges on the amino acids in a protein are changed, then the bonds that maintain the three-dimensional structure of a protein may be broken. If, for example, the amino acids become mainly positively charged, the repulsion between the charges forces the polypeptide to unfold. Some enzymes make use of this, changing their shape in response to variations in pH. In most cases, if the pH varies, the protein shape is altered so much that the enzyme becomes denatured and can no longer catalyse a reaction, or the substrate may change shape so it no longer fits into an active site.

Effect of substrate and enzyme concentration

The amount of substrate or enzyme present in a reaction mix can limit the reaction rate and the amount of product formed. Increased amounts of substrate will result in more products being made until all the enzyme molecules are working at their maximum capacity (Figure 3.12). This is called the saturation point.

When the amount of enzyme in a system is increased, then the yield of product increases exponentially until the product starts to inhibit enzyme action or the substrate is depleted. The rate of reaction is proportional to the enzyme concentration, provided there is excess substrate present.

▼ **Figure 3.12** The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At saturation, further increases in substrate concentration do not increase the rate of the reaction.



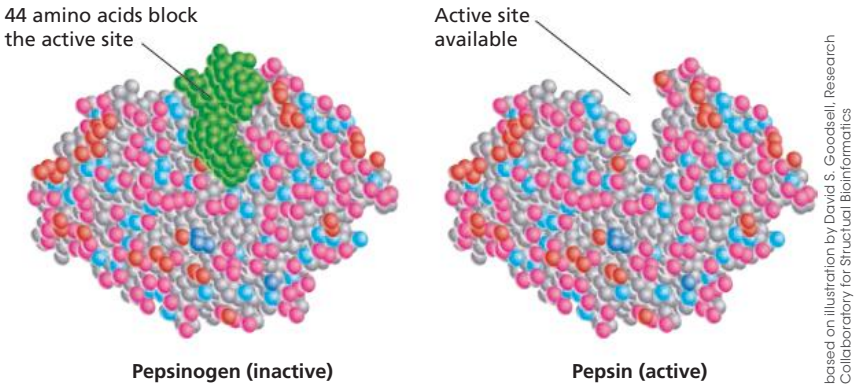


Figure 3.13 ▶
In the stomach, protein chains bind in the deep active site groove of pepsin so they are digested. Part of the polypeptide (shown in green) is removed from pepsinogen, the inactive form of pepsin. This then exposes the active site groove where proteins will bind.

Enzyme concentrations are regulated in response to the needs of a cell. This regulation is achieved by controlling the expression of the enzyme, the rate of degradation of the enzyme or by activating the enzyme in response to a stimulus. For example, pepsinogen is secreted as an inactive form of the enzyme pepsin. Pepsinogen is pepsin but with a 44-amino-acid extension that corks the enzyme's active site (Figure 3.13). Upon exposure to HCl in the stomach the extension is clipped off, revealing the active site. The pepsin is now activated to catalyse the digestion of proteins.

RECALL

- Enzymes are sensitive to temperature. Lower temperatures reduce activity and higher temperatures can denature enzymes, making them permanently inactive.
- Enzymes are sensitive to pH. Acidic environments can cause enzymes to gain hydrogen ions and alkaline environments can cause them to lose hydrogen ions.
- The relative concentrations of enzyme and substrate can affect the rate of a biochemical reaction.

RECAP 3.4

1 If an enzyme has become denatured, explain what has happened to the protein structure.
2 Outline two factors that can affect the activity of an enzyme.
3 A human protease works best at 37°C.
   a What would happen to the enzyme's activity at very low temperatures?
   b How does this differ from the activity of the enzyme at very high temperatures?
   c What has happened to the active site in both cases?

Inhibiting enzymes

Some enzymes have two or more active binding sites. These enzymes can move between their active and inactive state when inhibitor or activator molecules bind with them. The activity of many enzymes is regulated by **feedback inhibition**, in which the product of a reaction inhibits enzyme activity. If a large amount of product is present in the cell it will act as an inhibitor by binding to a site on the enzyme, other than the active site, thus slowing the rate of reaction. When the inhibitor binds to the enzyme, the active site of the enzyme changes shape so that it no longer has an affinity for its substrate. If the product is removed, then inhibition will be reduced, the substrate can enter the active site, and the product will again be formed. This helps cells keep the concentration

of products within a certain range. In this case the inhibitor does not bind with the enzyme's active site so it is said to be a **non-competitive inhibitor**.

Other inhibitors compete with the substrate for space in the active site and are said to be **competitive inhibitors** (Figure 3.14). For example, arsenic is an irreversible inhibitor that cannot be detected by the senses. Arsenate molecules resemble the phosphate substances used by cells for energy and signalling, and compete with them to bind to an enzyme's active site. Once arsenates are bound to an active site, the normal substrate is permanently excluded. Over time, less and less of the active enzyme remains to catalyse the reactions that produce energy for the cell. Thus, arsenic is called a chronic poison.

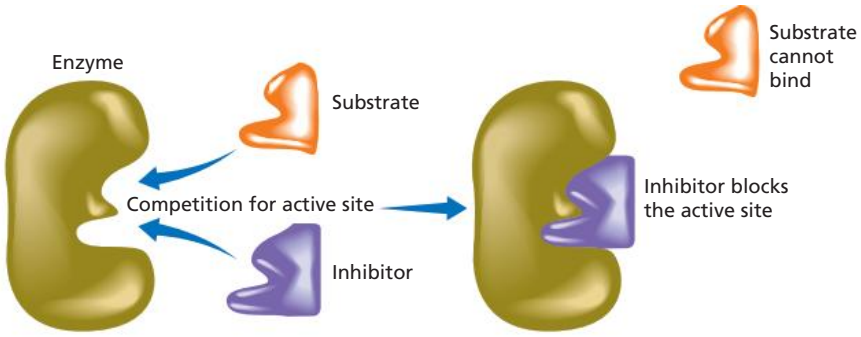


Figure 3.14 A competitive enzyme inhibitor blocks the active site of an enzyme so that the substrate can no longer fit in. Some inhibitors bind irreversibly so the enzyme can no longer perform its specific function.

RECALL

- A non-competitive inhibitor alters an enzyme's activity by changing its conformation without binding to its active site.
- A competitive inhibitor blocks the active site of an enzyme.
- The actions of inhibitors can be reversible or irreversible.

RECAP 3.5

1 How can the amount of product produced in a reaction affect an enzyme's activity?
2 Distinguish between a non-competitive inhibitor and a competitive inhibitor.
3 Why is arsenic called a 'chronic poison'?

Cycling energy in the biosphere

The biosphere is the collective term to describe all life forms on our planet. Nearly all life on Earth obtains energy directly or indirectly from the Sun, which bathes our planet with solar energy. Solar radiation is transformed into other types of energy that flow through the biosphere as organisms go about their daily business of living. Much of the energy is lost as heat energy: since chemical and physical processes do not convert one form of energy to another with 100% efficiency, the remainder is lost as heat. Living things within the biosphere can be grouped according to the way that they meet their energy and matter requirements. **Autotrophs** can manufacture organic material from inorganic material that they take in from their surroundings to meet their energy needs, whereas **heterotrophs** obtain their organic materials by feeding on other organisms and their products, which they then break down into simpler substances.

Autotrophs that capture solar energy are called **photoautotrophs**. They contain pigments that capture light energy, and use carbon dioxide as the sole source of carbon to produce organic molecules such as glucose in a process called photosynthesis. They include all plants, algae and photosynthetic bacteria (called cyanobacteria). However, not all autotrophs rely on solar energy as their primary energy source. Many bacteria, called **chemotrophs**, use various inorganic materials, including sulfur, hydrogen and iron, as their primary energy source.

Heterotrophic organisms ingest various organic molecules, such as carbohydrates, lipids and proteins. All animals, fungi, some protists and most bacteria are heterotrophs.

Both autotrophs and heterotrophs use organic molecules to fuel metabolic reactions. The organic molecules are broken down to release the stored energy in the process of cellular respiration. Thus, the energy stored in the chemical bonds is released to do work for the cell.

Generating chemical energy: photosynthesis

Photosynthesis is the process by which photoautotrophs capture light energy and use it to convert water and carbon dioxide to glucose, water and oxygen. In essence, the purpose of photosynthesis is to capture solar energy and convert it to chemical energy in the form of glucose, a sugar, which can later be used as fuel for the plant. Photosynthesis can be summarised by the following equation.

$$12H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C_6H_{12}O_6 + 6H_2O$$

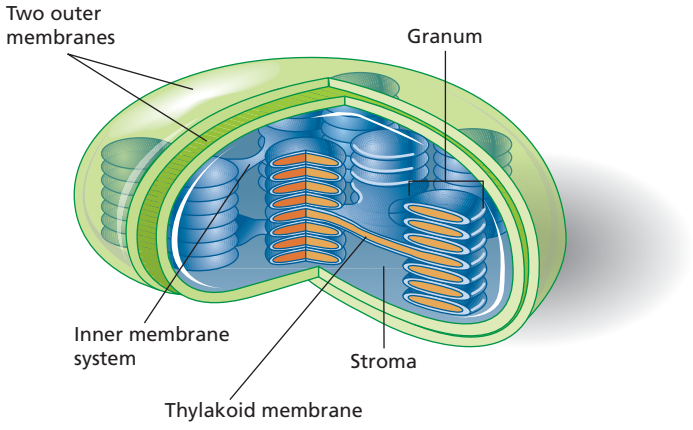
However, this equation shows only the initial reactants, the final products, and the need for light as an energy source that is captured by a pigment called **chlorophyll**. It does not tell the whole story. Photosynthesis occurs as a series of steps in a biochemical pathway, each catalysed by specific enzymes, which take place in specialised membrane-bound organelles called **chloroplasts**.

Chloroplasts

Chloroplasts (Figure 3.15) are eukaryotic organelles with a long evolutionary history. They are found in green plant cells and some protists, and are the sites of photosynthesis. Chloroplasts have an outer and an inner membrane. Enclosed by the inner membrane is the **stroma**, a gel-like matrix that is rich in enzymes. Suspended in the stroma is a third membrane system called the **thylakoid membranes**: these are flat, sac-like structures that are grouped together into stacks called **grana**.

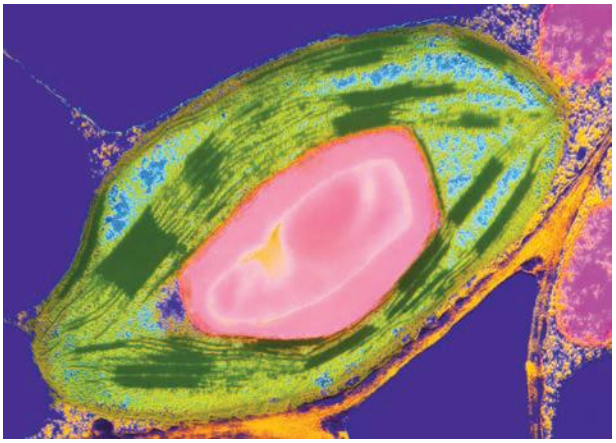
Figure 3.15 ▼
(**a**) Generalised sketch and (**b**) false colour transmission electron micrograph of a green chloroplast from a leaf of the plant *Coleus blumei*. The green, thread-like strands are thylakoid membranes. They pack together tightly to form grana. The large pink region is a starch grain, where the products of photosynthesis are temporarily stored after they have been produced in the light-independent reactions that take place in the stroma (magnification ×5000).

a



b

Coleus blumei (magnification ×5000)



Science Photo Library/Dr Jeremy Burgess

Chloroplasts contain their own genetic material (DNA and RNA) and ribosomes that are independent of those of the nucleus and cytoplasm of the cell. In fact, they have several characteristics in common with prokaryotic cells. This has led biologists to formulate the endosymbiotic theory, which suggests that chloroplasts evolved from ancient prokaryotic cells and subsequently established a symbiotic relationship with a much larger primitive nucleated host cell.

See page 95 for more on the endosymbiotic theory.

Photosynthesis can be divided into two distinct stages, the **light-dependent stage** and the **light-independent stage**. Each stage is confined to specific sites within the chloroplast.

RECALL

- Autotrophs are organisms that can produce their own food in the form of organic substances. They include phototrophs and chemotrophs.
- Heterotrophs obtain their food by consuming other organisms.
- Photosynthesis is the process by which phototrophs produce glucose as a food source. It occurs in chloroplasts and has a light-dependent and a light-independent stage.

RECAP 3.6

1 Distinguish between a photoautotroph and a chemotroph.

2 Recall the equation of photosynthesis.

3 Describe the features of chloroplasts.

The light-dependent stage

Light energy is absorbed by different **pigments** within the thylakoid membranes. These pigments include chlorophylls (green), carotenoids (orange) and xanthophylls (yellow). Chlorophylls absorb light with blue and red wavelengths. They reflect green wavelengths, which is why plant parts with an abundance of chlorophyll molecules appear green to us. All green algae and plants have chlorophylls as their major photosynthetic pigments.

When a chlorophyll molecule absorbs light energy, electrons within the molecule become energised and are ejected from the molecule. To replace the lost electrons, chlorophyll seizes them from water molecules. Consequently, the light energy splits water molecules into electrons, protons (H^+), and oxygen gas (Figure 3.16). Water, in this case, is an input of photosynthesis, whereas oxygen is a by-product.

The energised electrons are transferred to the **electron transport chain**, a series of molecules embedded in the thylakoid membrane (Figure 3.16). The electrons are relayed along the molecules of the electron transport chain, giving off energy at each step. The energy is used to pump protons from the stroma into the **thylakoid space**. At the end of the electron transport chain, spent electrons are conveyed to an acceptor molecule in the stroma. The acceptor molecule is a coenzyme called nicotinamide adenine dinucleotide phosphate (**$NADP^+$**), which also accepts protons. When $NADP^+$ receives a proton and a pair of electrons it becomes **NADPH**. The $NADP^+$ is an input and NADPH is an output of the light-dependent stage. The NADPH made in the stroma is subsequently used in the light-independent stage.

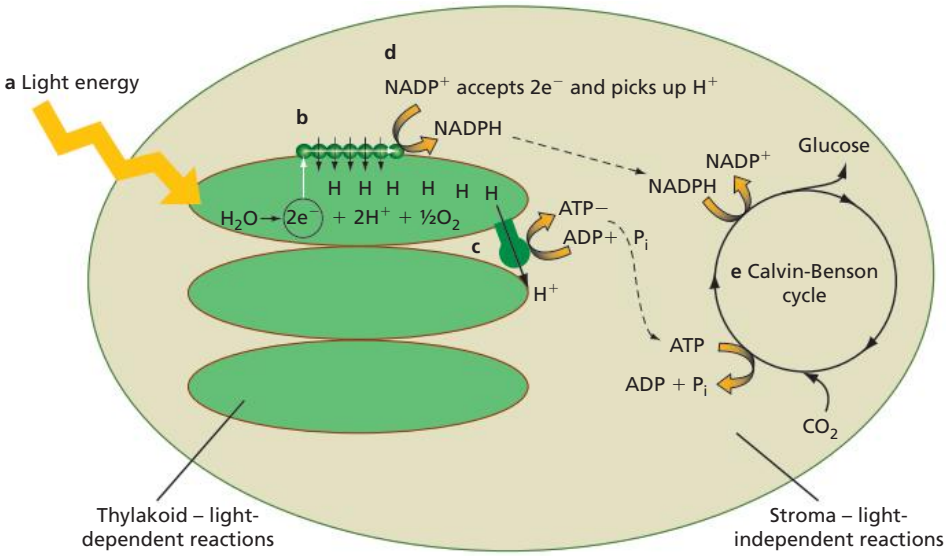


Figure 3.16 ▶ Light-dependent reactions occur in the thylakoid space. (**a**) Light energy absorbed by chlorophyll pigments, energising electrons, splits water molecules. (**b**) Electrons move along the electron transport chain (ETC). This generates energy to pump hydrogen ions into the thylakoid space, creating an ion gradient. (**c**) These ions move out of the thylakoid space through ATP synthase, generating ATP. (**d**) Electrons exiting the ETC are passed to $NADP^+$, which also picks up an H^+ ion to become NADPH. (**e**) The products of the light-dependent reactions (ATP and NADPH) are used to reduce carbon dioxide to carbohydrate in the Calvin-Benson cycle.

The activity of the electron transport chain packs the thylakoid space with protons. This creates an ion gradient across the thylakoid membrane. The high concentration of protons inside the thylakoid space causes a pressure build-up. The protons are all positively charged, so they mutually repel each other. To release the pressure, the protons need to escape down the concentration gradient back into the stroma. However, charged protons cannot cross the thylakoid membrane freely. The only available exit for the protons is an enzyme called **ATP synthase**. This enzyme is embedded in the thylakoid membrane. As electrons flow through ATP synthase, the energy from this movement of electrons is used to drive production of ATP from ADP and inorganic phosphate. ADP and inorganic phosphate are inputs, and ATP is an output of the light-dependent stage. ATP generated by this process is used in the light-independent stage to make sugar molecules.

RECALL

- During photosynthesis, green plants and algae use light to drive the synthesis of glucose from carbon dioxide and water, and oxygen is produced as a by-product.
- The light-dependent stage of photosynthesis is located in the chloroplast grana and utilises an electron transport chain and ATP synthase.
- Water, ADP and $NADP^+$ are inputs for the light-dependent stage.
- ATP, NADPH and oxygen gas are outputs of the light-dependent stage.

RECAP 3.7

1 Describe the role of chlorophyll in photosynthesis.
2 What is the water used for in the light-dependent stage of photosynthesis?
3 What drives ATP synthase in the light-dependent stage, and how does it generate ATP?

The light-independent stage

The light-independent reactions occur in the stroma of the chloroplast. In these reactions, sugar molecules are produced from carbon dioxide in a biochemical pathway called the **Calvin-Benson cycle** (Figure 3.17). This reaction requires a supply of carbon dioxide gas, hydrogen ions and chemical energy in the form of ATP. The loaded acceptor molecule NADPH, formed in the light-dependent stage, becomes the source of hydrogen ions. Likewise, ATP molecules formed in the light-dependent stage provide the chemical energy for the conversion of carbon dioxide to sugar molecules.

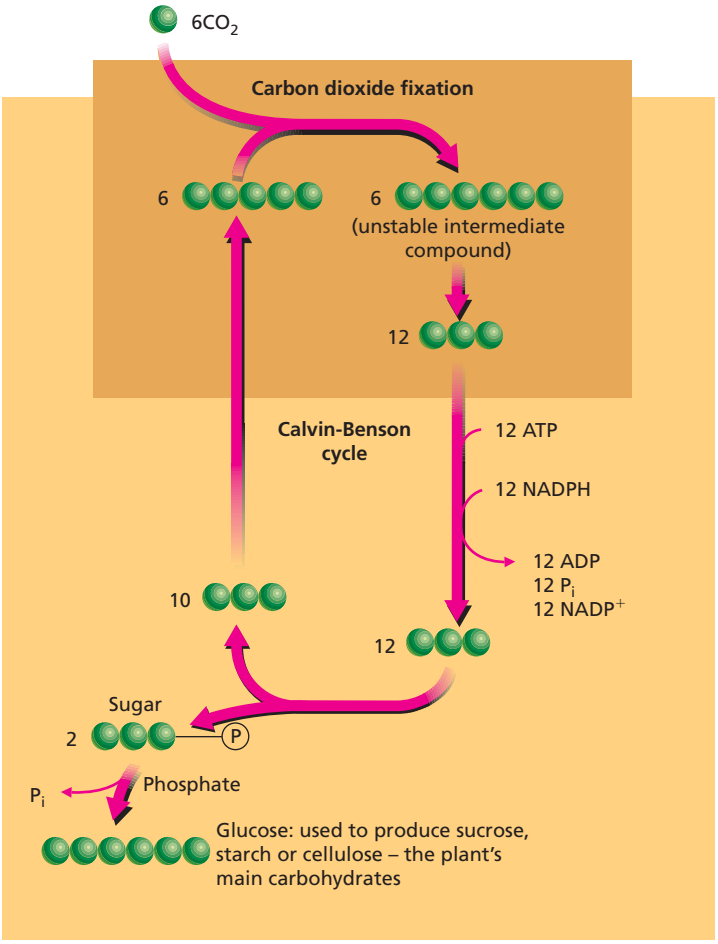


Figure 3.17
The Calvin-Benson cycle. The green spheres signify the carbon atoms of the key molecules. In this biochemical pathway, 12 ATP and 12 NADPH molecules are required in the fixation of carbon dioxide molecules to yield one glucose molecule.

The first step of the Calvin-Benson cycle involves attaching carbon dioxide to a five-carbon molecule called ribulose bisphosphate (RuBP). This step is called **carbon fixation** and it is catalysed by the most abundant enzyme on Earth, Rubisco. Carbon dioxide binds to the active site of Rubisco. Interestingly, oxygen can also bind to the same site and can decrease the rate of photosynthesis. When Rubisco fixes a carbon dioxide molecule, an unstable six-carbon compound is formed. This intermediate quickly splits into two three-carbon molecules. (The Calvin-Benson cycle is also known as C_3 photosynthesis because the first compound produced is a three-carbon sugar phosphate molecule.) The ATP produced in the light-dependent stage supplies the energy to drive the cycle. NADPH donates protons (H^+) to form a three-carbon sugar phosphate molecule. Some three-carbon sugar phosphate molecules exit the cycle and the remaining ones are used to regenerate RuBP to begin the cycle again.

The three-carbon sugar phosphate molecules are rearranged and reshuffled to build the plant's main carbohydrates. Sucrose, starch and cellulose are produced from sugar phosphate molecules in other biochemical pathways. During daylight hours, chloroplasts convert the newly formed sugar phosphate molecule to sucrose or starch. Of all plant carbohydrates, sucrose is the most easily transportable. Starch is the most common storage form. It is stored briefly in the stroma during the day. At night, cells convert starch to sucrose for export to other cells in leaves, stems and roots that are devoid of chloroplasts. Glucose is the monomer for polysaccharides, such as starch and cellulose. Sucrose is a disaccharide of glucose and fructose; the fructose is a rearranged form of glucose. As the key building block of the plant's carbohydrates, glucose is a vitally important output of photosynthesis.

RECALL

- In the light-dependent stage of photosynthesis, chlorophyll absorbs photons to split water, releasing hydrogen ions (protons) into the electron transport chain.
- The electron transport system pumps protons into the thylakoid space to create a concentration gradient that fuels production of ATP. It also transfers electrons and protons to $NADP^+$ to form NADPH.
- In the light-independent stage, the ATP and NADPH from the light-dependent stage are used to provide the chemical energy and protons to fix carbon in the Calvin-Benson cycle.

RECAP 3.8

1 What do all photosynthetic organisms have in common?
2 Distinguish between the light-dependent and the light-independent stages of photosynthesis in terms of location, requirements and products.
3 Which products or outputs of the light-dependent reaction are used as inputs in the light-independent reaction of photosynthesis?

The inputs and outputs of photosynthesis

The two stages of photosynthesis require certain inputs, such as water and carbon dioxide, and produce outputs, such as oxygen and glucose. Coenzymes also cycle between the two stages in loaded and unloaded forms. For the two stages to correspond in the number of coenzymes cycled between them, 12 cycles of the light-dependent reactions must occur for every six of the light-independent reactions. An account of these inputs and outputs is presented in Tables 3.1 and 3.2.

Table 3.1 Summary of inputs and outputs for the light-dependent stage. The data represent 12 cycles of the light-dependent reactions.

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Water (H_2O)	12	Oxygen (O_2)	6
$NADP^+$	12	NADPH	12
ADP	12	ATP	12
Inorganic phosphate	12		

Table 3.2 Summary of inputs and outputs for the light-independent stage. The data represent six cycles of the light-independent reactions.

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Carbon dioxide (CO_2)	6	Water (H_2O)	6
NADPH	12	$NADP^+$	12
ATP	12	ADP	12
		Inorganic phosphate	12
		Glucose	1

Comparing the inputs and outputs for both stages of photosynthesis emphasises how some components are recycled between the two stages. NADPH and ATP formed during the light-dependent stage are broken down during the light-independent stage. The $NADP^+$, ADP and inorganic phosphate produced during the light-independent stage are returned as inputs to the light-dependent stage. For this reason, these components are not shown in the photosynthesis equation.

Water is an output of the light-independent stage. This water is produced by rearrangement of the oxygen atoms in carbon dioxide. Half of the oxygen atoms from the carbon dioxide are incorporated into the carbohydrate, the other half into water. This water is therefore different from the water consumed as an input in the light-dependent stage. For this reason, water is represented on both sides of the photosynthesis equation.

Photosynthesis in C_3, C_4 and CAM plants

Many plants grow in areas with a high light intensity that is favourable for photosynthesis, but which are deserts or grasslands and so tend to be dry. To conserve water, these plants close their stomata when light and heat are intense. How do they get the carbon dioxide needed for photosynthesis with their stomata closed most of the day? C_4 and CAM plants have evolved ways to help overcome the problem of carbon dioxide uptake versus water loss by concentrating carbon dioxide in their tissues.

While all plants use the Calvin-Benson cycle to fix carbon in the final stage of photosynthesis, C_4 and CAM plants have evolved an extra carbon fixation step prior to the Calvin-Benson cycle. These adaptations have led to distinctive distribution patterns for these plants. C_3 plants are found in temperate and tropical climes and represent about 83% of the world's flora; C_4 plants are found in grassland areas and make up only 3% of the world's total flora; and CAM plants are found in deserts (with the occasional tropical appearance) and make up about 10% of the total flora.

All plants use the enzyme Rubisco to catalyse reactions that create organic carbon out of inorganic carbon dioxide, a key step in maintaining life on Earth. Rubisco is the most plentiful enzyme on Earth but it is highly inefficient. In C_3 plants, Rubisco fixes carbon dioxide by joining it to a five-carbon sugar. Then it cuts the new six-carbon sugar chain into two identical three-carbon molecules, and hence the name **C_3 plants**. On hot dry days, stomata close so that C_3 plants can conserve water, but then carbon dioxide cannot diffuse into the leaves. The carbon dioxide level drops and carbon fixation slows down. Rubisco begins to react with the oxygen that is building up in the leaves rather than with the small supply of carbon dioxide. This is called **photorespiration**, and results in the production of carbon dioxide by Rubisco.

In **C_4 plants**, carbon dioxide is 'harvested' in the mesophyll cells. It is joined to a three-carbon molecule to form a four-carbon molecule, and hence the name C_4 plants. This fixed carbon moves out of the mesophyll cells into specially adapted bundle sheath cells. Here the carbon dioxide is liberated and then fixed by Rubisco in the Calvin-Benson cycle. In this way the carbon dioxide gradient stays low in mesophyll cells so that it will continue to diffuse in from the outside, even when the stomata are almost closed. This partitioning also means that C_4 plants move the Calvin-Benson cycle into an area with a high carbon dioxide concentration. Why is this important? The enzyme Rubisco will spend more of its time fixing carbon dioxide in photosynthesis than fixing oxygen in photorespiration. In C_3 plants, photorespiration increases as the temperature rises, so carbon fixation by Rubisco slows. This does not happen in C_4 plants, so they are more competitive in high temperatures. C_4 plants include maize and sugar cane, which is renowned for its high glucose production.

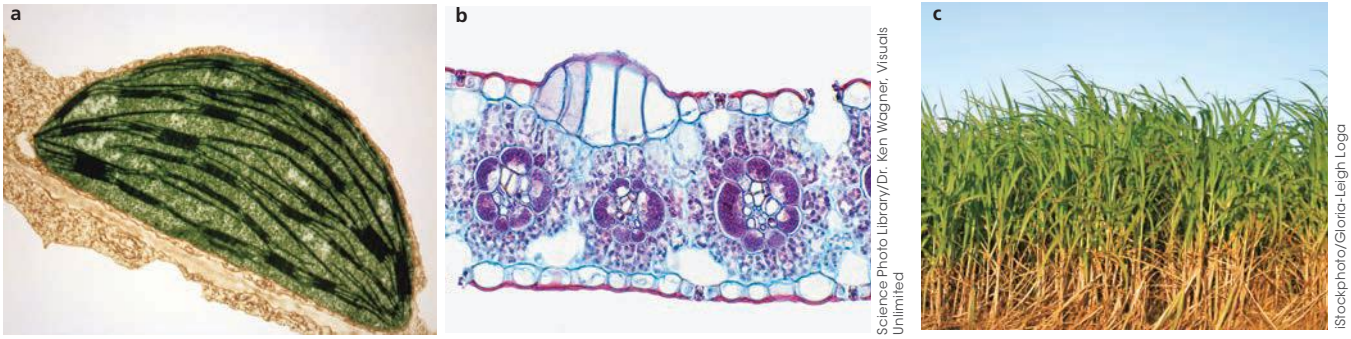


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Figure 3.18 ▲
(**a**) C_4 plants have two types of chloroplast. Thylakoid membranes stack in mesophyll chloroplasts, like the ones shown here, but do not stack in bundle sheath chloroplasts. (**b**) This cross-section of the leaf of a C_4 plant shows that the concentration of Rubisco is higher in the chloroplasts of bundle sheath cells (bright purple) than in those of mesophyll cells. (**c**) Sugar cane is an example of a C_4 plant.

In **CAM (crassulacean acid metabolism) plants**, carbon dioxide is 'harvested' at night and fixed to form malate. This again is a four-carbon molecule, but CAM plants differ from C_4 plants. CAM plants do not transport malate away from the mesophyll cells but store it during the night and then liberate carbon dioxide from these molecules and use it in the Calvin-Benson cycle during the day. This use of malate requires four more ATP molecules than the C_3 pathway, so these plants tend to grow more slowly than other plants. They lose up to 95% less water than C_3 plants as they only open their stomata at night. This is very useful for plants living in arid zones, such as the desert. Interestingly, CAM plants can swap to the C_3 pathway if there is a period of rainfall, giving them a sudden growth spurt. They can also keep their stomata closed all night and day during drought conditions, and exist by fixing carbon dioxide that is released from respiration reactions within the plant. The pineapple is an example of a CAM plant.

RECALL

- In C_3 plants (the majority of plants), Rubisco fixes carbon dioxide by joining it to a five-carbon sugar, producing a six-carbon sugar chain that it then cuts into two identical three-carbon molecules.
- In hot weather, stomatal closure causes a build-up of oxygen. This can react with Rubisco, causing photorespiration and preventing photosynthesis.
- C_4 plants fix carbon into a four-carbon molecule in mesophyll cells, which is then shuttled into bundle sheath cells where the Calvin-Benson cycle takes place. This maintains a high concentration of carbon dioxide in the bundle sheath cells.
- CAM plants only open their stomata at night, fixing carbon dioxide in the form of malate, which is then used as a source of carbon dioxide for the Calvin-Benson cycle during the day.

RECAP 3.9

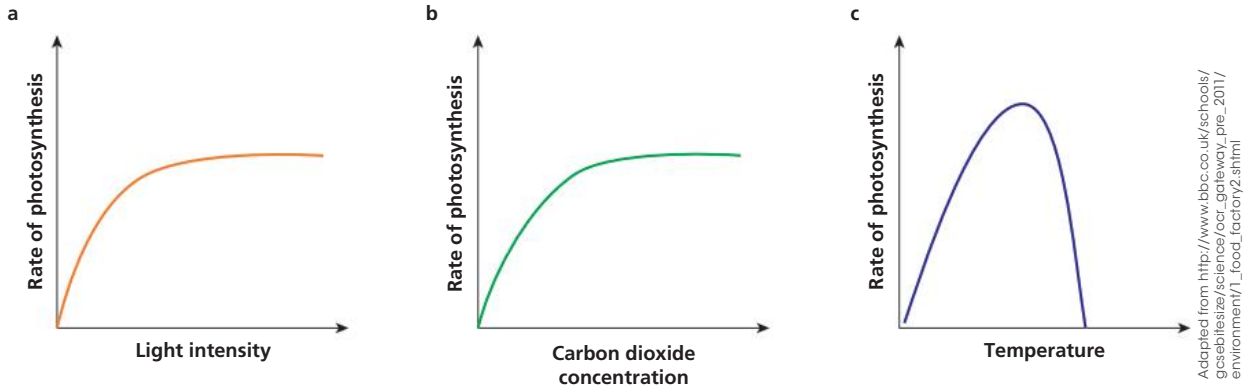
1. Give examples of C_4 and CAM plants and link their carbon fixation strategies to their distribution patterns.
2. Describe the action of the enzyme Rubisco in C_3 plants.
3. Why is Rubisco described as an inefficient enzyme (i.e. what happens when carbon dioxide is in low supply)?
4. Why do C_4 plants have a competitive edge over C_3 plants in high temperatures?
5. How do CAM plants overcome the problems of having to close stomata during the day?

Factors that affect photosynthesis

Like all biochemical reactions, photosynthesis is affected by many factors. The main factors affecting photosynthesis are environmental: light, carbon dioxide and temperature, although other factors such as the presence of inhibitors and changes in pH can also change the rate of photosynthesis.

Light

In the light-dependent phase, photons are needed to excite the chlorophyll molecules. These then split water molecules, freeing electrons to enter the electron transport system. The amount of light available is therefore an important factor that limits the rate of the first reaction in photosynthesis. A light saturation point is reached when adding more light does not increase the rate of photosynthesis (Figure 3.19). This happens when more ATP and NADPH are produced in the light-dependent stage than can be used in the light-independent stage.



▲ **Figure 3.19** Factors that affect the rate of photosynthesis include (**a**) light intensity, (**b**) carbon dioxide concentration, and (**c**) temperature.

Carbon dioxide

The availability of carbon dioxide is usually the main limiting factor in photosynthesis. Ambient carbon dioxide concentrations are relatively low, at ~0.04%. During the light-independent stage, carbon dioxide is the substrate for Rubisco, which converts it to an unstable six-carbon compound that splits into two three-carbon compounds. These form the building blocks for glucose. As the concentration of carbon dioxide increases, the rate of photosynthesis can also increase as long as enough enzyme active sites are available to catalyse the photosynthetic reactions, and the ATP and NADPH supply from the light-dependent stage is adequate to fuel the light-independent reactions. An increasing carbon dioxide concentration also raises the light saturation point, because

then the light-independent reactions can use up more ATP and NADPH and the rate of photosynthesis can increase overall.

Temperature

The third main factor that influences the rate of photosynthesis is temperature. As with all chemical reactions, increasing temperature increases the rate of the reaction. This is because the molecules involved have greater kinetic energy, causing them to collide and interact more frequently. This results in a higher rate of interaction between an enzyme and its substrate, giving more opportunities for the enzyme to catalyse the reaction and generate the products. However, as with all proteins, above a certain temperature an enzyme becomes denatured and the reaction essentially stops. As the ambient temperature rises, it is essential for plants to maintain their temperature within their tolerance limits, or adapt to the increasing temperature.

RECALL

- The rate of photosynthesis is affected by factors such as light intensity, carbon dioxide concentration, and temperature.

RECAP 3.10

1 In the context of enzyme activity, explain why the rate of photosynthesis levels off at high carbon dioxide concentrations.

2 What occurs at the light saturation point of photosynthesis? Identify this point on the graph in Figure 3.19a.

EXPERIMENT 3.1

INVESTIGATING FACTORS AFFECTING PHOTOSYNTHESIS

Conversion of light energy into chemical energy by photosynthesis is affected by many factors. The main environmental factors are light, temperature and carbon dioxide concentration. This experiment tests the effects of light and carbon dioxide availability on the accumulation of starch, a storage form of glucose in plants.

Aim

To test the effects of light and carbon dioxide availability on photosynthesis by measuring starch accumulation

Materials

- potted geranium plants of similar sizes and in the same soil conditions
- dark paper, card or foil
- paper clips
- conical glass flasks, stands and clamps
- sodium hydroxide solution – see risk assessment box
- sodium hydrogen carbonate (sodium bicarbonate) solution or carbonated water
- water
- cotton wool
- beaker and test tube
- Bunsen burner, tripod and gauze or electrically heated water bath
- industrial methylated spirit – see risk assessment box
- Petri dishes
- iodine solution

What are the risks in this experiment?	How can you manage these risks to stay safe?
Sodium hydroxide solution is corrosive	Avoid contact with skin and eyes. If spilt or splashed, rinse affected area immediately with plenty of water and report any accidents to your teacher. Eye wash facilities and equipment should be readily available.
Industrial methylated spirit is highly flammable and is irritating to eyes and respiratory system	Keep the container tightly closed. Keep away from all sources of ignition. Extinguish all Bunsen burners before opening container. Do not breathe vapour and avoid contact with skin.
Iodine is irritating to skin, eyes and respiratory system	Avoid contact with skin and eyes. If spilt or splashed, rinse affected area immediately with plenty of water and report any accidents to your teacher. Eye wash facilities and equipment should be readily available.

Procedure

Destarching the plant

Since a variable measured in this experiment is the production of starch, the first step forces the plant to use its reserves of starch without allowing its replacement by photosynthesis.

1. Water the plant.
2. Place the plant in a dark place (e.g. cupboard) for 24–48 hours.
3. Test a leaf of the plant for starch (see below). If starch remains, return the plant to the dark. If no starch is present, proceed to treating the plant.

Testing a leaf for starch

The first step in testing for the presence of starch is decolourisation of the leaf. **Ensure strict safety precautions are used due to the flammability of industrial methylated spirit.**

Decolourisation

If using a Bunsen burner:

1. Place a beaker of water on a tripod and gauze standing over a Bunsen burner (Figure 3.20).
2. Check that the beaker is stable, then light the burner and bring the water to a boil.
3. Place the leaf in the boiling water for 1–2 minutes until it becomes flaccid.
4. **Turn off the Bunsen burner.**
5. Pour some methylated spirit into a test tube, place the leaf into the test tube, then place the tube into the beaker (Figure 3.21).
6. Allow the test tube to warm gently in the heated water for several minutes.

If using an electrically heated water bath:

1. Place the leaf directly into a water bath at 100°C for 1–2 minutes.
2. Transfer the leaf to a test tube of methylated spirit, then place the tube in a water bath set to 78°C for several minutes.

Iodine staining for starch

7. Immediately after treating the leaf with methylated spirit, immerse the whole leaf briefly in cold tap water.
8. Spread the leaf on a Petri dish.
9. Add a few drops of iodine solution to the leaf and wait for the colour to develop.

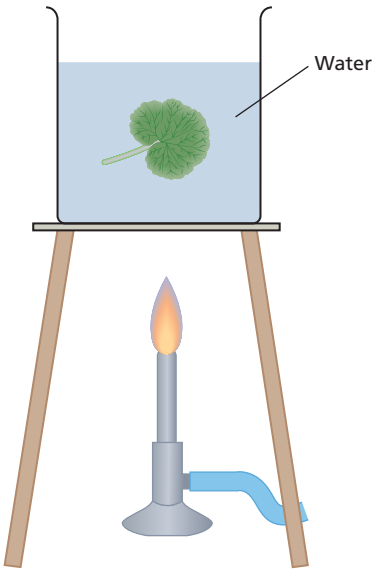


Figure 3.20 ▲
Set-up for heating the leaf

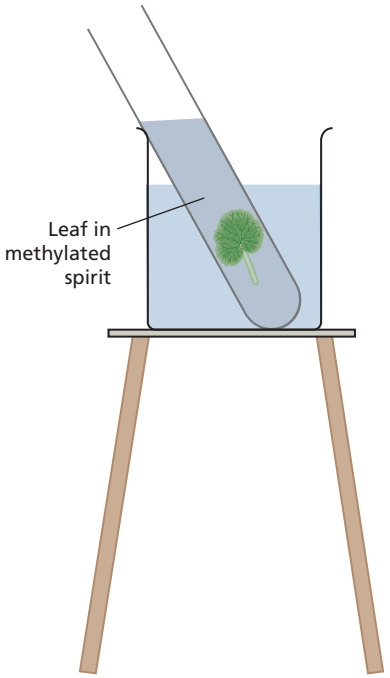


Figure 3.21 ▲
Set-up for warming the methylated spirit

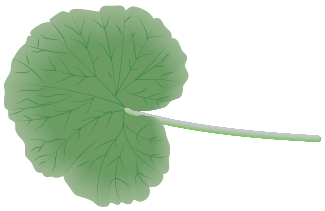


Figure 3.22 ▲
Leaf appearance before decolourisation

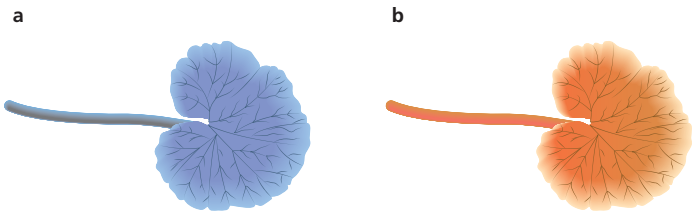


Figure 3.23 ▲
After decolourisation and staining (**a**) grey-blue-black areas represent starch, and (**b**) yellow-orange-red areas represent no starch. It may be necessary to hold the dishes over white paper to see differences.

Treatments

The following treatments are applied to leaves that are attached to the plant.

The effect of light

10 Cut a distinctive shape out of a piece of dark paper, card or foil. Cover a section of leaf on both sides with the card, holding it in place with paper clips if necessary.

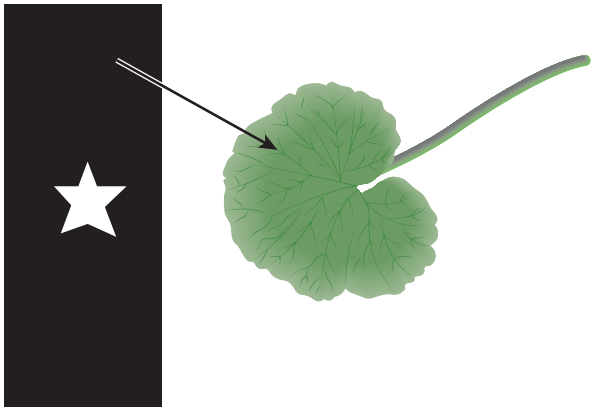


Figure 3.24 ▲
Cover the leaf (still attached to the plant) with a piece of card in which you have cut a distinctively shaped hole.

The effect of carbon dioxide

11 Fill four flasks with the following liquids:

- **a** sodium hydroxide solution – caution: corrosive!
- **b** sodium hydrogen carbonate (sodium bicarbonate) or carbonated water
- **c** water
- **d** no liquid.

12 Place each flask over a leaf and support it at an angle using stands and clamps. Do not let the liquid touch the leaf.

13 Create an airtight barrier around the petiole (leaf stalk) at the opening of each beaker to seal in the liquid vapour. This barrier may be cotton wool carefully kept saturated with water from a washbottle. Alternatively you may use split corks or bungs with plenty of petroleum jelly.

14 Keep the plant well watered and evenly exposed to good light.

15 After a couple of days or more, remove the leaves for testing. It is important to maintain a way of identifying the leaves during the testing process, for example by making small distinctive cuts in each one.

16 Test the leaves for starch content using the decolourisation and iodine staining procedure described above.

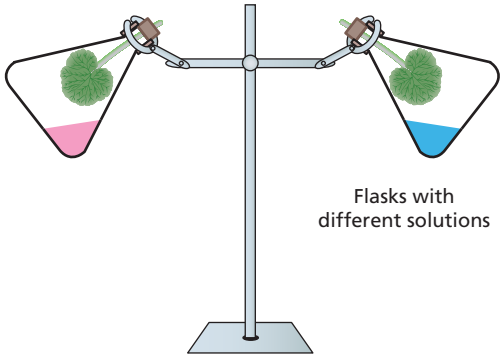


Figure 3.25 ▲
Cover four leaves (still attached to the plant) with flasks containing the test solutions. Do not let the leaves touch the solutions, and seal the flasks around the leaf petioles.

Results

1 Draw the outlines of the leaves from your five treatments (leaf covered with card plus the four leaves in the enclosed flasks).

2 Draw the outlines of the areas that stained positively and negatively for starch.

3 Compare your results with those of others in your class.

Discussion

1 Explain how light affected photosynthesis in this experiment. Discuss the pattern of starch accumulation in the presence and absence of light, and what this may mean for movement of starch within a leaf.

2 Sodium hydroxide reacts with carbon dioxide to produce water and sodium carbonate, which forms a high pH (alkaline) solution:

$$CO_2 + 2NaOH \rightarrow Na_2CO_3 + H_2O$$

   a Why is the beaker containing sodium hydroxide set up so that the liquid does not touch the leaves?
   b Where in the experimental set-up does the above reaction take place?
   c Draw a schematic diagram of photosynthesis and annotate it to explain how the reaction would be altered in the presence of sodium hydroxide.

3 Sodium hydrogen carbonate is a source of carbon dioxide. Explain how this treatment affected starch deposition using the terms photosynthesis, carbon fixation, light-dependent and light-independent.

4 What were the controls in this experiment?

5 Why use a beaker filled with water and one with no liquid?

6 How would your experiment be affected if the leaves had not been destarched before the experiment was conducted?

7 An alternative model for measuring the rate of photosynthesis is by measuring the rate of bubble formation by the pondweed *Elodea* submerged in a beaker of water.
   a Reflect briefly on how light and carbon dioxide availability would affect bubble production by *Elodea*.
   b Why is starch production a more reliable way of measuring photosynthesis?
   c What benefits does the *Elodea* system have over the system used here?

Conclusion

Propose conclusions about how photosynthesis is affected by the factors you have investigated.

Taking it further

Design an experiment to investigate the effects of different wavelengths of light on starch formation using photographic filters. Ensure your experimental design maintains a carefully controlled environment to minimise variables.

Cellular respiration

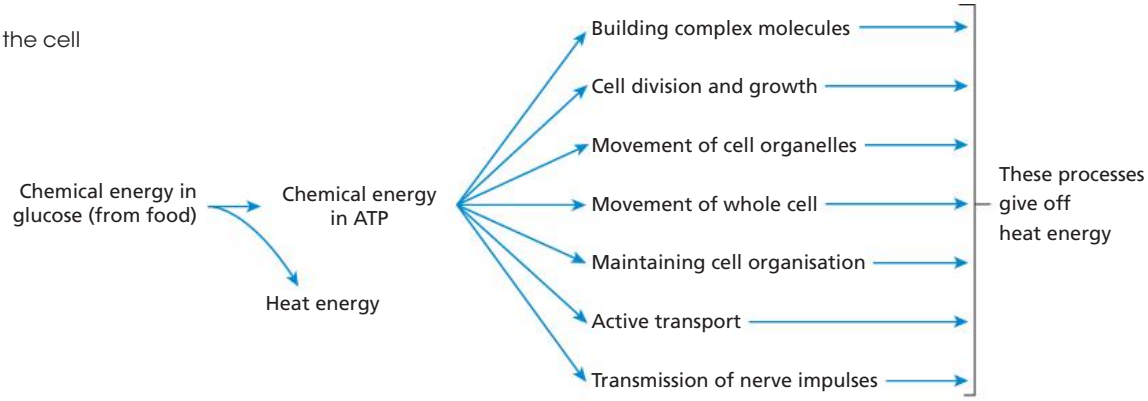
Organisms require energy to carry out the processes associated with life. ATP is the primary source of free energy in all living organisms, and the process of cellular respiration allows cells to capture the energy stored in chemical bonds of glucose to create ATP ready for use by the cell (Figure 3.26).

All organisms, with the exception of the Archaea, use glucose as the primary source of energy to drive cellular metabolism. The chemical bonds in glucose are broken, resulting in more stable products and the release of free energy. This is an exergonic reaction in which electrons are transferred from the glucose molecule to a final electron acceptor, such as oxygen. This reaction can be summarised by the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + 36ATP$$

This chemical process is known as aerobic cellular respiration. The word 'aerobic' is used when oxygen is the electron acceptor. The equation above simply shows the initial reactants and the final products. In fact, there are about 20 reactions that contribute to this biochemical pathway, each catalysed by specific enzymes. Most animals, plants, protists, fungi and bacteria are aerobes: they all require oxygen for cellular respiration.

Figure 3.26 ▶
Uses of energy in the cell



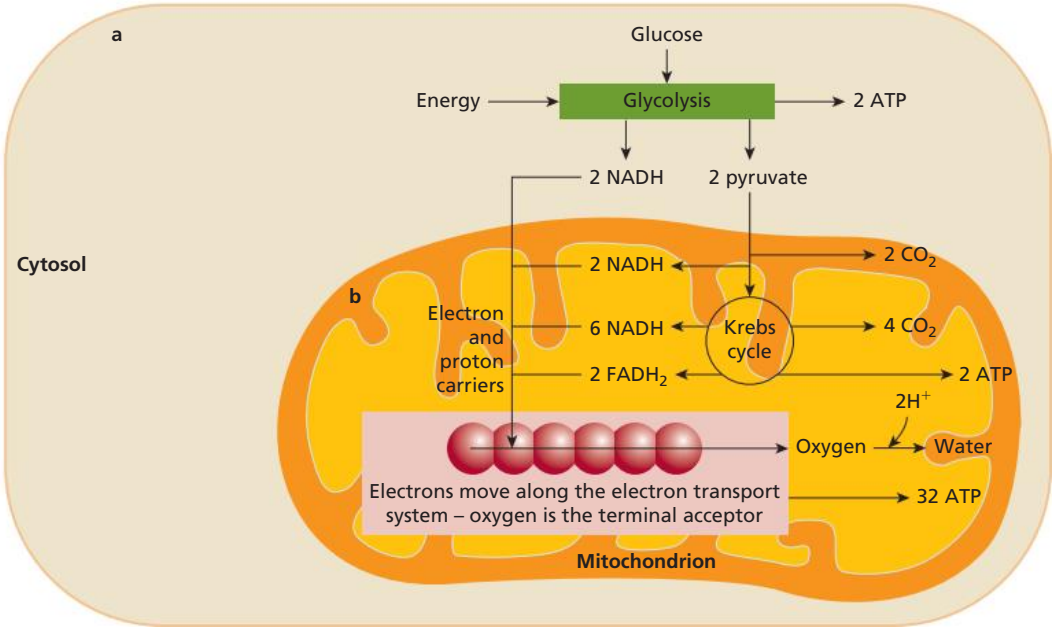
However, oxygen is not always the final electron acceptor, and many micro-organisms use other molecules as electron acceptors. These organisms are called anaerobes, and they need to live in an environment devoid of oxygen.

For all organisms, the oxidation of glucose to supply the cell with available energy, regardless of whether oxygen is present or not, starts with a biochemical pathway called **glycolysis**.

Glycolysis

Glycolysis takes place in the cytosol of cells. The biochemical pathway is made up of ten reactions, with each step controlled by a specific enzyme. The initial reactant is glucose and the final product is two molecules of a three-carbon compound called **pyruvate**. Two unloaded acceptor molecules, **NAD^+**, are also loaded with hydrogen atoms in glycolysis to form two loaded **NADH** molecules. The glycolysis pathway also produces a net two ATP molecules that may be used by the cell as a source of energy (Figure 3.27). The net yield of two ATP molecules, which can be used by the cell immediately, may be sufficient for the needs of certain micro-organisms, but it is not sufficient for multicellular organisms. The fact that all organisms carry out glycolysis,

Figure 3.27 ▶
(**a**) Glycolysis, the first stage of cellular respiration, occurs in the cytosol. In glycolysis, glucose partially breaks down to pyruvate. (**b**) Pyruvate enters the mitochondria. In the presence of oxygen, the Krebs cycle and electron transport system generate ATP molecules (see next section).



either as their sole source of energy or as the first step in more elaborate pathways to gain sufficient ATP for their needs, points to glycolysis being one of the earliest reactions to produce energy for the cell. But what occurs after glycolysis in both prokaryotic and eukaryotic cells? What happens next depends upon whether oxygen is present or absent.

RECALL

- The purpose of cellular respiration is to use the energy stored in glucose to create ATP, which can provide energy to fuel cellular reactions.
- Glycolysis occurs in the cytosol. It involves conversion of glucose to two pyruvate molecules, two ATP and two loaded acceptor molecules, NADH.

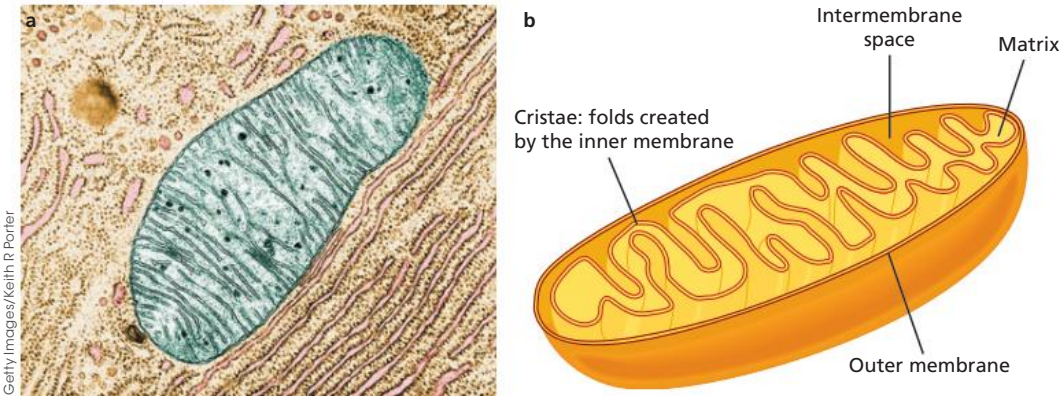
RECAP 3.11

1 Write the equation for cellular respiration.
2 What is the initial substrate in the glycolysis pathway and what is the final product?
3 Where does glycolysis take place in all cells?

Cellular respiration with oxygen

In eukaryotic cells that are supplied with oxygen, the two molecules of pyruvate formed in glycolysis enter an organelle called the **mitochondrion**. Mitochondria are often described as the 'energy powerhouse' of the cell because large numbers of ATP molecules are produced in them in a biochemical pathway called the **Krebs cycle** (also called the **citric acid cycle**). Before we consider how ATP molecules are produced, and why oxygen is an essential requirement, we will take a closer look at the structure of mitochondria.

Mitochondria are small organelles that are found scattered throughout the cytosol of eukaryotic cells. Each mitochondrion consists of an outer smooth membrane and a highly folded inner membrane. The folds in the inner membrane, called **cristae**, protrude into the inner space of the mitochondrion, a protein-rich fluid called the matrix. The space between the outer and the inner membranes is also filled with fluid and is called the intermembrane space (Figure 3.28b).



Getty Images/Keith R Porter

Figure 3.28 (**a**) Electron micrograph and (**b**) generalised sketch of a mitochondrion in longitudinal section. Stalked particles on the surface of the cristae are the site of ATP synthesis.

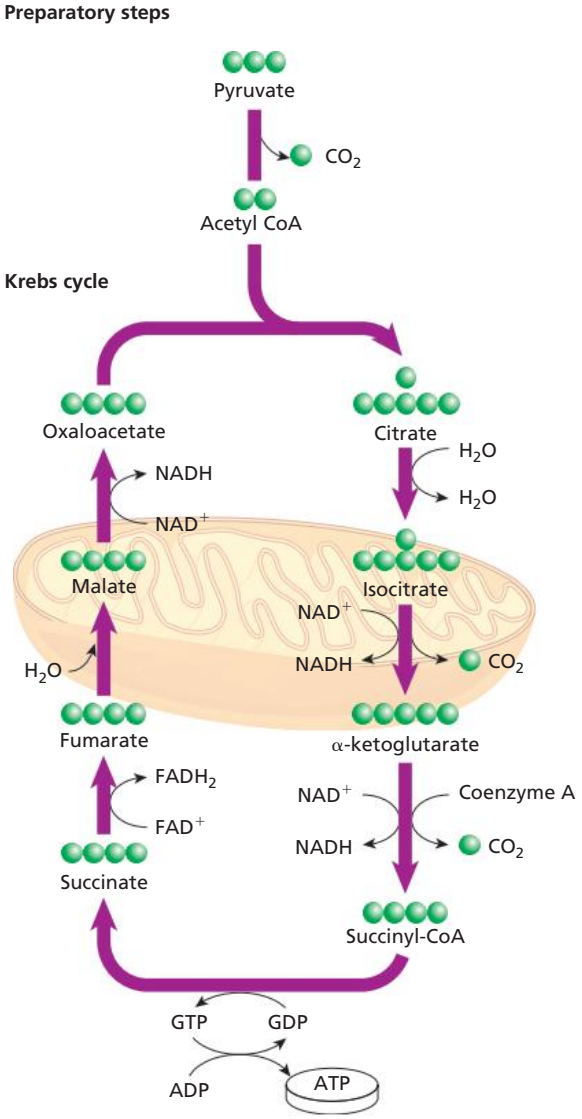


Figure 3.29 ▲
Second stage of aerobic respiration – the Krebs cycle. For each three-carbon pyruvate molecule entering the cycle, three carbon dioxide molecules and one ATP molecule are formed. The steps shown proceed twice.

Mitochondria, like chloroplasts, have their own genetic material (mtDNA and RNA) and ribosomes. Like chloroplasts, they are also capable of division independently of the cell's nucleus, so they make copies of themselves and they have many characteristics that are similar to prokaryotic cells. This evidence points to their bacterial origins.

The two pyruvate molecules formed in glycolysis are transported into the mitochondria. Pyruvate undergoes a reaction that involves a coenzyme, called Coenzyme A. This results in the formation of a loaded coenzyme called **acetyl CoA**. Carbon dioxide molecules are produced as a by-product of this reaction. The acetyl CoA enters a cyclic biochemical pathway called the Krebs cycle (see Figure 3.29). This cycle produces more carbon dioxide, ATP molecules and loaded coenzymes (e.g. NADH, $FADH_2$). The loaded coenzymes enter an electron transport chain associated with the inner mitochondrial membrane. Here, electrons are transferred through a series of compounds called **cytochromes** until they are finally accepted by oxygen. This process is called the electron transport system.
If oxygen is not present, the Krebs cycle and electron transport chain come to a sudden stop. The energy released from the electron transport chain is used to pump protons (H^+) from the loaded coenzyme molecules into the mitochondrial intermembrane space. As the protons flow down the concentration gradient back into the matrix, they drive the production of ATP by ATP synthase. From all the reactions associated with aerobic cellular respiration, it is possible to produce a net 36 ATP molecules in one cycle. Figure 3.30 summarises the process of aerobic respiration.

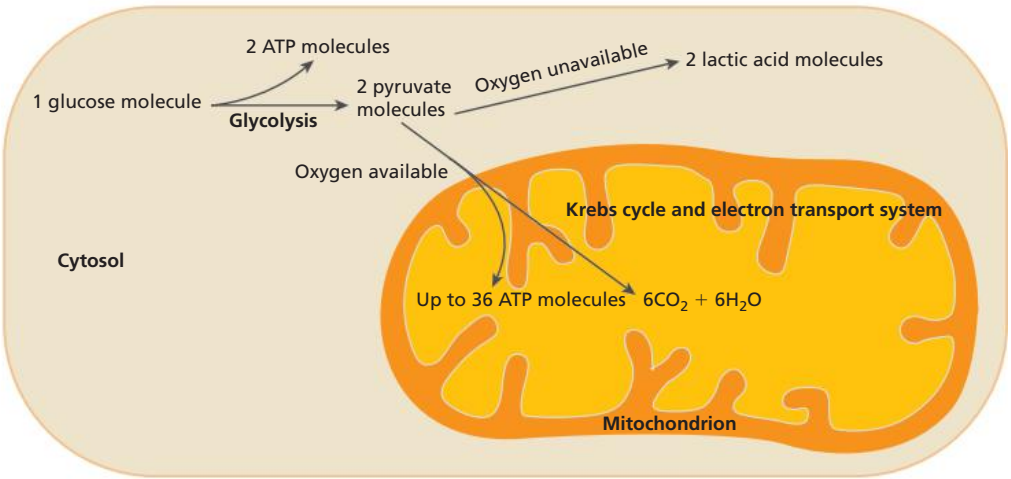


Figure 3.30 ▲
Summary of aerobic and anaerobic respiration in an animal cell

RECALL

- In the presence of oxygen, pyruvate enters the mitochondrion where it is converted to acetyl CoA.
- Acetyl CoA enters the Krebs cycle in which it produces loaded coenzymes NADH and $FADH_2$, plus ATP and carbon dioxide.
- The loaded coenzymes transfer electrons to the electron transport system, which sets up a proton gradient that is used to drive ATP formation.

RECAP 3.12

1 What is the role of oxygen in aerobic cellular respiration?
2 What is the source of the by-product carbon dioxide in aerobic cellular respiration?
3 How many net molecules of ATP are produced in one cycle of aerobic cellular respiration? In practice, the number is somewhat less. Suggest a reason for this.

Cellular respiration without oxygen

The first energy-releasing pathways evolved around 3.8 billion years ago when there was not much free oxygen in the atmosphere. The process was essentially anaerobic in that it was able to run to completion in the absence of oxygen. Many bacteria and protists still live in places where oxygen is absent or not always available, and they produce ATP using anaerobic pathways. Such organisms have evolved biochemical pathways that allow glycolysis to continue in the cytosol by using molecules other than oxygen as the final electron acceptor. Prokaryotes have evolved many anaerobic pathways but eukaryotes commonly use two pathways, referred to as **alcoholic fermentation** and **lactic acid fermentation**.

Oxygen is required for aerobic respiration in eukaryotic cells. If oxygen is absent, the Krebs cycle in the mitochondria shuts down. The cell then relies entirely on glycolysis to maintain a supply of ATP for its energy needs. However, without the Krebs cycle to syphon them off, the products of glycolysis build up in the cytosol. These products are pyruvate and the loaded coenzyme NADH. The problem is that NADH must be continually unloaded, forming NAD^+, to sustain glycolysis. Eukaryotic cells have solved the problem by reacting NADH with the pyruvate in the processes of either alcoholic fermentation or lactic acid fermentation.

Alcoholic fermentation

Many micro-organisms, including yeast and some bacteria, carry out alcoholic fermentation. The glycolysis pathway produces two net ATP molecules and two NADH loaded acceptor molecules. Still in the cytosol, a carbon dioxide molecule is removed from the pyruvate molecules produced in glycolysis to produce acetaldehyde. NADH donates a proton to acetaldehyde and ethanol is formed. Hence, the products of alcoholic fermentation are carbon dioxide and ethanol, an alcohol. The overall summary for alcoholic fermentation is given below.

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \rightarrow \underset{\text{(ethanol)}}{2CH_3CH_2OH} + \underset{\text{(carbon dioxide)}}{2CO_2} + \underset{\text{(adenosine triphosphate)}}{2ATP} + \underset{\text{(210 kJ)}}{\text{energy}}$$

Humans make use of these metabolic waste products in the production of wine, beer and bread (Figure 3.31). Plants, however, cannot use ethanol. It cannot be reconverted into carbohydrate, nor can it be broken down in the presence of oxygen. Alcohol

Shutterstock.com/HLPhoto

Figure 3.31 ▲
Plants and yeast produce ethanol and carbon dioxide in the anaerobic respiration process called alcoholic fermentation. This reaction has been used by industry to produce bread and wine. In bread, the alcohol is baked out.

is toxic to cells and cannot be allowed to accumulate. Many plants (or parts of plants) can respire anaerobically for a short time, such as germinating seeds and roots living in water-logged soil, where there is little oxygen. However, before the concentration of ethanol reaches a certain level they must revert to aerobic respiration, otherwise they will be poisoned by the ethanol.

This is also true for yeast. Yeast is a classic example of a life form that employs anaerobic respiration, and it is used in the brewing and wine-making industries. However, it grows much better in aerobic than in anaerobic conditions. If too little oxygen is present, the ethanol concentration rises so much that the yeast cells are killed. The secret in making beer and wine is not to let conditions become too anaerobic. It is commercially beneficial to develop new strains of yeast that are tolerant of high concentrations of ethanol. This is a major occupation of microbiologists working for brewing companies.

Lactic acid fermentation

Lactic acid, or lactate, is the end product of anaerobic respiration in animals. After strenuous exercise, muscle soreness and cramps may develop. In 1929, Archibald Hill proposed that these symptoms were the result of lactic acid building up in the muscle tissue. However, the latest theory gaining widespread acceptance in the scientific world suggests that these symptoms are a result of an increase in extracellular potassium ion concentration, which leads to the observed muscle fatigue cramps.

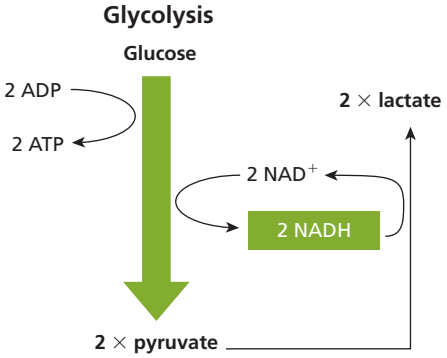


Figure 3.32 ▲
Lactic acid fermentation occurs in muscle cells. In the process, NADH is oxidised to NAD^+, allowing glycolysis to continue.

Lactic acid fermentation is an important pathway for energy production. This anaerobic respiration in animal cells is represented by the equation below.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH + 2ATP + \text{energy}$$

(glucose) (lactic acid) (adenosine triphosphate) (150 kJ)

Aerobic respiration produces 2880 kJ of energy. In anaerobic respiration, glucose is not broken down as completely as it is in aerobic respiration and so less energy is released as a consequence. A lot of energy still remains locked up in the ethanol or lactic acid molecules.

In animals, the energy trapped in the lactic acid molecule can be released by conversion of the lactic acid back into pyruvate, which may then be broken down to carbon dioxide and water via the Krebs cycle.

RECALL

- Eukaryotes have two pathways for producing ATP using pyruvate in the absence of oxygen.
- Yeasts, plants and some bacteria use alcoholic fermentation and animals use lactic acid fermentation.
- The products of anaerobic respiration are toxic at high concentrations and so anaerobic respiration is only a short-term solution for obtaining chemical energy.

RECAP 3.13

1 List two differences between aerobic respiration and fermentation.

2 Compare the products of anaerobic respiration with those of aerobic respiration in:
   a animal cells
   b plant cells.

Factors affecting the rate of cellular respiration

The rate of cellular respiration is influenced by many environmental and physiological factors. Temperature and the concentrations of glucose and oxygen have important effects on cellular respiration. As with all biochemical reactions, as the concentration of substrates increases the rate of respiration increases up to a saturation point, at which other factors limit the reaction rate. These other factors may include the amount of enzyme produced and degraded by the cell, the pH of the environment, and the presence of cofactors and coenzymes, as well as competitive and non-competitive inhibitors of the enzymes involved.

Yeast provides an accessible model for measuring the effect of various factors on the rate of cellular respiration. When mixed with a sugar food source and water, yeast undergoes cellular respiration, producing carbon dioxide that forms foam. Several independent variables can be altered in this system, including temperature, sugar sources and the availability of oxygen, and foam can be measured as a dependent variable.

Temperature

The rate of cellular respiration rises as the temperature increases. However, at a certain temperature, the enzymes involved begin to become denatured and cellular respiration is inhibited. Organisms reach the upper limit of their tolerance range and cells and tissues start to shut down.

At lower temperatures, the kinetic energy of all the molecules involved is reduced and this affects the efficiency of the reaction. Plants have lower growth rates and some animals go into hibernation so that their energy requirements are minimised.

Glucose

Cellular respiration depends on an ongoing supply of glucose and cells must constantly replenish their glucose stores. Cells can do this in a number of ways. Photosynthetic cells can produce their own glucose by photosynthesis. Alternatively, cells can transport extracellular glucose into the cell. In multicellular organisms, glucose can be stored in specialised cells and released and transported, as required, to the rest of the tissues. In plants, as discussed earlier, starch grains in chloroplasts store glucose for future use. In animals, glucose is accumulated and stored as **glycogen** in liver cells. The need for glucose must be communicated between the cells that demand it and the cells that store it. If glucose stores become depleted, the body draws on alternative sources to meet the energy needs of its cells. Pyruvate, lactic acid and lipids may be recycled to generate the glucose molecules required for cellular respiration.

Oxygen

The availability of oxygen affects a cell's switch from aerobic to anaerobic respiration. During anaerobic respiration, the ATP is supplied purely by glycolysis. The switch to anaerobic respiration therefore results in a decrease in the efficiency of ATP production. Far less ATP is generated from each glucose molecule during anaerobic respiration than aerobic respiration (two versus 36 ATP molecules).

On the other hand, ATP production by anaerobic respiration is faster than that of the more complex set of reactions involved in aerobic respiration. Consequently, if the demand for ATP outstrips the supply from aerobic respiration, eukaryotic cells supplement demand by shifting towards anaerobic respiration. This occurs, for example, in the muscle cells of athletes such as sprinters when they perform brief bursts of strenuous exercise. The switch is only a temporary solution, and the accumulation of acid, as lactic acid, in the cells inhibits metabolism. It must be removed to restore normal cell functioning and this takes time to achieve.

Putting photosynthesis and aerobic cellular respiration together

Photosynthesis and aerobic cellular respiration are closely related and interdependent – that is, the outputs of one are the inputs of the other (Figure 3.33). In plants and other autotrophs, the two processes occur in the same individual cells when both

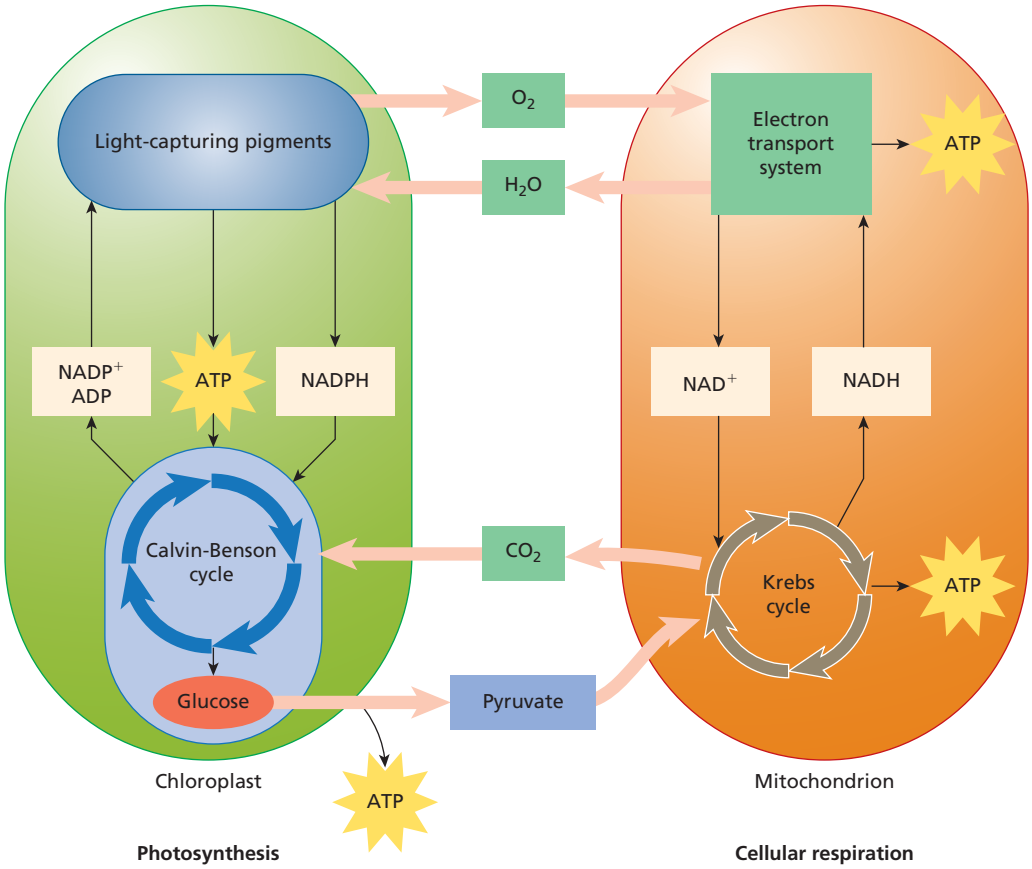


Figure 3.33 ▶ Photosynthesis uses the products of cellular respiration and cellular respiration uses the products of photosynthesis.

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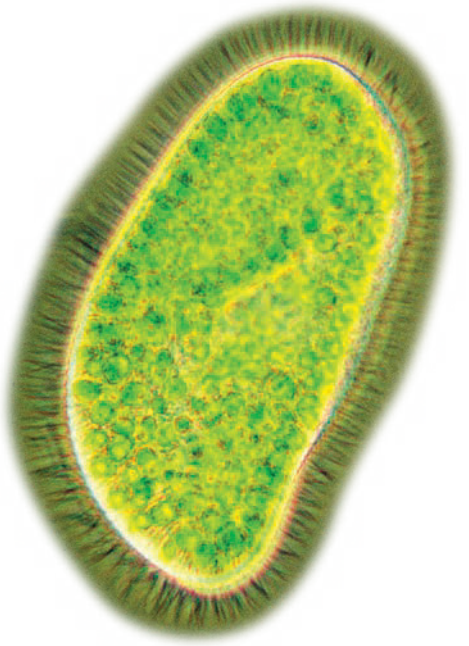
chloroplasts and mitochondria are present. But many cells in green plants, such as root cells, do not have chloroplasts. These cells and those of heterotrophs depend on the products of photosynthesis to carry out cellular respiration. Thus, there is a dependency between autotrophs and heterotrophs. Interestingly, both types of eukaryotic cells have come to rely on two ancient prokaryotic cells that we today call organelles: chloroplasts and mitochondria. These organelles are integral to their host cell's survival strategy as they carry out high-risk energy transformations in the form of electron transfer reactions.

The origins of chloroplasts and mitochondria: the endosymbiotic theory

The predatory way of life of a single-celled eukaryote can help explain how cells may have acquired mitochondria and chloroplasts. Scientists believe that mitochondria and chloroplasts evolved through **endosymbiosis**, where one species lives inside another in a mutually beneficial relationship. The larger host cell would have ingested the smaller cells by phagocytosis, where they escaped digestion and proliferated along with the host cell. To this day mitochondria and chloroplasts make copies of themselves and split in two, like bacteria do when they reproduce by binary fission. Both mitochondria and chloroplasts have two membranes. The outer one is probably derived from the host membrane when it engulfed the bacterium, and the inner one is probably the membrane of the ingested bacterium.

Mitochondria are similar in size to small bacteria and they have their own genome, which, like that in bacteria, is contained on a circular DNA molecule and lacks histones. They contain unique ribosomes that differ from those found in the cell cytosol, and they have their own transfer RNA molecules so that they can make their own proteins. It is now almost certain that an ancestral eukaryotic cell engulfed an aerobic eubacterium that managed to escape digestion. The eubacteria would have evolved with the eukaryotic cell, receiving protection and organic molecules, such as sugar, in return for the ATP molecules they produced for their hosts. Because of the endosymbiotic relationship, the host cell would have been more productive and successful than cells lacking eubacteria. It would have been more competitive and able to survive to reproduce even though other cells perished. This relationship was probably established when the Earth's atmosphere first became oxygen-rich about 1.5 billion years ago.

Many eukaryotic cells also contain chloroplasts. Scientists believe that these arose from cyanobacteria that were ingested by eukaryotic cells that already contained mitochondria about one billion years ago (Figure 3.35). This explains why not all eukaryotic cells contain chloroplasts but they all contain mitochondria. Chloroplasts have their own DNA, RNA and ribosomes that are similar to those of prokaryotes. They harness energy from light, storing it in the bonds of organic molecules such as glucose. These organic molecules provide energy to the host, and the host environment provides inorganic compounds, such as carbon dioxide and water, and protection from drying out and from predators. A eukaryotic cell that contains chloroplasts no longer needs to engulf other cells to obtain food. This may explain why plant cells have lost the ability to change shape rapidly to engulf other cells by phagocytosis. Instead, they have developed a tough and protective cell wall.



▲ **Figure 3.34** Light micrograph of *Paramecium bursaria*, a single-celled protozoan. The smaller green cells within the *Paramecium* are unicellular green algae, which live endosymbiotically in the organism.

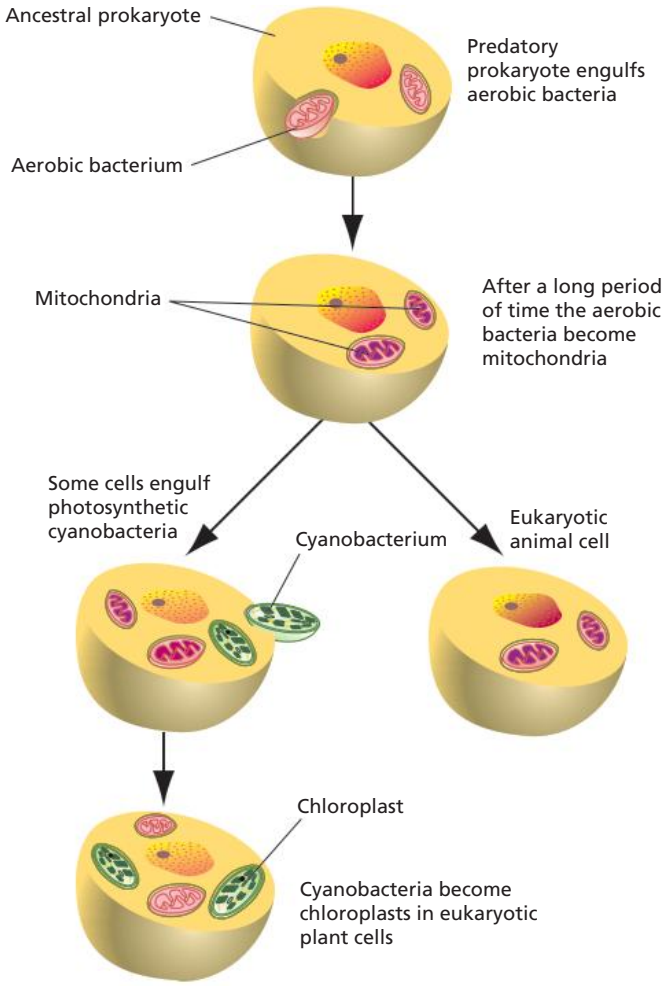


Figure 3.35 ▶
The theory of endosymbiosis explains how cells may have acquired mitochondria and chloroplasts.

RECALL

- The rate of cellular respiration is affected by temperature, glucose and oxygen availability as well as other factors.
- Photosynthesis and cellular respiration are linked in that the inputs of one are the outputs of the other.
- The endosymbiotic theory provides an explanation for how mitochondria and chloroplasts came to be within eukaryotic cells.

RECAP 3.14

1 Describe how cellular respiration is altered when
   a temperature is very high or low
   b oxygen is very high or low
   c glucose is very high or low.

2 Draw a simple schematic diagram to show how photosynthesis and cellular respiration are linked.

3 List the evidence that has led scientists to believe that mitochondria and chloroplasts were once free-living organisms.

Biological knowledge and society: Transgenic canola as a sustainable omega-3 supply

Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is needed for healthy plasma membranes, but it cannot be synthesised by humans in adequate amounts so it must be supplied in the diet. This is achieved by eating oily fish, which is high in DHA, or by taking fish oil dietary supplements. The fish oil industry is big business and there are issues concerning the sustainability of ocean ecosystems. Some companies are fermenting farmed algae to supply the infant formula and medical grade omega-3 market, but this is an expensive process. A cheap, sustainable source of omega-3 fatty acids is needed.

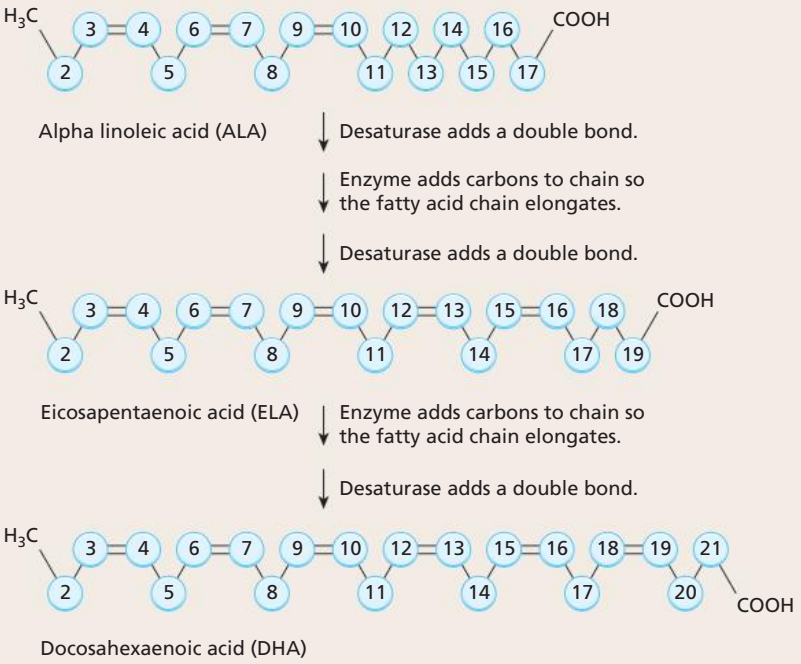
For more information about the issues surrounding the fish oil industry, refer to the science and society box in Chapter 1.

Can science help solve this issue?

A group of scientists working with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) have used DNA technologies to produce the omega-3 fatty acid DHA in canola. Canola is an oilseed crop widely grown in Australia for the cooking oil industry. It can produce alpha linoleic fatty acid (ALA) naturally, but not DHA fatty acids. The scientific team have spent years genetically engineering a strain of canola so it will produce DHA from ALA. Because it was engineered to contain a set of new, introduced genes required for DHA production from its precursor substrates, the genetically modified DHA canola is a *transgenic* crop variety. This is in contrast to other modes of genetic modification, including *knocking out* a gene or *knocking a gene in*to a specific locus. (See Chapter 11 for more details on these terms.)

Discovering genes required for DHA synthesis

Various species of marine algae use biochemical pathways to produce DHA from ALA (Figure 3.36). They have genes for enzymes that control steps in this biosynthesis pathway. Canola does not have the enzymes to produce DHA from ALA, so to get canola to synthesise DHA in its seeds the team had to identify the genes for the DHA conversion enzymes in marine algae and then introduce them into the canola plant. They also had to ensure coordinated expression of all of the introduced genes so the entire pathway would be switched on in DHA canola seeds.



▲ **Figure 3.36**
A biochemical pathway for synthesising the omega-3 polyunsaturated fatty acid DHA from ALA. Land plants can only synthesise ALA because they lack the enzymes found in this pathway.

For more information on the use of endonucleases and ligases to generate recombinant plasmids refer to Chapter 11.

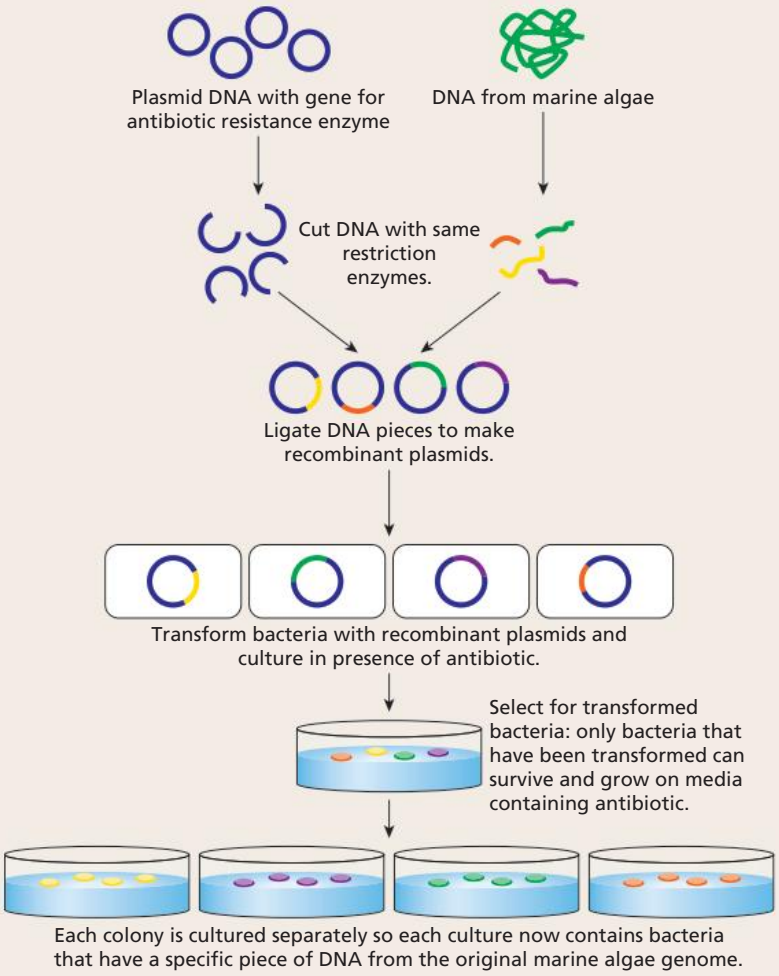


Figure 3.37 ▲
To identify the genes responsible for DHA synthesis in marine algae, scientists use gene cloning to generate a DNA library of the marine algae genome.

The first step is to identify the genes coding for enzymes in the DHA biosynthesis pathway in marine algae. To do this, scientists use a technique called gene cloning to generate a DNA library for the marine algae genome, as outlined in Figure 3.37. Next, scientists transform eukaryotic yeast cells with marine algae DNA from the DNA library. Each transformed yeast culture is incubated in a solution containing the substrate ALA. The scientists test each culture to see if it can produce DHA. Any culture producing DHA from ALA must contain genes from the marine algae library that are required in the final steps of the DHA synthesis pathway.

Engineering canola to express genes for DHA synthesis

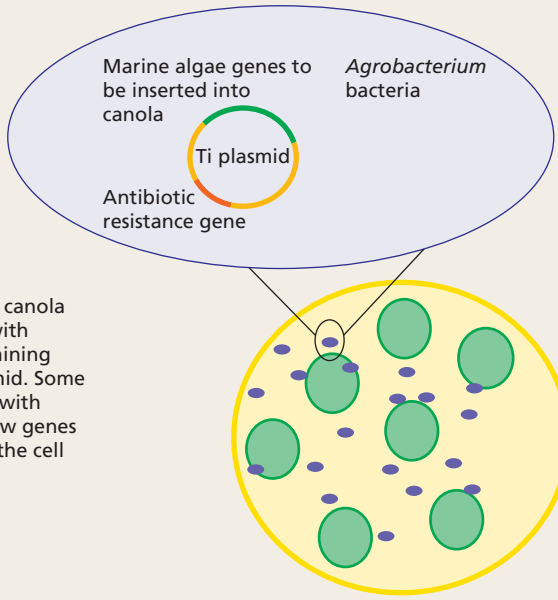
Once the genes of interest have been discovered, scientists need to insert them into canola plant cells. This process takes advantage of a plant pathogen called *Agrobacterium tumefaciens*. This soil bacterium naturally infects plants, causing gall disease. *Agrobacterium* contain a plasmid called tumour inducing plasmid (Ti plasmid). This plasmid contains genes that code for proteins that hijack the plant cell machinery so plasmid genes are integrated into the plant cell's genome. Scientists can remove the disease-causing genes from the Ti plasmid and replace them with the gene of interest for insertion into the plant genome. As a result, the plant will not get gall disease. Instead it will get a new set of one or more genes inserted into its genome, and so it becomes transgenic.

Figure 3.38 outlines the steps used to produce transgenic DHA canola. The genes coding for enzymes required for the DHA synthesis pathway are inserted into Ti plasmids. *Agrobacterium* are then transformed with the recombinant Ti plasmid. These transformed *Agrobacterium* are incubated with leaf discs cut from canola leaves and placed onto a growth medium containing antibiotic. The antibiotic acts as a selective agent: only cells that have been infected by *Agrobacterium* can divide in the presence of the antibiotic because the Ti plasmid carries an antibiotic resistance gene. The Ti plasmid also carried the DHA synthesis genes so these plant cells will also have the DHA synthesis genes inserted into their genome. The plant cells that have the new DNA are said to be transfected. All cells arising from these transfected cells have the new genes in their genome.

In the growth medium, the dividing cells form a cell mass called a callus. The callus is transferred to shooting growth media so shoots develop. The shoots are then transferred to rooting media to develop roots. The transgenic plants are then grown in secure glasshouses. At maturity, their seeds are tested for DHA content.

All activities involving genetically modified organisms (GMOs) in Australia require authorisation from the Office of the Gene Technology Regulator (OGTR). If a GMO is grown in a laboratory or in the

◀ **Figure 3.38**
The steps involved in producing transgenic canola plants that produce DHA



1 Cut leaf discs from canola plant and incubate with *Agrobacterium* containing recombinant Ti plasmid. Some cells will be infected with *Agrobacterium* so new genes will be inserted into the cell genome.

Alamy/age fotostock

2 Antibiotic in growth media lets only cells transfected with Ti plasmid divide and grow into a callus.

Getty Images/Sinclair Stammers

3 Transfer callus of transfected cells onto shooting media, then transfer growing plants onto rooting media.

amanaimages/Chris Crewell/ZUMA Press

4 Grow plants in greenhouse and test seeds for DHA production.

field it will require a licence. The OGTR uses risk analysis to decide whether or not to issue a licence, and monitors compliance with these licences. The OGTR identifies possible risks by considering how a new property of a GMO may cause harm to people or the environment. Because any organism can potentially cause harm, the OGTR compares the risk of a GMO against the risk of harm from the 'parent' organism. There also needs to be a legitimate benefit to society and/or the environment in producing the GMO.

Over to you

1. What is the issue?
2. Identify the relevant biology that relates to this issue.
3. Distinguish between genetically modified organisms and transgenic organisms.
4. Construct a flow chart to show the steps taken to enable canola to produce DHA. In your flow chart, show the biology and the tools and techniques used to manipulate DNA.
5. Imagine that you work in the OGTR. Scientists have applied for approval to release DHA canola commercially. Construct a list of the risks, benefits and impacts on society and the environment if this transgenic canola is approved for release. Use the following questions as a guide to consider risk, benefit and equity.
   - a Does DHA canola represent a solution to one or more current issues?
   - b Does it present unacceptable risks to the environment?
   - c Does it present unacceptable risks to animal and/or human health?
   - d Will all farmers have equitable access to this technological advance?
   - e Does this technological advance negatively impact any other industries?

CONCEPT SUMMARY

Cellular metabolism

- The metabolic rate differs between organisms, affecting their radiated heat and lifespans
- Anabolic reactions building molecules, endergonic
- Catabolic reactions – breaking down molecules
- ATP → ADP + Pi + energy

Key terms

- Substrate
- Product
- Active site
- Lock-and-key model
- Induced fit model
- Cofactors
- Coenzymes (loaded and unloaded)

Enzymes

Organic catalysts that control steps of a biochemical pathway and act by reducing the activation energy of a reaction

Affected by

- Temperature
- pH
- Substrate concentration
- Non-competitive inhibitors
- Competitive inhibitors

Energy sources for organisms

Autotrophs

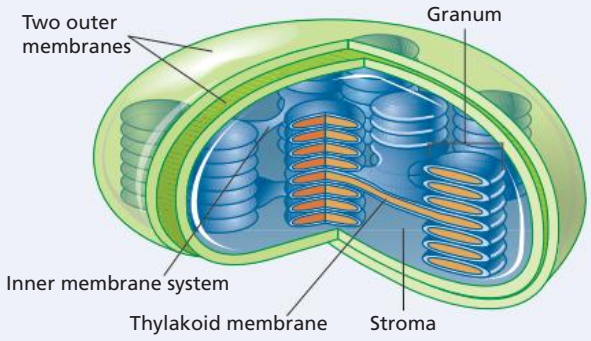
- Photoautotrophs
- Chemotrophs

Photosynthesis

$$12H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C6H_{12}O_6 + 6H_2O$$

Photosynthesis is affected by light, CO_2 and temperature

CHLOROPLAST STRUCTURE



Stages of photosynthesis

The light-dependent stage

- Light energy is absorbed by pigments, especially chlorophylls.
- Electrons within the molecule are energised and ejected from chlorophyll. These enter the electron transport chain.
- To replace the lost electrons, chlorophyll splits water molecules into electrons (seized by chlorophyll), protons (H^+; pumped into thylakoid space), and oxygen gas (a product of photosynthesis).
- $NADP^+$ receives a proton and a pair of electrons and becomes NADPH.
- The protons build up in the thylakoid space and exit through ATP synthase, driving formation of ATP from ADP and inorganic phosphate.

The light-independent stage

- The Calvin-Benson cycle occurs in the chloroplast stroma.
- This series of reactions requires carbon dioxide, hydrogen ions (from NADPH) and ATP.
- The 3-carbon sugar phosphate molecules are used to build glucose, which can be converted to sucrose, starch and cellulose.
- Starch is produced and stored in the stroma during the day. At night, cells convert starch to sucrose for export to cells in other plant tissues.

Cellular respiration

Affected by:

- temperature
- concentrations of glucose and oxygen
- the amount of enzyme produced and degraded by the cell
- the pH of the environment
- the presence of cofactors and coenzymes
- competitive and non-competitive inhibitors

Aerobic cellular respiration

$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + 36ATP$

- The process occurs in the mitochondrion.
- Pyruvate and coenzyme A react to form a loaded coenzyme, acetyl CoA, plus carbon dioxide.
- Acetyl CoA enters the Krebs cycle (also called the citric acid cycle), which produces more carbon dioxide, ATP and loaded coenzymes (e.g. NADH, $FADH_2$).
- The loaded coenzymes transfer electrons to an electron transport chain, which involves cytochromes in the inner mitochondrial membrane.
- Oxygen is the final electron acceptor molecule in the electron transport system.
- The electron transport chain pumps protons (H^+) from the loaded coenzyme molecules into the mitochondrial intermembrane space.
- As the protons re-enter the matrix, they drive the production of ATP by ATP synthase. A net 36 ATP molecules are made per cycle.

Anaerobic cellular respiration

Anaerobic pathways react the NADH with pyruvate, formed in glycolysis. This unloads the NADH of a proton, forming NAD+, allowing glycolysis to continue.

Alcoholic fermentation

- A carbon dioxide molecule is removed from the pyruvate molecules produced in glycolysis to produce acetaldehyde.
- NADH donates a proton to acetaldehyde and ethanol is formed.
- $C_6H_{12}O_6$ (glucose) $\rightarrow 2CH_3CH_2OH$ (ethanol) + $2CO_2$ (carbon dioxide) + 2ATP
- Energy (210 kJ) is formed in this process.

Lactic acid fermentation

- NADH is oxidised to NAD^+, allowing glycolysis to continue.
- Lactic acid can be converted back into pyruvate, which may then enter the Krebs cycle.
- $C_6H_{12}O_6$ (glucose) $\rightarrow 2CH_3CH(OH)COOH$ (lactic acid) + 2ATP
- Energy (150>kJ) is formed in this process.

CHAPTER GLOSSARY

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

activation energy the energy required to initiate a reaction

active site the place on the surface of an enzyme molecule where substrate molecules attach

adenosine diphosphate (ADP) a low-energy compound composed of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound composed of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

anabolic reaction a reaction that builds up complex molecules from more simple ones

anaerobic without oxygen

ATP synthase an enzyme that provides energy for the cell through synthesis of ATP

autotroph an organism capable of making its own food from inorganic substances using light (through photosynthesis) or chemical energy (through chemosynthesis); green plants, algae and certain bacteria

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to form a final product

C_3 plant a plant that carries out a type of photosynthesis in which a three-carbon compound, phosphoglycerate, is the first stable product of carbon fixation; this occurs during the first step of the Calvin-Benson cycle. Most plants are C_3 plants

C_4 plants plants such as tropical grasses in which a four-carbon compound, malate, is the first stable product of carbon fixation; carbon dioxide is later drawn out of the malate and into the Calvin-Benson cycle rather than directly from air

Calvin-Benson cycle a biochemical pathway in which sugar molecules are produced from carbon dioxide

CAM plant (crassulacean acid metabolism) a plant that uses an enzyme to fix carbon dioxide at night, generating the four-carbon compound malate; this is broken down during the day to release carbon dioxide, which then enters the Calvin-Benson cycle

carbon fixation the use of atmospheric carbon dioxide and its conversion into carbohydrates; this process occurs in the stroma of chloroplasts in eukaryotic cells

catabolic reaction a reaction, such as cellular respiration, that involves the breakdown of complex molecules to simpler products

catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change

cellular metabolism the sum of metabolic reactions in a cell

chemotroph an organism that synthesises its own food from inorganic substances using chemicals as the primary energy source

chlorophyll the green pigment found in chloroplasts; it is able to absorb light energy, making it available for photosynthesis

chloroplast a membrane-bound organelle containing the green pigment chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates

citric acid cycle see **Krebs cycle**

coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction

cofactor an ion that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction

competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity

cristae the folding of the inner membrane into the matrix of a mitochondrion, thus increasing the total surface area of the inner membrane

cytochrome a membrane-bound protein that carries out electron transport; cytochromes are located in the mitochondrial inner membrane and in chloroplasts

electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the phosphorylation of ADP to yield ATP

endergonic reaction a chemical reaction requiring energy

endosymbiosis a mutually beneficial relationship between two single-celled organisms in which one of them lives inside the other

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

exergonic reaction a reaction that releases energy

feedback inhibition the cellular control mechanism in which an enzyme that catalyses the production of a particular product is inhibited by the product, therefore balancing supply and demand of a product for a cell

glycogen an energy-storage polysaccharide in animals that is composed of glucose

glycolysis an energy-yielding process occurring in the cell cytosol in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

heterotroph an organism that cannot synthesise its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

induced-fit model a model to explain that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl coA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

lactic acid a product of anaerobic cellular respiration in animals

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll; water molecules split to produce oxygen and hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions carbon dioxide, hydrogen ions and ATP produce carbohydrate

loaded describes coenzymes that are attached to the specific group of atoms they transfer

lock-and-key model a model suggesting that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

NAD^+ unloaded form of the coenzyme nicotinamide adenine dinucleotide; has a role in cellular respiration

NADH loaded form of the coenzyme nicotinamide adenine dinucleotide; has a role in cellular respiration

$NADP^+$ unloaded form of the coenzyme nicotinamide adenine dinucleotide phosphate; has a role in photosynthesis

NADPH loaded form of the coenzyme nicotinamide adenine dinucleotide phosphate; has a role in photosynthesis

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

optimum pH the pH at which an enzyme works at its fastest rate

optimum temperature the temperature at which an enzyme works at its fastest rate

phosphorylation the addition of a phosphate group to a protein or other organic molecule

photoautotroph an organism that synthesises its own food from inorganic substances using light as its primary energy source

photorespiration an alternative pathway for Rubisco, the carbon-fixing enzyme in photosynthesis, in which oxygen is consumed and carbon dioxide is produced, decreasing the rate of photosynthesis; generally occurs when stomata close and the concentration of oxygen in the leaf exceeds that of carbon dioxide

photosynthesis anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose

pigment a molecule that absorbs certain wavelengths of light and reflects all others

psychrophile an organism that lives in extremely cold conditions

pyruvate the three-carbon molecule that is the end product of glycolysis

stroma the jelly-like, semifluid interior of a chloroplast

substrate a substance upon which an enzyme acts; a reactant for an enzyme-controlled reaction

thermophile an organism that lives in high-temperature environments

thylakoid membrane the interconnected, folded membrane within a chloroplast

thylakoid space the space inside a thylakoid membrane

unloaded describes coenzymes that are not attached to the specific group of atoms they transfer

CHAPTER REVIEW QUESTIONS

Understanding

1 Identify each of the following as either an anabolic or a catabolic process. Justify your choice in each case.
   - **a** Protein synthesis
   - **b** Digestion
   - **c** DNA synthesis
   - **d** Photosynthesis
   - **e** Cellular respiration

2 What is meant by phosphorylation? What is its significance to maintaining a cell's energy supply?

3 Enzymes are responsible for production of both sperm and male sex hormones in the testicles of human males. Some of these enzymes have an optimal temperature of 33°C, which is about 4°C lower than body temperature. If this temperature is increased or lowered, sperm and testosterone production is adversely affected.
   - **a** Why would an increase in temperature affect sperm production?
   - **b** What anatomical feature helps the testicles to maintain a lower temperature?

Applying

4 Many cut fruits will brown quickly when exposed to air. This is caused by the naturally occurring enzyme polyphenol oxidase. If the freshly cut fruit is rubbed with lemon juice, it can prevent the brown discoloration. Explain why this happens.

5 Cyanide binds to the enzyme cytochrome oxidase, preventing it from transferring electrons to the final acceptor molecule in aerobic cellular respiration.
   - **a** What is the final acceptor molecule?
   - **b** Where in the cell would cyanide target this enzyme?
   - **c** Explain why cyanide is such a fast-acting poison that results in the death of the organism.

6 Describe two pieces of evidence that support the view that chloroplasts and mitochondria were once free-living prokaryotic organisms that formed an association with a larger host cell. How could this relationship be mutually beneficial?

7 Describe two structural differences and two structural similarities between chloroplasts and mitochondria.

8 The pH of human blood and body fluids (excluding gastric juices) is about 6.8–7.0. Explain why maintaining this level of pH is important.

9 During a heart attack, blood flowing to the heart muscle is interrupted by a blockage of a coronary artery. How would you expect the metabolism in the heart to change?

10 After a heart attack, people often have small amounts of lactate in the blood, which comes from the injured heart muscle. Suggest an explanation for this observation.

11 Are photosynthesis and cellular respiration exact opposites? Explain.

12 Draw a labelled diagram of a chloroplast. Note on the diagram the sites where the light-dependent and the light-independent stages of photosynthesis occur.

13 Discuss the factors that may affect the rate of photosynthesis in a plant exposed on a hilltop from sunrise to sunset during the course of a hot summer's day.

Analysing

14 You are given two test tubes containing two types of yeast cells that are the same in every way except that one can carry out only aerobic respiration and the other can carry out only anaerobic respiration. The tubes are labelled A and B. Yeast in tube A grows rapidly, whereas the yeast in tube B grows slowly. Which tube contains the cells capable of performing only aerobic respiration? Justify your choice. Devise an experiment to explain the test result.

Evaluating

15 Organisms such as the bacterium *Thermophilus* can thrive in hot springs at about 80°C. Use resource materials to find out why some enzymes are more heat stable than others.

16 If a test tube containing an aqueous suspension of chloroplasts is kept in the dark, what substances would you have to add to the suspension for a three-carbon sugar phosphate molecule to be formed by photosynthesis? Explain.

Creating

17 Investigate the use of a commercial enzyme and record your findings under the following subheadings. Some trade names of commercial enzymes are Neutrase, Lipolase, Lactozyme and Termamyl.
   - **a** Source of the enzyme
   - **b** Properties or action of the enzyme
   - **c** Industrial or commercial applications


Shutterstock.com/James AP

CHAPTER 4

CELLULAR SIGNALS AND APOPTOSIS

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

KEY KNOWLEDGE

Cellular signals

- the sources and mode of transmission of various signalling molecules to their target cell, including plant and animal hormones, neurotransmitters, cytokines and pheromones
- the stimulus–response model when applied to the cell in terms of signal transduction as a three-step process involving reception, transduction and cellular response
- difference in signal transduction for hydrophilic and hydrophobic signals in terms of the position of receptors (on the membrane and in the cytosol) and initiation of transduction (details of specific chemicals, names of second messengers, G protein pathways, reaction mechanisms or cascade reactions are not required)
- apoptosis as a natural, regulatory process of programmed cell death, initiated after a cell receives a signal from inside (mitochondrial pathway) or from outside (death receptor pathway) the cell resulting in the removal of cells that are no longer needed or that may be a threat to an organism, mediated by enzymes (caspases) that cleave specific proteins in the cytoplasm or nucleus (details of specific cytoplasmic or nuclear proteins are not required)
- malfunctions in apoptosis that result in deviant cell behaviour leading to diseases including cancer.

KEY SCIENCE SKILLS

Analyse and evaluate data, methods and scientific models

- organise, present and interpret data using schematic diagrams and flow charts, tables, bar charts, line graphs, ratios, percentages and calculations of mean

Getty Images/Time Life Pictures

Figure 4.1 ▶
Nineteenth century revolutions in medicine: William Morton administering ether to a patient, enabling the painless removal of a neck tumour.

It is 1846 in Boston and an operation is about to be performed in an amphitheatre with medical professionals looking on. The surgeon, John Collins Warren, is about to remove a tumour from his patient's neck. In the absence of painkiller, the patient is strapped down to prevent excessive movement. William Morton, a local dentist, strides in holding a glass inhaler that contains ether. He asks the patient to breathe in the gas. The patient loses consciousness and feels no pain throughout the entire operation. This was the first successful public demonstration of the use of an anaesthetic. The ether, by disrupting **cell signalling** in the nervous system, rendered the patient insensitive to pain.

Throughout each cell's life it releases and receives signals that regulate and coordinate everyday **cellular processes** for the benefit of the whole organism. These processes control whether a cell should move or stay in stasis, which molecules and structures to build and which ones to digest, whether to grow, what to consume, and what to secrete. Modern research scientists use an understanding of cell signalling pathways and of molecular structures to design highly specific medicines to treat disease and pain. Understanding the underlying mechanisms that cells utilise to produce, receive and respond to chemical signals is providing scientists with major breakthroughs in the areas of health, agriculture and the environment, which help to change the way we live and behave. This chapter is all about how cells communicate to coordinate cellular activities using chemical signalling.

Evolution of cell signalling systems

Ever since the first prokaryotic cell appeared on Earth billions of years ago, there has been a need for cells and organisms to detect and respond to external environments to keep their internal environment safe. These early single-celled organisms bathed in a sea containing calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}) and chloride (Cl^{-}) ions. These charged ions are very small but they cannot cross plasma membranes. To survive in these seas, the cells evolved **ion channels** to control the passage of ions across their plasma membrane.

Calcium ions (Ca^{2+}) were integral to cell functioning in prokaryotes, but high concentrations were lethal. These primitive bacteria had to keep their **intracellular** Ca^{2+} levels 10 000 times lower than the **extracellular** environment. Through evolution, proteins arose to be highly sensitive to changes in Ca^{2+} concentrations. Small increases in intracellular Ca^{2+} levels could change the activity of the proteins inside the cell. This set the stage for the evolution of calcium signalling systems that are now found in all organisms. Ca^{2+} gradients are involved in regulating exocytosis, muscle contraction, gene regulation and more. **Ion gradients** have evolved to be enormously important in cell signalling.

Unicellular organisms socialise for group work

Wikipedia/Bruno in Columbus

▲ **Figure 4.2** A gathering of the social slime mould, *Dictyostelium*. Single cells can be seen migrating towards a central point by chemotaxis.

Communication systems continued to evolve as cells became more complex. Prokaryotes evolved the capacity to detect signals secreted by other cells. They started to socialise and coordinate group work, enhancing the survival of the species. Some scientists believe that this **intercellular** signalling may have paved the way for the evolution of multicellular organisms. For example, the social slime mould (*Dictyostelium*) spends most of its time as a unicellular organism; however, when it is challenged by starvation the cells secrete a signalling molecule that attracts other slime mould cells by **chemotaxis**, or directional cell movement. This coordinates thousands of cells so that they group together to form a multicellular 'slug', capable of moving around in search of a new food source (Figure 4.2).

Cell chatter in multicellular organisms

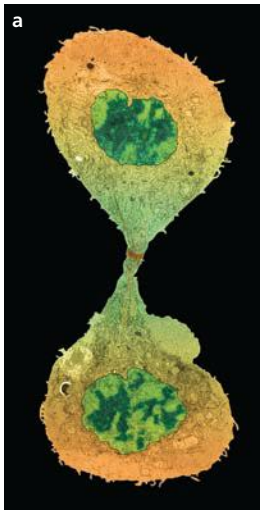
Becoming multicellular requires the coordination of many cells for survival. Cells become specialised to perform particular functions and group together to form tissues, and ultimately organs and coordinated body systems. This type of complexity requires constant monitoring and adjusting and, therefore, cell communication.

Cells specialise to perform particular functions

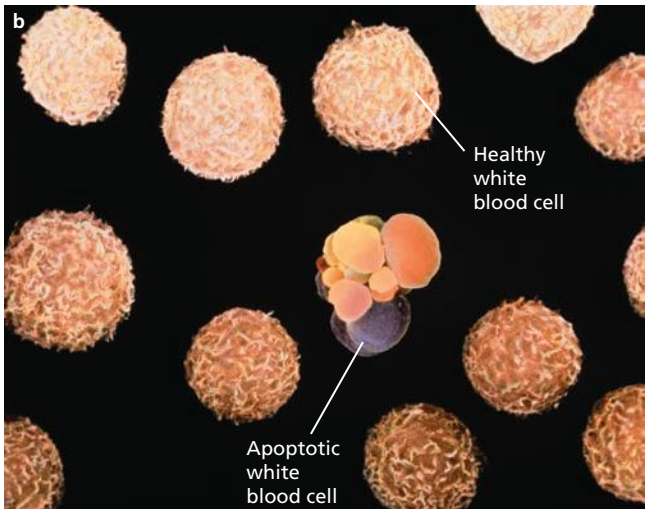
There are approximately 200 different types of cells that perform particular jobs in the human body. All of these cells arise from **stem cells**, precursor cells that receive signals that stimulate **cell differentiation**, so that they develop particular characteristics enabling them to perform specialised functions in our tissues. For example, many different types of blood cells arise from stem cells produced in the bone marrow.

Keeping cell numbers in balance

The length of a cell's life varies. White blood cells live for approximately 13 days, red blood cells 120 days and liver cells for 18 months. Every second, your body is producing more than one million red blood cells, so it follows that old and damaged red blood cells must be removed to balance the numbers. Chemical signalling controls the balance of cell numbers in multicellular organisms. Signals stimulating cell division increase numbers and those stimulating **apoptosis** (programmed cell death, discussed in greater detail on page 132 of this chapter) decrease numbers.



Science Photo Library/Steve Gschmeissner



Science Photo Library/Dr Gopal Murti

◀ **Figure 4.3** Regulating cell numbers through chemical signalling. (**a**) A skin cell undergoing cell division to increase cell numbers; (**b**) A damaged white blood cell undergoing apoptosis so it will be removed from the organism

Signalling molecules are the key to controlling cell activities

Signalling molecules are effective in minute amounts and play important roles in homeostasis, growth and development, reproduction, behaviour and energy production, storage and use. These chemical signals can come into contact with many cells. But only specific **target cells** respond. What drives this specificity? For chemical signals to stimulate a response, they must bind to **receptors**, proteins that are located inside or on the surface of target cells. Just as you need the right-shaped key to open a lock, a specific signalling molecule can bind only to a specific receptor. Its shape and chemical properties allow it to fit into the **binding site** of the receptor protein. Once binding takes place, the receptor is activated and this triggers a cascade of processes inside the cell (Figure 4.4). The processes triggered depend on the receptor that has been activated. In this way the release of chemical signals controls specific responses in specific cells, such as those seen in Table 4.1.

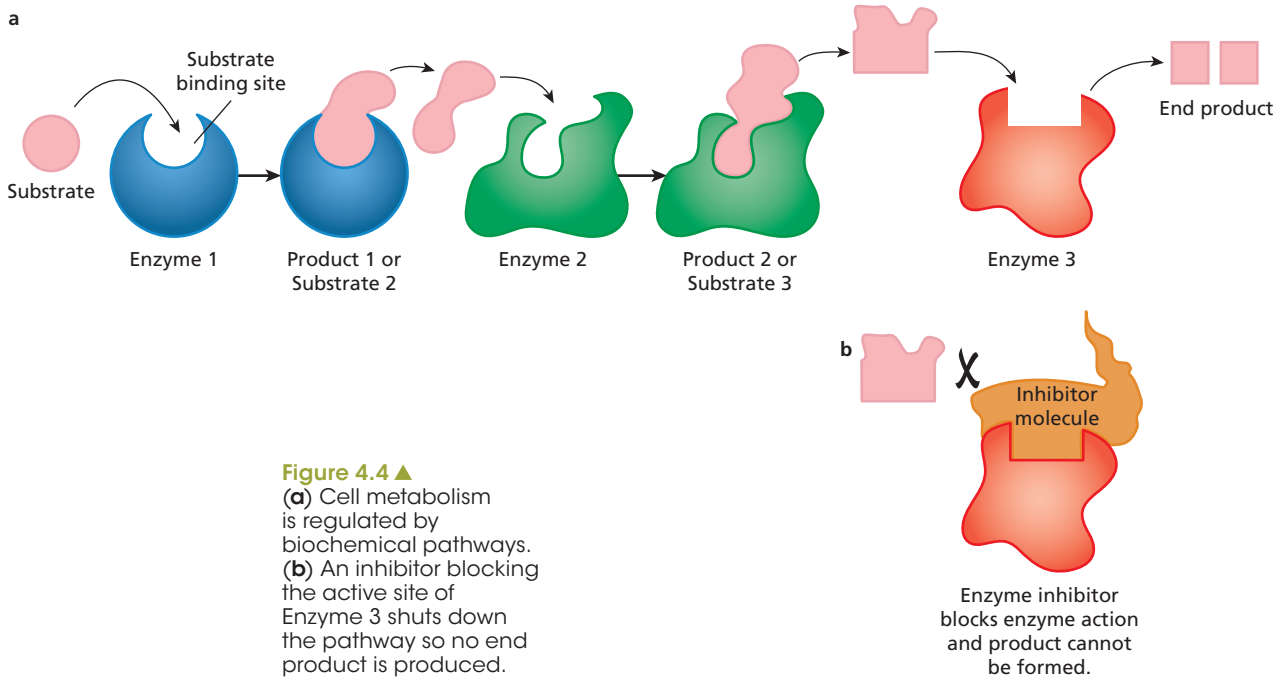


Figure 4.4 ▲
(**a**) Cell metabolism is regulated by biochemical pathways. (**b**) An inhibitor blocking the active site of Enzyme 3 shuts down the pathway so no end product is produced.

Table 4.1 Cellular processes regulated by chemical signalling

Cellular process	Description of process
Gene expression	Proteins are the cellular machinery, carrying out diverse functions in cells. Cells regulate when a particular protein is produced by switching gene expression on and off.
Metabolism	The biochemical reactions required to maintain life are organised into metabolic pathways allowing for the transformation of molecules in steps. Catabolic (digestion) reactions provide energy for anabolic (synthesis) reactions.
Programmed cell death (apoptosis)	Allows removal of damaged and diseased cells and is involved in maintaining cell numbers.
Reproduction	Cells replicate for growth and development via mitosis and produce gametes via meiosis. In order to do this, DNA replication must take place.
Cell communication	Cells need to communicate to coordinate functioning of the whole organism.
Molecule transport	Nutrients and molecules are transported between organelles and into and out of a cell through osmosis, diffusion, active transport, endocytosis and exocytosis.

These cellular processes ultimately rely on **cellular metabolism**, the collective biochemical reactions occurring in a cell to maintain homeostasis. As you can see in Figure 4.4, reactions are organised in **biochemical pathways**, with each reaction or step in the pathway controlled by an enzyme. At each step, substrate molecules are joined together, separated, or rearranged to produce new products. These reactions are monitored and balanced to meet supply and demand. Often a pathway is **up-regulated** (increased) in response to increasing concentrations of substrate or to meet demand for a product. When the substrate becomes limiting, the pathway may be **down-regulated** (decreased). Sometimes the buildup of products of biochemical reactions causes down-regulation of a pathway in a process called **feedback inhibition**.

Cells regulate metabolism by controlling the production and activity of enzymes. To increase the amount of enzyme, the gene for that enzyme must be expressed. The activity of an enzyme can be altered by changing the conformation of the enzyme; for example, by adding a phosphate group. **Inhibitors** bind to enzymes, changing the conformation and rendering the enzyme inactive. When the inhibitor is removed, the enzyme becomes active once more. In a similar fashion, **activators** bind to enzymes to change their conformation so that they become active. By managing enzyme production and activity, and thus biochemical pathways, the cell can respond to changes in its environment and regulate its metabolism.

RECALL

- The evolution of ion pumps to maintain internal ion gradients relative to external ion gradients was integral to the development of cell signalling systems.
- Unicellular organisms evolved to have intercellular signalling systems to coordinate group work.
- Multicellular organisms have complex signalling systems that coordinate cell processes for the survival of the whole organism.

RECAP 4.1

1 Name four charged ions utilised by cells in chemical signalling.
2 Identify the cellular processes that maintain cell numbers in multicellular organisms.
3 How did the evolution of ion pumps contribute to the development of cell signalling systems?
4 Name three ways that the outputs of an enzyme can be regulated.

Communication through chemical signals

Multicellular organisms use a variety of chemical and electrical signals in a communication network to coordinate individual cells to support the organism as a whole. Animal communication networks involve two systems: the nervous system and the endocrine system. Rather than being two separate systems, they are integrally related and follow the same principles of cell communication. They both rely on extracellular signalling molecules that are produced and released by cells to signal to other cells that will ultimately respond by changing their behaviour. Plant communication relies on chemical signalling molecules to transfer messages between cells.

Some chemical signals can be produced ahead of time and stored for later use, while others cannot be stored and must be produced on demand. Similarly, cells can receive

signals that will stop the production and/or release of signal molecules. Signal molecules continue to stimulate target cells until the signal is deactivated or removed. Some signals degrade quickly so their effect is very short, while others have a prolonged effect.

Many signal molecules are secreted from cells by exocytosis while others move across the plasma membrane through passive or facilitated transport. The chemical signal then diffuses through the extracellular fluid or is transported in the circulatory and lymphatic systems to arrive at target cells around the body.

Chemical signals in animals

Chemical signals can be classified according to the distance over which they travel to exert their effect (Figure 4.5). Some signal molecules remain tightly bound to the surface of the cell producing them, so they can only influence other cells through direct cell-to-cell contact. Autocrine and paracrine signalling molecules are secreted into the interstitial fluid to stimulate cells locally, and endocrine hormones travel in the blood and lymph to arrive at and stimulate distant cells.

Contact-dependent signalling

As the name implies, **contact-dependent** signalling requires direct cell-to-cell contact (see Figure 4.5a). The signal molecule is anchored in the membrane of a cell and interacts with a receptor molecule located in the membrane of another cell. This type of contact-dependent signalling provides the cross talk between cells that is integral for guiding embryonic development, maintaining the size and architecture of adult organs, and apoptosis.

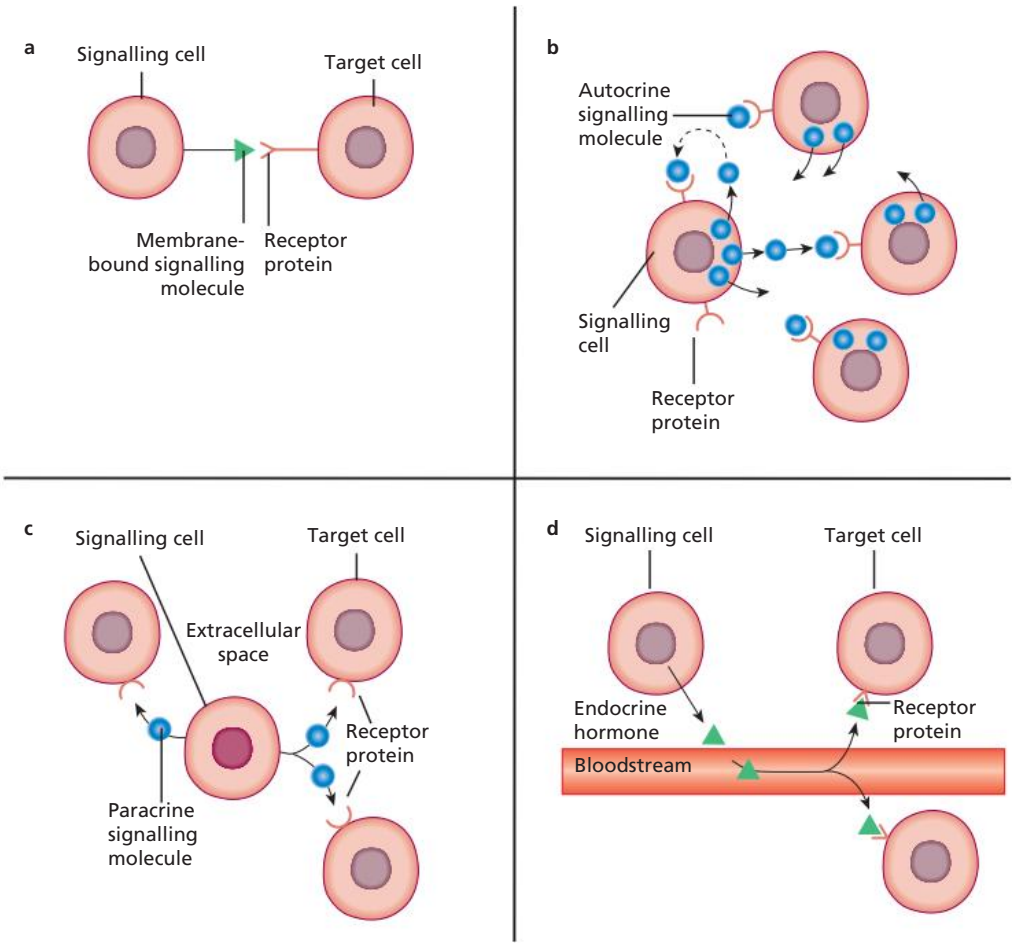


Figure 4.5 Chemical signals can be classified by the distance over which they travel to their target cells. (**a**) Contact-dependent signalling requires cells to be in direct contact. (**b**) Autocrine signals act on the cell that secreted them or cells of the same type. (**c**) Paracrine signals act locally on cells in the neighbourhood of the signalling cell. (**d**) Endocrine signals travel in the blood to reach target cells throughout the body.

In 1953 Abercrombie and Heaysman were interested in observing the social behaviour of cells. They grew chicken cells in culture dishes. They noticed that when cells collided they did not crawl over one another. Rather, they behaved like dodgem cars, changing direction after a collision. As the number of cells in the dish increased, collisions became more frequent, causing cell replication to slow and stop and resulting in the cells forming a monolayer. This demonstrated that cells have a spatial awareness signalling system that operates through **contact inhibition**. Contact inhibition is mediated by a group of membrane proteins called cadherins, **adhesion proteins** that influence the dynamics of the cell cytoskeleton required for a cell to move and replicate.

Contact inhibition of cell growth is essential for wound healing, to ensure cells move in and fill a wound. It is also necessary for maintaining the size and shape of organs. In **cancer** cells, signalling pathways for contact inhibition become disrupted and cell division continues even when cells come in contact with neighbouring cells. Cells no longer change direction or stop dividing on contact so they start to pile up and grow over each other, forming a tumour. Cells can also leave the tissue of origin and invade neighbouring tissues or travel to new organs around the body. This is called **metastasis**. These malignant cells acquire motility as they stop responding to contact inhibition signals exerted by neighbouring cells.

Cancer is discussed more on page 136.

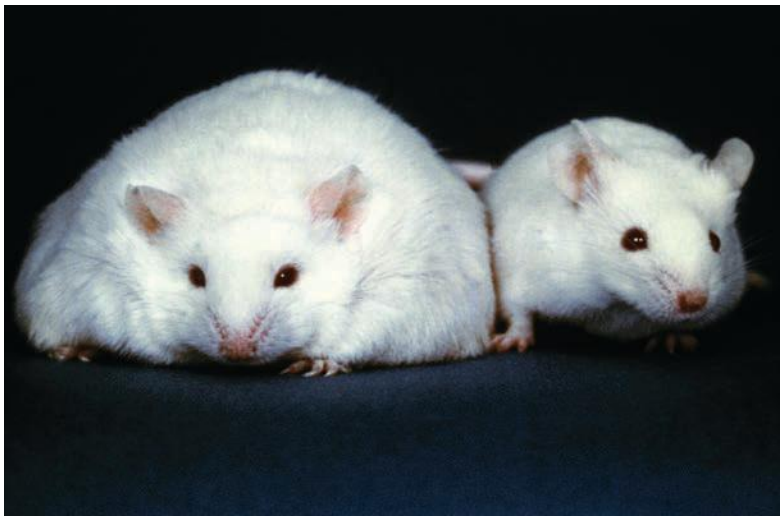
Local communication using autocrine and paracrine hormones

Autocrine signals are secreted by cells and act directly on the same cell, or on cells of the same type (see Figure 4.5b). Thus, they use a feedback mechanism to regulate their own activity. **Paracrine** signals are local regulators that are used for communication between neighbouring cells of different types (see Figure 4.5c). This signalling occurs when a cell secretes regulatory substances (signalling molecules) into the surrounding interstitial fluid, affecting only nearby target cells.

Prostaglandins are autocrine and paracrine molecules made from fatty acids by many different nucleated cell types. They are involved in regulating the contraction and relaxation of smooth muscles lining the blood vessels, gut, respiratory tract, bladder, uterus and eye. They induce a variety of signalling outcomes, including the relaxation of blood vessels to allow for greater flow of blood when needed, and the constriction of uterine muscles to induce labour. Prostaglandin action has also been implicated in less desirable outcomes, such as sensitising neurons to pain and causing the constriction of bronchioles observed in asthma.

Long-distance communication using endocrine signalling

In many cases, the cells that produce the signal are not in the same part of the body as those that respond. Thus, information or signals must be transmitted to the cells that need to respond. This can be achieved through the activities of two long-distance signalling systems: neurotransmission, an electrical signalling system discussed on page 118, and the **endocrine** system, a system of long-distance chemical signalling mediated by **hormones**. The endocrine system comprises a collection of glands that secrete chemical signals into the blood. The circulatory system and lymphatic system are used to transport hormones throughout the body, but the hormones exert their effect only on specific target cells (see Figure 4.5d and Table 4.2). Leptin is an endocrine signalling molecule produced and secreted by fat cells in adipose tissue. It travels in the blood to reach the brain, where it helps to regulate food intake and body weight. Figure 4.6 shows the result observed when a mouse cannot produce leptin.



Science Photo Library/Oak Ridge National Laboratory

Figure 4.6 ▶
The larger mouse on the left has inherited two copies of a mutant allele for an obesity gene, so it cannot produce the hormone leptin.

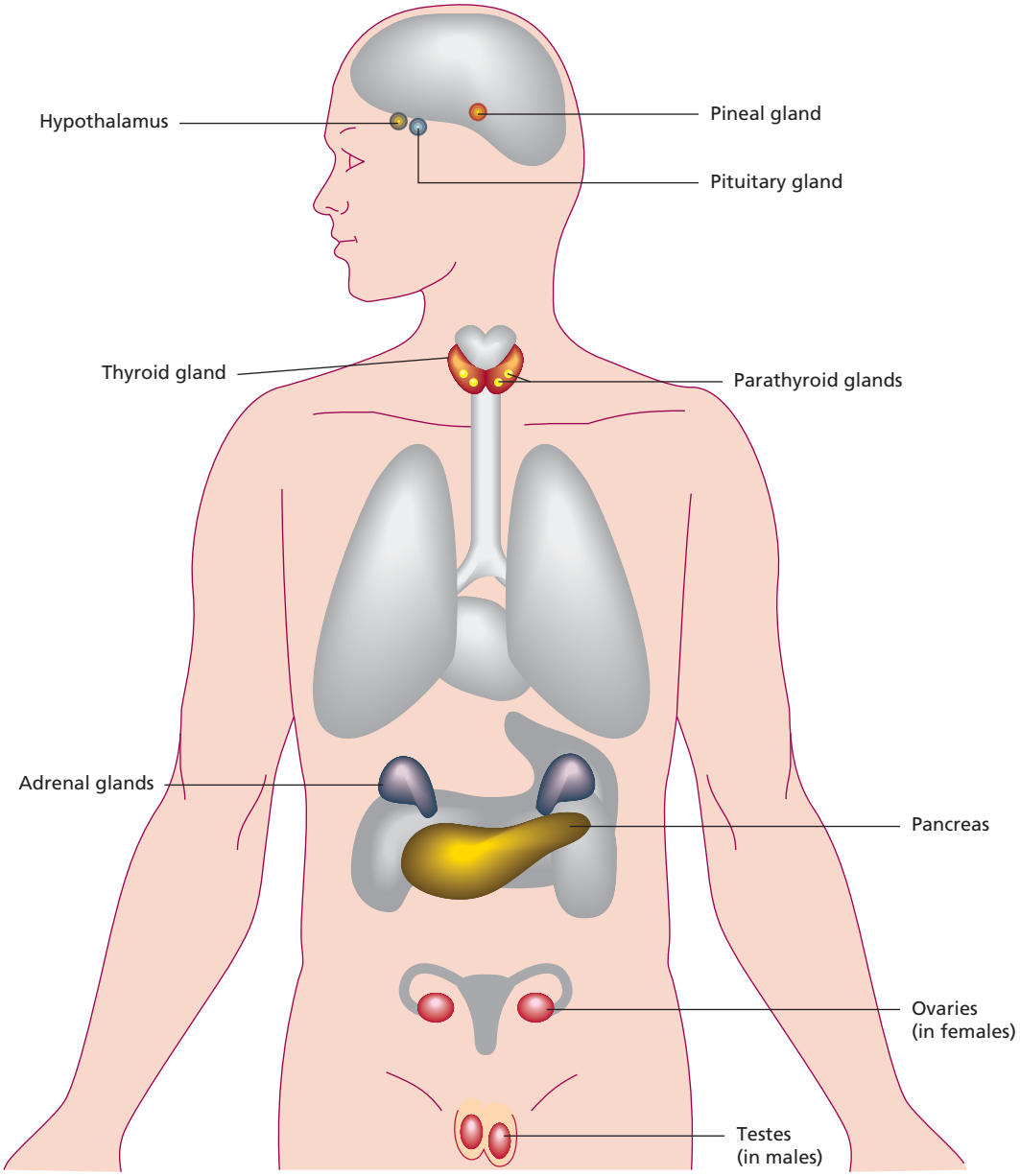
Although minute quantities of a hormone are produced, they have considerable impact. Some effects are temporary, such as when adrenaline signals the release of glucose and increased heartbeat in the 'fight or flight' response. Regulatory mechanisms, such as those in foetal development, can have a longer lasting effect.

Glands are organs that secrete substances including hormones into the circulation (Table 4.2). A target tissue may be a long way from the gland that secretes the hormone (Figure 4.7). For example, antidiuretic hormone (ADH) is secreted from the pituitary gland in the brain and exerts its effects on the kidneys. It stimulates the reabsorption of water, helping maintain an appropriate water balance in the body.

Coordination of activities associated with the endocrine system is often connected to the pituitary gland. The pituitary gland is sometimes referred to as the master gland because it produces many hormones that stimulate or inhibit the production of other hormones by other endocrine glands.

Table 4.2 Examples of human endocrine glands, a hormone they secrete, and its function

Endocrine gland	Hormone secreted	Target tissue/ organ	Function
Posterior pituitary	Antidiuretic hormone	Kidney	Stimulates reabsorption of water
Adrenal	Adrenaline	Kidneys, liver, blood vessels	Constricts blood vessels in kidney and liver; stimulates liver to release more glucose; prepares for 'fight or flight'
	Cortisol	Many tissues	Responsible for most of the body's physiological responses to stress
Thyroid	Thyroxine	Nearly all tissues	Increases metabolic rate, therefore increases oxygen consumption and heat release
Beta cells of pancreas	Insulin	Most body cells	Lowers blood sugar level, increases glycogen storage by liver, stimulates protein synthesis
Alpha cells of pancreas	Glucagon	Liver	Stimulates conversion of glycogen to glucose and its release



◀ **Figure 4.7**
Location of the main endocrine organs in the human body

Refer to Chapter 1 for a reminder of the secretory pathway by which a protein hormone is produced and secreted.

How a hormone is produced and what it is made from will govern its properties and behaviour. **Hydrophilic hormones** are water soluble and insoluble in lipids. **Hydrophobic hormones** (also called lipophilic hormones) are water insoluble and soluble in lipids. The solubility of a hormone determines how it is produced and secreted by cells, how it travels in fluids and how it exerts its effects at the target cell. As discussed in Chapter 1, each target cell is surrounded by a plasma membrane, a phospholipid bilayer that serves as a boundary and restricts the entry of large or hydrophilic hormones. The location of receptors on or within the cell varies depending upon the size of its binding hormone and whether the hormone is hydrophilic, or whether it is hydrophobic and can readily pass through the plasma membrane.

Table 4.3 shows how scientists categorise hormones into three groups based on their chemical structure: **steroid hormones**, **amine hormones** and **peptide hormones**.

The problem with this chemical classification system of hormones is how to fit newly discovered chemical signals into existing categories. There is now mounting evidence that a fourth group of signalling molecules should be added to this list: the dissolved gases including nitric oxide, carbon monoxide and hydrogen sulfide. Scientists are still debating how to categorise this group of signalling molecules.

Table 4.3 Hormones categorised into three groups based on their chemical properties and behaviour

Steroid hormones	Amine hormones	Peptide hormones
Synthesised from cholesterol in the gonads and adrenal gland.	Synthesised from amino acids through the action of enzymes. Iodine is required for synthesis of thyroxines so it must be supplied by the diet.	Chains of amino acids synthesised by ribosomes and packaged into secretory vesicles by the Golgi apparatus.
Hydrophobic, so they are lipid soluble and can cross plasma membranes. They bind to intracellular receptors, travel in fluids attached to carrier proteins and cannot be stored, so are secreted as they are produced.	Most are hydrophilic and bind to extracellular receptors. The exception is the thyroxines, which are hydrophobic and bind to intracellular receptors.	Hydrophilic, so they cannot cross plasma membranes. They bind to extracellular receptors.
The adrenal gland produces cortisol and aldosterone. The testes and ovaries produce testosterone and oestrogen.	Hydrophilic examples include adrenaline (known as epinephrine in the United States) produced by the adrenal gland and by some nerve cells, dopamine produced by some nerve cells, and melatonin produced by the pineal gland. They also include a group of hydrophobic hormones called thyroxines that are produced by the thyroid gland.	Produced and secreted by exocytosis from cells in a range of tissues and glands including leptin by fat tissue, insulin by the pancreas, growth hormone by the pituitary, and antidiuretic hormone (ADH) produced in the hypothalamus and secreted from the pituitary.

RECALL

- Signalling between cells and tissues may occur in a contact-dependent manner, or through autocrine, paracrine or endocrine signalling.
- Autocrine signalling molecules act on cells of the same type as to that which produces the signal; paracrine signalling molecules act on nearby cells of a different type to those that produced the signal.
- Endocrine signalling involves hormones, which are classified according to their properties – hydrophobic hormones include steroid hormones and some amine hormones (the thyroid hormones, thyroxines); hydrophilic hormones include peptide hormones and most amine hormones.

RECAP 4.2

1. Name three instances where contact-dependent signalling is involved.
2. Compare and contrast the properties of hydrophilic and hydrophobic signalling molecules.
3. Name an example of a steroid hormone, an amine hormone and a peptide hormone, and state where these are produced.

Cytokines

Cytokines are a large family of chemical messengers that coordinate the movement and behaviour of cells and tissues of the immune system. They are produced by cells of the immune system or by normal body cells that are damaged by injury or infection. They can stimulate the movement of immune cells to the affected area, prepare neighbouring cells for infection, promote the maturation or specialised functions of immune cells and switch off an immune response when the infection is cleared.

Cytokines are generally small, hydrophilic proteins that bind to a specific receptor on the plasma membrane. Like hormones, only the cells that express a specific cytokine receptor will be responsive to that particular cytokine. Cytokines can signal in a contact-dependent manner, or have autocrine or paracrine effects. They can also diffuse throughout the body to have systemic, or whole-body, effects. There are many mechanisms to degrade cytokines and counteract their functions so that immune responses last only as long as they are needed to clear the danger.

Pheromones

Pheromones are a type of chemical signal that are secreted or excreted from the body. They trigger a response in other members of the same species, usually by binding to specific receptors in the olfactory system. Pheromones can signal that the organism that released the pheromone is a potential mate, or ready to feed her young in the case of a mother with nursing offspring. A pheromone may signal that the organism that released it is alarmed, or occupies a territory, or has created a trail that the recipient of the signal should follow (such as in ants). In moths, pheromones can guide males towards females, even if the female is hundreds of metres away and if a wind is blowing to disperse the female's pheromones. A wide 'cone of detection' is used by the male to hone in on the female, based on only tiny amounts of pheromone detected. Many aquatic organisms use pheromones to coordinate the synchronised release of eggs and sperm during spawning.

In humans, pheromones are still not well understood but there is growing evidence that they are used to transmit information between individuals. Humans can detect whether a person is anxious by smelling their perspiration. Newborns are attracted to the scent of milk at the mother's breast. In one study, women were attracted to the scent of men whose HLA genes, which determine how well an individual can fight different types of infections, were most different from their own. This can result in a match that produces children with an ability to fight a wider range of infections.

RECALL

- Cytokines are chemical messengers of the immune system, acting in a contact-dependent, autocrine, paracrine or systemic manner.
- Pheromones are chemical messengers that relay a signal between individuals of a species.

RECAP 4.3

1. Compare and contrast cytokines and pheromones.
2. Construct a table indicating how chemical signals can be classified by the distance they travel to reach target cells. Include some examples for each category in your table.

Plant hormones

Hormones are just as important in plants as they are in animals. In plants, hormones are often produced in one part of the plant and act upon another part to change the way the plant grows or responds to its environment. Often multiple hormones are acting at the same time and the outcome is determined by the relative concentrations of the different hormones. There are five different groups of plant hormones: auxins, cytokinins, gibberellins, ethylene and abscisic acid.

Auxins

Auxins are plant hormones that have three main effects: **apical dominance**, root growth and cell elongation. Apical dominance occurs when the tip of the plant suppresses sideways, or lateral, growth from buds lower down on the plant. Auxins are usually produced in the highest tip of a plant in a small cluster of cells called the **apical meristem**. They are then transported down through the stem, inhibiting lateral bud growth to cause apical dominance. Cutting off the shoot tip removes the apical meristem, allowing lateral buds to grow until a new apical meristem is formed. Painting the cut tip with synthetic auxins, which are widely used in gardening, can restore apical dominance and is useful in some situations such as ornamental tree shaping.

Auxins also promote the initiation of root growth as well as elongation and branching of existing roots. Synthetic auxins are often used to stimulate root growth in cuttings. Auxins also cause plant cell walls to become flexible and cell elongation to occur. **Tropisms** are patterns of growth of plants in a particular direction, in response to a particular stimulus. For example, roots show tropism towards gravity and shoots towards light. In **phototropism**, plant shoots grow towards the sun or another source of light. Sunlight triggers the breakdown of auxins in cells, so that the concentration of auxin along a length of shoot is greater in the side facing away from the sun. This causes cell elongation on that side, causing the shoot to grow towards the sun (Figure 4.8).

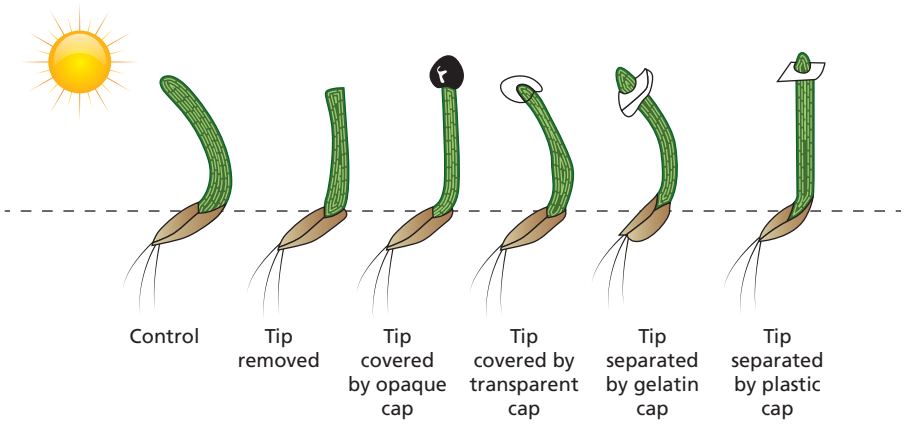


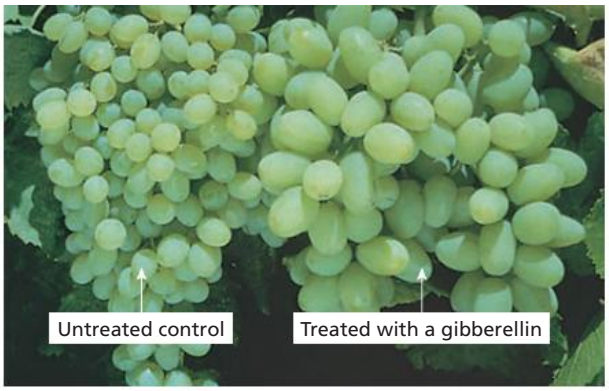
Figure 4.8 ▶
An experiment showing the effects of auxin on phototropism

Cytokinins

Cytokinins are plant hormones that are synthesised from the nucleotide adenine. They promote cell division, chloroplast formation and growth of lateral shoots and leaves. They are produced in developing shoots and roots as well as fruits and seeds of plants. Cytokinins inhibit root growth and appear to have opposing functions to auxins. It is the balance of the two that determines the outcome for the plant, although these two hormones can also act together to cause different responses in plants.

Gibberellins

In the 1920s, Japanese scientists discovered that plants infected with the *Gibberella* fungus grew much taller than non-infected plants because of a substance called **gibberellin** that was produced by the fungus. Plants make this substance naturally in small amounts and it promotes vertical growth of the plant. Gibberellins are used widely for commercial purposes. For example, seedless grapes are naturally very small, but spraying them with gibberellins increases fruit size and the spacing of the fruit along the stem (Figure 4.9).



▲ **Figure 4.9** Gibberellin induces growth in Thompson's seedless grapes. The bunch on the left is an untreated control. The bunch on the right was sprayed with a gibberellin during fruit development.

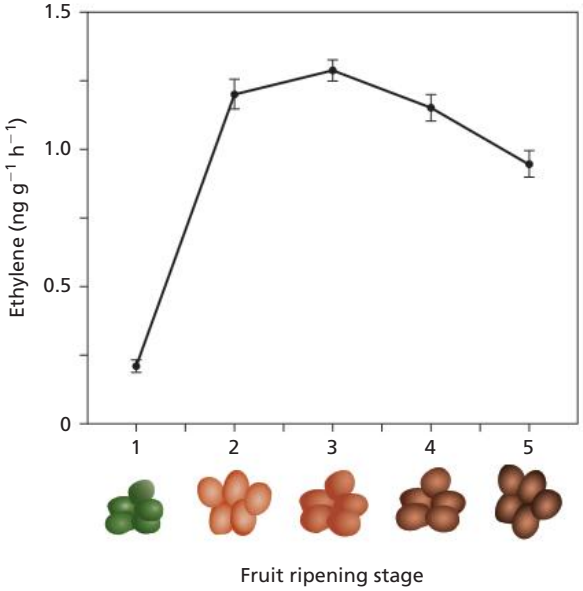
Ethylene

Unlike the other groups of plant hormones, **ethylene** is a gas at room temperature and easily diffuses through the air. Some fruits produce it as they ripen and it stimulates many of the changes that are associated with ripening, including softening and colour changes (Figure 4.10). The saying 'One bad apple spoils the barrel' refers to the effects of ethylene produced by over-ripened or rotting fruit.

Ethylene is widely used for commercial purposes, including to ripen tomatoes and bananas and to stimulate cherries and walnuts to detach more easily from the tree when they are harvested by shaking branches. Oranges and lemons can remain naturally green when they are ripe, but are exposed to ethylene to trigger breakdown of the green pigment and make their colour more appealing to customers.

Abscisic acid

Abscisic acid can inhibit some of the functions of other hormones and can promote a state of dormancy in plants. It will maintain dormancy in seeds and buds until the conditions are right for growth to begin. During drought, abscisic acid concentrations rise in leaves and cause potassium ions to be pumped out of guard cells. Water follows by osmosis and the stomata close, preventing further water loss.



▲ **Figure 4.10** Ethylene production in *Coffea arabica* coffee beans during ripening.

RECALL

- Hormones are essential chemical messengers in plants. Multiple plant hormones usually interact to control plant growth and behaviour.
- Auxins are responsible for apical dominance, promoting shoot growth and inhibiting lateral bud growth. Cytokinins stimulate growth of lateral buds and roots.
- Other plant hormones include gibberellins, which promote vertical growth and have many commercial applications; ethylene, which is involved in fruit ripening; and abscisic acid, which promotes dormancy and water retention during drought.

RECAP 4.4

1 Define apical dominance and give an example of how it may be used in gardening practices.
2 List the plant hormones and their major functions.
3 Describe the mechanism of phototropism.

Communication through electrical signals

While plants rely on chemical signals to transmit messages and coordinate functions, higher order animals also have a second major signalling system. The nervous system is a circuitry of specialised cells that transmit electrical impulses around the body. Peripheral nerves pick up messages and transmit them to the central nervous system (CNS) for processing. They also deliver messages to muscles lining glands, skeletal muscles, hairs and ducts in a response coordinated by the CNS. The brain alone contains one trillion **neurons** that are connected to form circuits. To function within this circuit a neuron must receive, process and relay signals to other neurons. Neurons are connected in an intricate network, with particular pathways coordinating particular responses in the body. The pathway followed depends upon whether a neuron is an excitatory neuron or whether it is an inhibitory neuron.

Sensory neurons convey a signal from the periphery to the CNS. Interneurons relay the signal within the nervous system, and motor neurons transfer the signal to peripheral effector tissues such as muscle fibres.

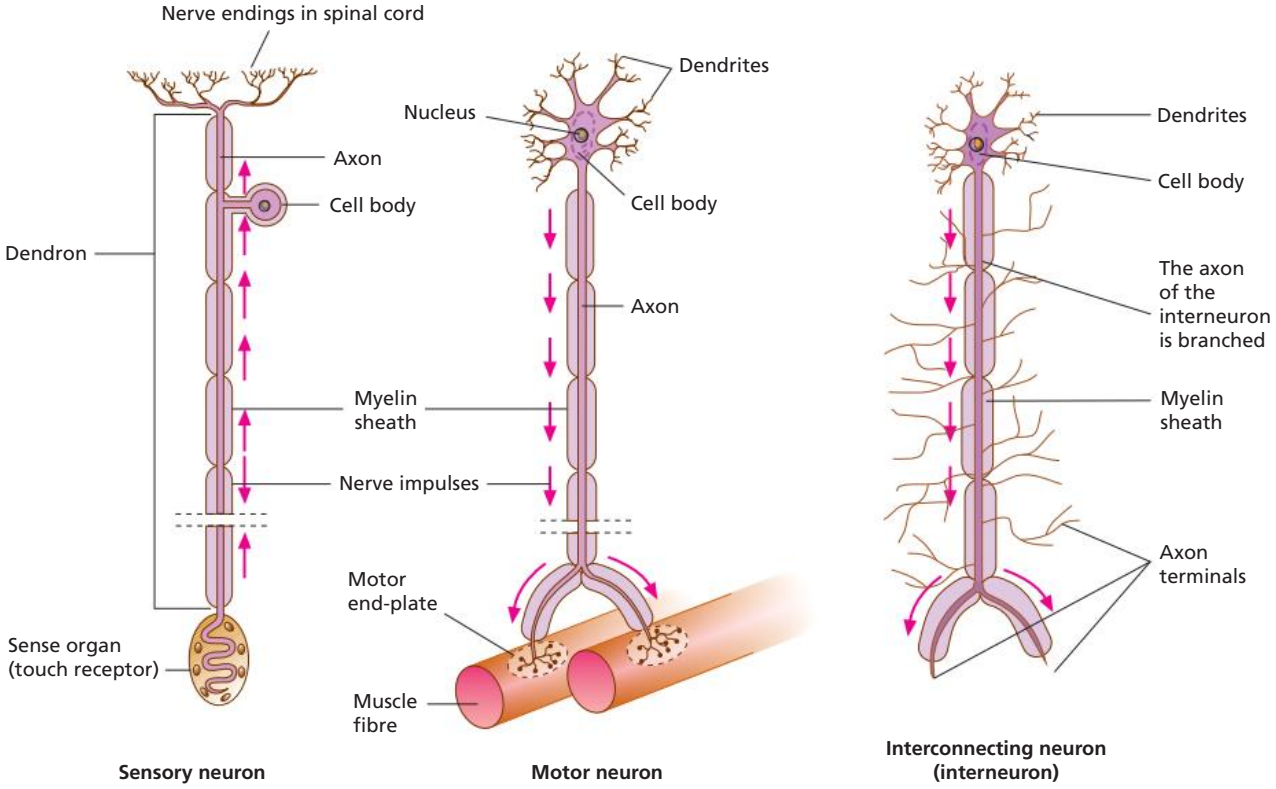


Figure 4.11 ▲
The generalised structure of sensory, motor and interconnecting neurons. A variety of shapes occur within each class of neuron.

The signal: initiating the electrical message

Many stimuli can initiate an electrical message. Stimulation can come from an external receptor organ, certain chemicals or even physical stimulation, such as a pinch. In all cases, the message is initiated by opening up ion channels in the plasma membrane, allowing the movement of sodium and potassium ions across the membrane. This causes an electrical impulse that moves along an axon.

The plasma membrane of an axon is polarised; that is, there is a difference in charge between the inside and the outside of the cell. An axon that is at rest (not transmitting an impulse) is negatively charged inside relative to the outside. This is called a **resting potential**. To attain this polarised state, **sodium–potassium pumps** in the plasma

membrane actively pump sodium ions out of the cell and potassium ions into the cell. The pump works in such a way that three sodium ions are expelled for every two potassium ions pumped in. Consequently, the number of positive charges outside the cell becomes greater than the number inside the cell. This difference in number of charges results in the inside being negative relative to the outside.

What happens when an electrical impulse arrives? The membrane suddenly becomes permeable to sodium ions. These, being about 10 times more concentrated on the outside than the inside, diffuse in rapidly. This depolarises the membrane, reversing the resting potential. The inside of the axon is now positive relative to the outside. This charge reversal is called an **action potential** and takes place in a millisecond. In summary, the action potential is the mode of transmission of electrical signals along the axon of a neuron.

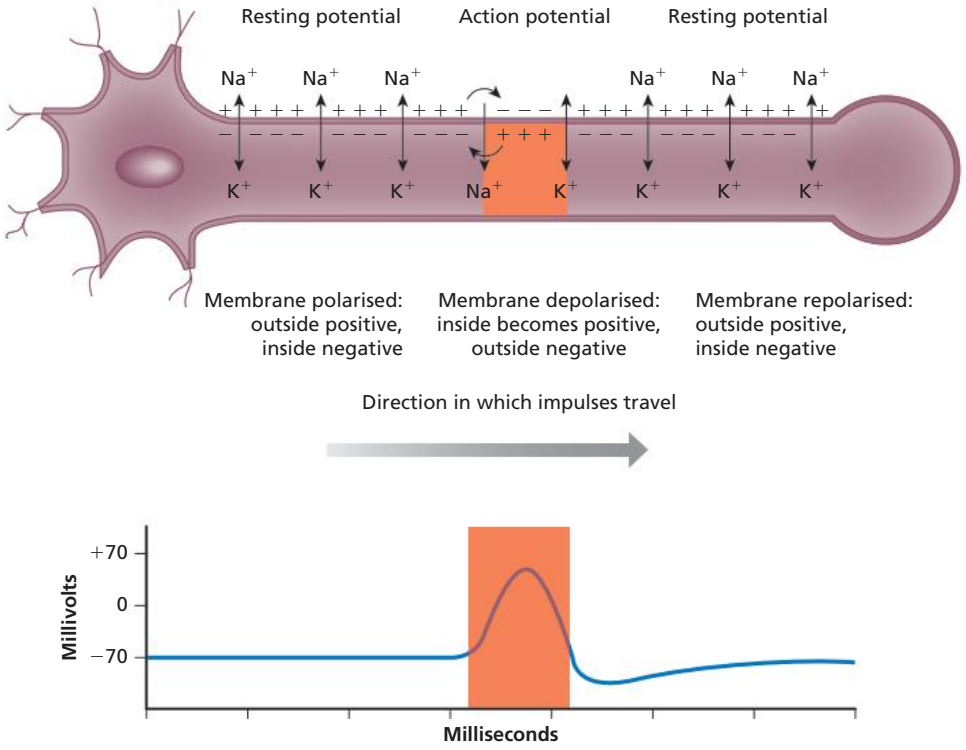


Figure 4.12
Transmission of impulses along a neuron

On depolarisation, specific channel proteins in the next part of the plasma membrane open up, so sodium ions diffuse into the cell. Once depolarisation is complete, the channels close and the influx of sodium ions ceases. Thus, depolarisation moves progressively down the axon. As the sodium ions enter, the potassium ions begin to leave: this is the start of the recovery process. The sodium–potassium pump mechanism now restores polarisation by pumping out sodium ions and pumping in potassium ions. As a result, the distribution of ions that normally exists when the axon is at rest is reinstated; that is, the axon is returned to its resting potential.

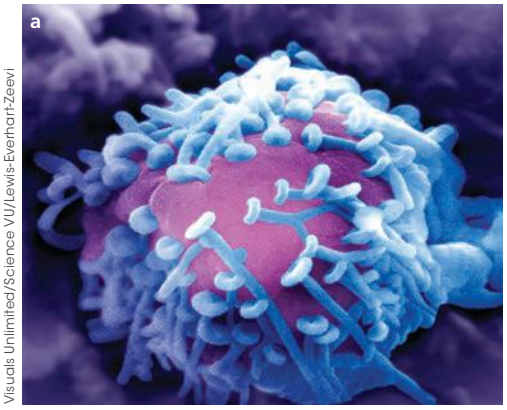
When axons are protected by a **myelin sheath**, the depolarisation wave jumps from one gap of exposed axon membrane to the next. These gaps between Schwann cells (in the peripheral nervous system or PNS) or oligodendrocytes (in the CNS), called nodes of Ranvier, are the only part of the membrane that become depolarised. Because of this jumping between nodes, the impulse along a myelinated axon travels much faster.

Neurotransmitters: bridging the gaps

An impulse travels to the end of the axon, but neurons do not actually touch; there is a gap between them known as the **synaptic cleft**. The electrical message is transmitted to the next neuron through the use of chemical messengers called **neurotransmitters**.

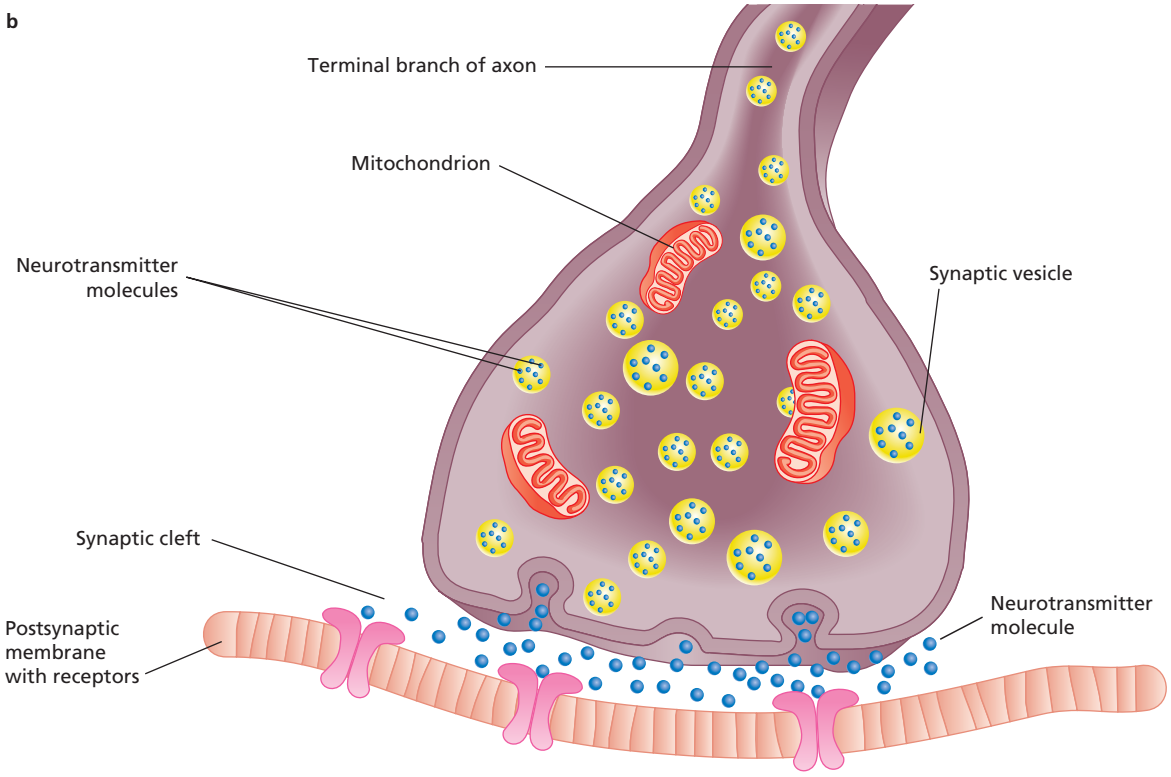
The end of each axon is rounded to form a synaptic knob (Figure 4.13). The end of the synaptic knob is called the presynaptic membrane, the gap between the synaptic knob and the adjoining neuron is called the synaptic cleft, and the membrane on the far side is called the postsynaptic membrane. The cleft is approximately 20 nm across.

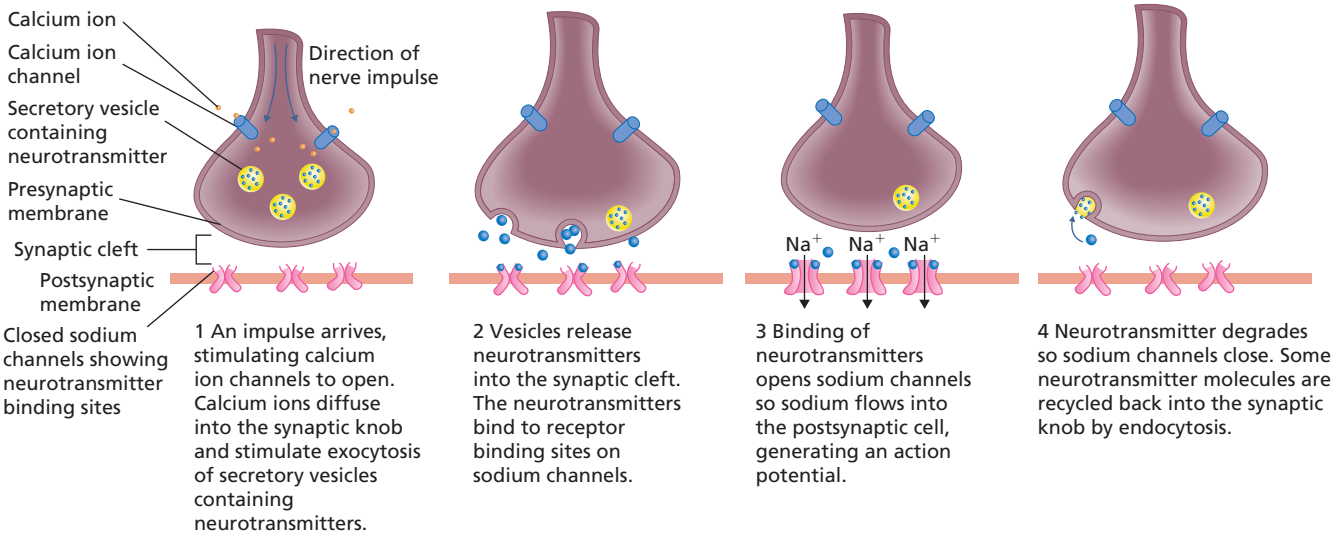
The presynaptic membrane contains numerous mitochondria and secretory vesicles that contain neurotransmitters. When an impulse arrives at the synaptic knob, it causes calcium ion channels in the plasma membrane to open. Calcium ions diffuse into the synaptic knob from surrounding tissue fluid and stimulate exocytosis of secretory vesicles containing neurotransmitters. These vesicles merge with the presynaptic membrane and release their contents into the synaptic cleft. The neurotransmitters then diffuse across the cleft to bind with specific receptors in neighbouring neurons. This causes ion channels to open up, allowing sodium ions to diffuse from the cleft into the postsynaptic neuron, causing partial depolarisation. If sufficient channels are opened, an action potential will be initiated. Once the neurotransmitter has activated the protein channels, it is important that it does not continue to stimulate the postsynaptic neuron. Thus, excess neurotransmitter in the synaptic cleft is deactivated by enzymes and recycled into the synaptic knob of the presynaptic neuron. This is summarised in Figure 4.14.



Visuals Unlimited/Science VU/Lewis-Everhart-Zeevi

Figure 4.13 ▶
Neurons connect with each other by synapses. (**a**) Scanning electron micrograph of synaptic knobs; (**b**) The structure of a synapse





▲ **Figure 4.14** Signal transduction across a synapse

Neurotransmitters can control and coordinate responses

When neurons are excited, they usually fire through several **synapses** at once. A single synapse will not produce enough neurotransmitter for an action potential to be generated in the postsynaptic neuron.

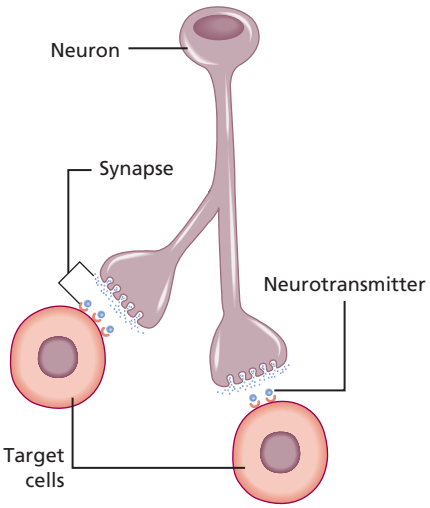
Synapses are also sensitive to the amount of stimulation they receive. Some have limited amounts of neurotransmitter available. Once it has been secreted, supplies have to be restored before further impulses can be transmitted. During this period they will not work. Different synapses vary in how quickly they become fatigued.

Not all synapses are involved with transmitting an electrical impulse. Some are there to hinder the message, using neurotransmitters that cause the inside of the postsynaptic membrane to become more negative than usual and therefore make it harder for it to become depolarised. This means that the nervous system is able to finely control any adjustments that need to be made to respond to a signal. For example, to maintain homeostasis a gland may be stimulated to secrete more of a substance, and secretion of the substance by the gland is inhibited when balance is restored.

Types of neurotransmitters

Scientists have isolated hundreds of neurotransmitters, many of them in the brain, and the list is still growing. A common neurotransmitter is **acetylcholine**, which is found in synapses and nerve–muscle junctions in the peripheral nervous system. Acetylcholine is normally deactivated by the enzyme cholinesterase. Many insecticides work by destroying the enzyme cholinesterase. In this way, the insect's muscle cells receive continuous signals, resulting in muscle spasms.

Paralysis ticks, tiger snakes and other venomous animals produce chemicals that block the production or action of neurotransmitters at synapses. Some pain-killing tablets take effect because they block the transmission of impulses from pain receptors. Naturally occurring endorphins in the CNS relieve pain when they are released at times of stress.



▲ **Figure 4.15** Neurotransmitters act like paracrine hormones, affecting cells in their close proximity.

Neurotransmitter or hormone?

How different is nervous signalling from endocrine signalling? Both systems rely on chemical signalling molecules: hormones and neurotransmitters. Like paracrine signals, neurotransmitters only affect cells in their local neighbourhood. Some neurotransmitters are very similar to hormones. For example, the neurotransmitter noradrenaline is chemically almost identical to the endocrine hormone adrenaline. Noradrenaline is associated with the sympathetic nervous system, and both adrenaline and noradrenaline are responsible for preparing our body to respond to stress. So, why is noradrenaline considered to be a neurotransmitter and not a hormone? The criteria defining a neurotransmitter are that it is released in response to presynaptic depolarisation and that its specific receptors are located on the postsynaptic membrane. Some chemical signals can act as both a neurotransmitter and a hormone. ADH and oxytocin, two peptide hormones secreted into the blood by the pituitary gland, can also be found functioning as neurotransmitters at some synapses.

The endocrine and nervous systems are inextricably linked through the hypothalamus and pituitary gland. The hypothalamus, which is made up of nervous tissue, is located in the brain and connected to the pituitary gland via both nerves and blood. An example of the interplay between the endocrine and the nervous system is seen in the regulation of the concentration of water in the blood plasma. Osmoreceptors, sensory neurons that detect the water content of the blood, are located near the hypothalamus. If the water content of the blood is low, nerves of the hypothalamus are stimulated to produce ADH and secrete this from their axons into the pituitary. The pituitary then secretes ADH into the bloodstream, where it travels to affect cells in the kidneys.

RECALL

- Generation of an action potential in a neuron results in an electrical impulse being relayed along its axon.
- The impulse is transmitted to other neurons or glands by neurotransmitters that are released into the synaptic cleft and bind with receptors in the postsynaptic membrane.
- A neurotransmitter is a chemical signal that is released in response to depolarisation at a presynaptic junction and binds specifically with receptors in the postsynaptic junction.

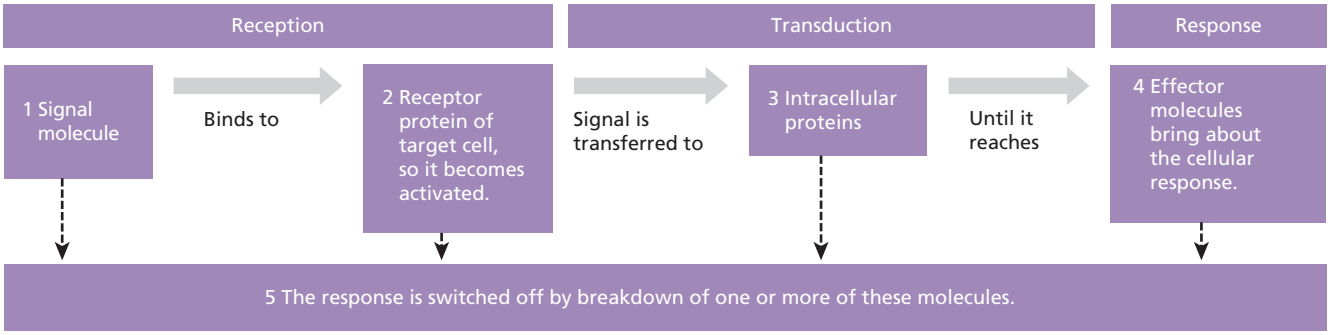
RECAP 4.5

1. Define what makes a molecule a neurotransmitter and distinguish it from a hormone.
2. Describe the role of the sodium–potassium pump in conveying action potentials along an axon.
3. Draw and label a synaptic junction and the features that surround it.
4. Name three ways that signalling across a synapse can be regulated.

Modelling the cellular reaction: the stimulus–response model

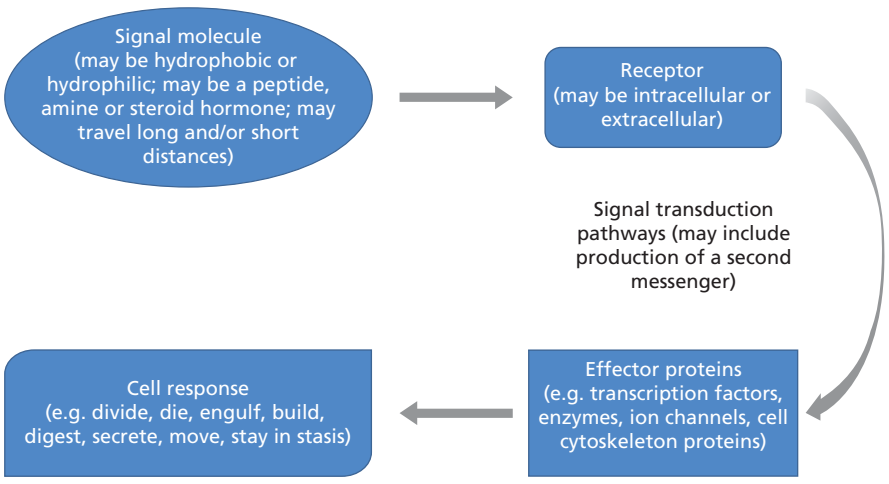
The activation of extracellular receptors, whether by contact-dependent signals, hormones, cytokines, pheromones or neurotransmitters, leads to a response by a cell, but how is the signal relayed within the cell? Binding of a ligand to its receptor triggers a cascade of modifications of cellular molecules and proteins, activating or inactivating them in a process called **signal transduction**. This series of events results in the activation of effector molecules that cause a specific cellular response (Figure 4.16). The cellular response is influenced by the local environment of the cell and the state of the cell, and is strictly regulated to ensure that its magnitude and duration are no greater than is necessary to elicit the desired outcome.

▼ Figure 4.16
The principles of signal transduction



A stimulus–response model, which has three components, can be used to describe signalling pathways in cells. First, a stimulus is *received* by a specific receptor that binds with lock-and-key specificity to the signalling molecule. Next, the signal is *transmitted* through a series of molecular events that form a signal transduction pathway. Finally, the signal transduction pathway causes the activation of effector proteins that bring about a particular *response* to the signal (Figure 4.17).

◀ Figure 4.17
Stimulus–response models can be used to represent signalling at the cellular level.



Receiving the signal

A chemical signal is received when it binds to its specific receptor protein. Receptors can be intracellular or extracellular and, like enzymes, bind with a high level of specificity to their ligands. It is the expression of particular receptors that determines the signalling molecules to which a cell is responsive.

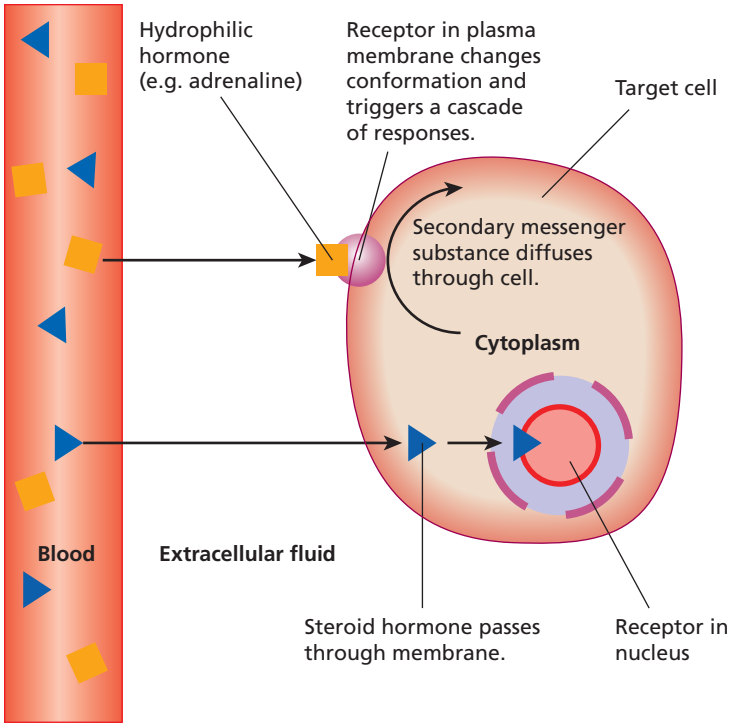


Figure 4.18 ▲
Hydrophilic signalling molecules such as adrenaline are detected by extracellular receptors. Hydrophobic signalling molecules, including steroid hormones, can bind to intracellular receptors.

Intracellular receptors for hydrophobic signalling molecules

Hydrophobic hormones such as steroid and thyroid hormones, and some hydrophobic pheromones, pass through plasma membranes and bind to and activate intracellular receptors found in the cytoplasm and nucleus (Figure 4.18). These intracellular receptors are often transcription factors, which become active only when the hormone is bound. They can then bind to DNA and regulate gene expression.

Have you ever considered how carnivores get the glucose required to fuel respiration pathways when they do not eat starchy foods? Or how a fasting person can continue to respire when their glucose reserves have been depleted? During starvation, the steroid hormone cortisol is produced by the adrenal gland. This hormone exerts a range of actions to increase and maintain blood glucose levels. It stimulates glucose synthesis from amino acids and glycerol in the liver, stimulates release of fatty acids from adipose tissue, and inhibits glucose uptake by muscles and adipose tissue. How does cortisol exert so many different effects on different cells of the body?

In liver cells, cortisol binds to a receptor that is also a transcription factor, changing the conformation of the receptor. This causes it to become an active transcription factor with the ability to regulate the expression of genes. In this example the genes code for enzymes controlling biochemical pathways for building glucose from amino acids and glycerol. The transcription factor enters the nucleus and binds to its specific DNA region to switch on gene expression. Enzymes are then produced and the pathway is up-regulated (Figure 4.19). While cortisol switches on one particular set of genes in the liver, it binds to different transcription factors in adipose tissue. Therefore a different set of genes is switched on, a different set of enzymes is made, and a different biochemical pathway is up-regulated. As a result, the response of adipose cells is different from the response of liver cells.

The ability of hydrophobic signalling molecules to traverse the plasma membrane and bind directly to a receptor that is a transcription factor means that signal transduction pathways for these molecules are relatively simple. Little or no involvement from other signalling molecules is needed to help transmit the signal from outside the cell to inside the nucleus, where gene transcription can be regulated to bring about a cellular response.

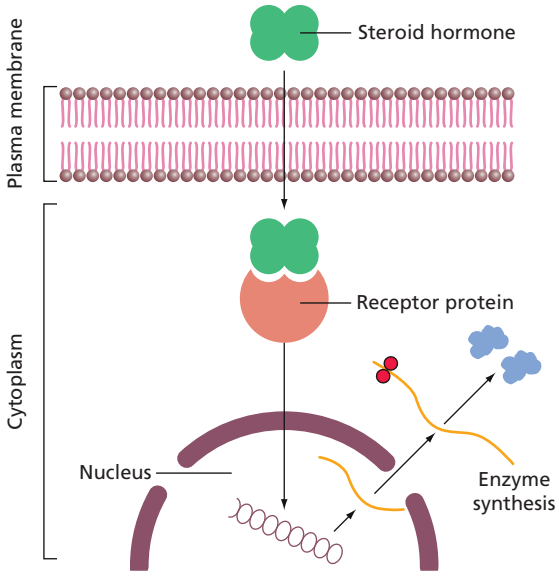


Figure 4.19 ▲
The steroid hormone cortisol binds to a transcription factor in the liver cell, activating gene expression. The result is the production of a set of proteins needed to make glucose from amino acids and glycerol.

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Hydrophilic signalling molecules bind to extracellular receptors

Peptide hormones, hydrophilic amines, cytokines and hydrophilic pheromones are signals that cannot pass across the plasma membrane because of their charge and large size. Instead, they bind to **extracellular receptors** that are embedded in the plasma membrane. These receptors have extracellular ligand-binding domains as well as a structure that anchors them to the plasma membrane. This is either a transmembrane domain or a lipid attached as a post-translational modification. Extracellular receptors must have some way of transmitting the signal on the cytoplasmic side of the membrane, and this mechanism determines the way that receptors are classified.

Extracellular receptors are generally grouped into three categories based on how they transfer a message into a cell, although some receptors cannot be categorised in this way.

1. *Ion channel receptors:* When a signal binds to an ion channel protein, it changes conformation and the channel opens so that ions can be transported across the plasma membrane. This response is fast because the cell responds quickly to changes in ion concentrations.
2. *G protein-coupled receptors:* These receptors have a protein called a G protein attached to them on the inside of the plasma membrane. When a signal binds, the receptor changes conformation and the G protein is released and activates other proteins in the signal transduction pathway.
3. *Tyrosine kinase receptors:* These receptors are inactivated enzymes embedded in the plasma membrane. Binding of a signal activates these enzymes by changing their conformation, usually by removing a phosphate group from an adenosine triphosphate (ATP) molecule and adding it to a protein (**phosphorylation**), producing adenosine diphosphate (ADP) as a by-product.

Protein phosphorylation is an important post-translational modification of signalling proteins that can rapidly change the conformation and activity of proteins. It is a common way of switching proteins on or off in signal transduction pathways.

Transduction of the signal

When a signal molecule binds to its receptor protein the receptor becomes activated, usually because of a change in its conformation. The activated receptor alters the activity of intracellular proteins, setting off an intracellular signalling cascade so that the message is transmitted through the cell by signal transduction. The message is transmitted to **effector** proteins in the cell, which are stimulated to elicit a response.

How a cell responds to a signal depends on the particular proteins it expresses, including receptors, enzymes and other types of proteins involved in signal transduction. The net effect of all of these signals is to regulate the function and behaviour of the cell.

RECALL

- The stimulus–response model of cell signalling has three main steps: receiving the signal, transduction of the signal, and a cellular response to the signal.
- Hydrophobic signalling molecules have intracellular receptors that are often transcription factors, activated by the presence or absence of the ligand.
- Binding of a hydrophilic signalling molecule to a receptor causes a change in conformation, triggering signal transduction to elicit a cell response.

RECAP 4.6

1 Draw and annotate a diagram of two different receptor proteins to indicate how the shape and properties of the binding site are complementary to their specific signal molecules.
2 Recount why signalling molecules can exert an effect only on target cells.
3 Recall the principles of signal transduction.
4 Distinguish between a receptor and an effector.

Second-messenger systems relay and amplify the signal

The binding of hydrophilic hormones to extracellular receptors stimulates rapid production of a **second messenger** within the cytoplasm of the cell. With a domino effect, this second messenger then stimulates the activity of specific proteins within the cell that bring about its response.

Why is such a system advantageous to a cell? Imagine your neighbour's house is on fire. What do you do? Ring 000! The operator will then contact fire fighters, police and paramedics, who will come to the scene. The second messenger in this scenario (the 000 operator) serves to spread the message to all the parties necessary to put out the fire and prevent further harm. Compare this to the responding cell. Only a small amount of signalling molecule (maybe just one molecule) is necessary to notify the second messenger, which then leads to activation of a range of chemicals in the cell.

Second messengers are usually small molecules. The first one to be discovered was cyclic adenosine monophosphate (**cyclic AMP** or **cAMP**) produced in response to adrenaline binding to a G protein-coupled receptor on liver cells. On binding, the conformation of the receptor changes, leading to the activation of adenylate cyclase. This enzyme begins generating large amounts of cyclic AMP from ATP. The cAMP second messengers rapidly diffuse away to broadcast the signal to proteins in other parts of the cell so glucose is released from glycogen stores. cAMP is now known to be a second messenger in many signalling pathways, generating responses that can include switching genes on and off, moving vesicles around the cell or activating enzymes.

Hormones are effective at very low concentrations, as binding of hormone to receptor initiates production of second messengers that amplify the response in a cell. Just one hormone molecule can result in the production of many second messengers. This amplifies the response, which explains why hormones are active in such small quantities. The response triggered by second messengers depends upon the proteins that they interact with. In the case of cAMP, it activates enzymes called protein kinases that add phosphate groups to proteins. The cell response depends upon the proteins being phosphorylated. In this way, the same second-messenger system can produce a different response in one cell compared to another.

There are three major second-messenger systems now recognised in cells: phospholipid-derived molecules (e.g. inositol triphosphate), ions (e.g. Ca^{2+}) and cyclic nucleotides (e.g. cAMP).

Effector proteins direct the cellular response

Activation of target proteins will lead to the cellular response, which can occur by one of three fundamental mechanisms: changing the activity or amount of proteins within the cell, or changing the expression of genes within the cell so that certain proteins are produced or not produced (Figure 4.20). Effector proteins that elicit a response include gene regulators, ion channels, enzymes, and parts of the cytoskeleton.

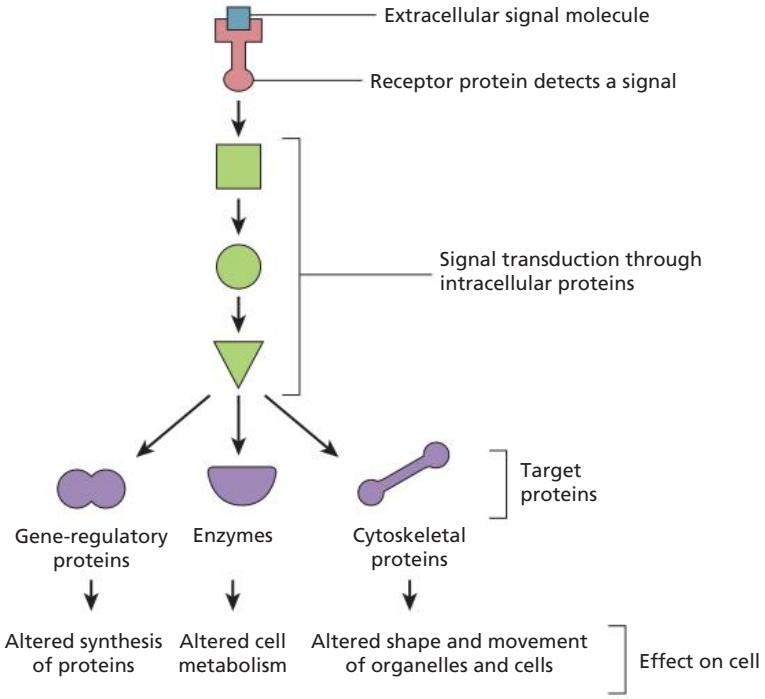


Figure 4.20
Signal transduction pathway through a cell

Signals also act collectively to influence cell activity. Different combinations of signals activate different responses for maintenance and survival of the cell, cell division and differentiation. Most of a cell's functions occur in response to specific signals, and withdrawal of some signalling molecules can induce apoptosis (Figure 4.21).

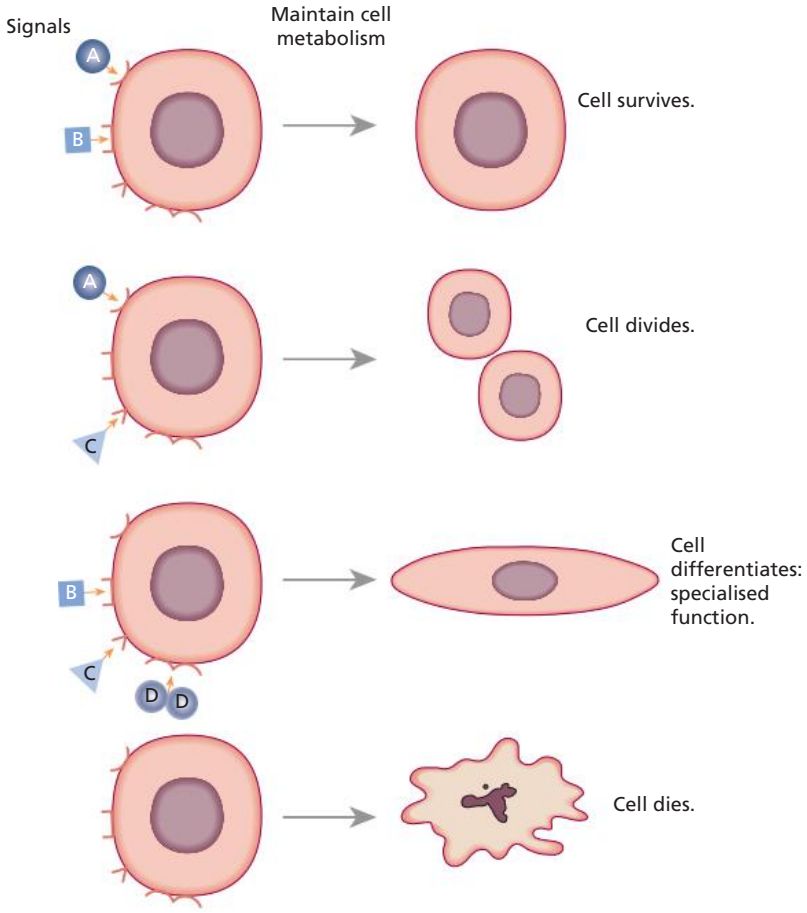


Figure 4.21
Signals act collectively to produce different outcomes of cell signalling, including cell survival, division, differentiation and death.

The signal is terminated

After signalling has been initiated and the message has been transduced to bring about a response in the cell, the message must be switched off. This is important for cells to maintain their responsiveness and to avoid overstimulation that can lead to disease. One way of shutting off the response is to quickly clear up the second messenger. Another way is to dephosphorylate proteins by removing phosphate groups. Phosphatases are a group of enzymes that remove phosphate groups from proteins.

An illustration of the stimulus–response model

Let's explore the stimulus–response model and its three main components by following the example of the signal transduction pathway induced when antidiuretic hormone (ADH) reaches a target kidney cell.

ADH is secreted from the pituitary gland when the hypothalamus senses a drop in blood volume. ADH travels in the blood to the kidneys where it acts to increase water absorption so blood volume increases. Let's follow the stimulus–response pathway stimulated by ADH in cells of the kidney collecting duct (Figure 4.22).

1 The stimulus, ADH, binds to an extracellular receptor, a G protein-coupled receptor called V2.

2 This binding causes a change in receptor conformation that leads to activation of an enzyme, adenylate cyclase, which is located on the inner side of the plasma membrane. It starts to convert ATP molecules to cAMP in the cytoplasm. Many cAMP second messengers are produced here. The message is amplified.

3 The cAMP molecules (second messengers) initiate a signal transduction cascade by activating protein kinases. These enzymes phosphorylate target proteins that stimulate exocytosis of vesicles containing aquaporin channels.

4 These water channels are inserted into the plasma membrane so water leaves the kidney and moves into the blood. After the cell has responded, intracellular enzymes degrade the cAMP and, over time, the aquaporin channels break down.

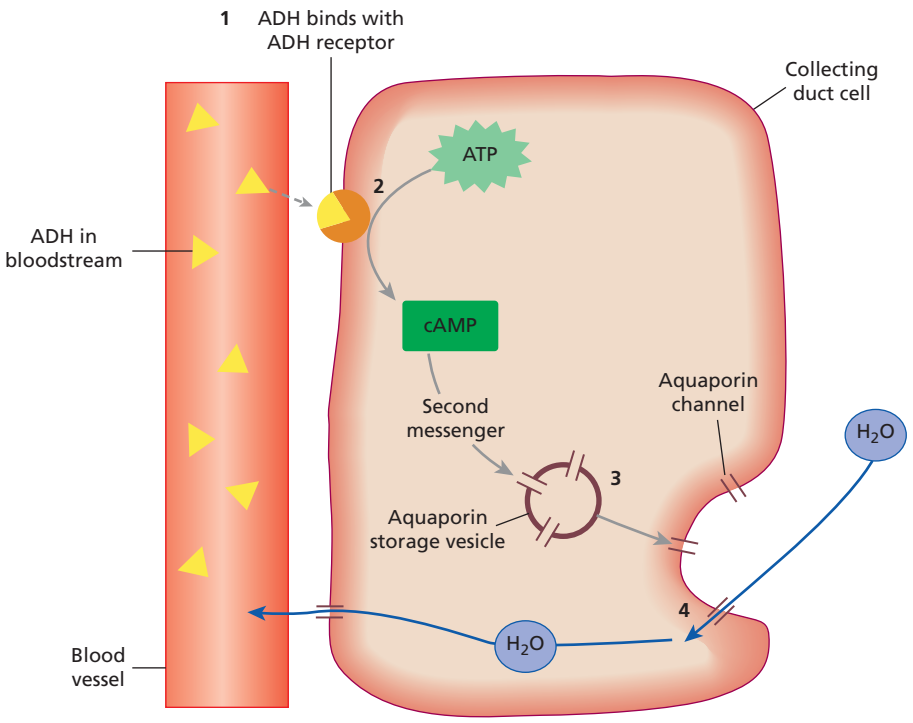


Figure 4.22 ▶ Antidiuretic hormone signals to cells in the collecting ducts of the kidney to reabsorb more water into the blood. Aquaporin channels are stored in vesicles in collecting tubule cells of the kidney. The signal transduction pathway is elicited when ADH binds to its receptor.

RECALL

- Second messengers are small molecules that amplify and transmit the signal between proteins or organelles within the cell. They can be ions or derivatives of nucleotides or phospholipids.
- Signal transduction results in the activation of effector proteins in the cell. The cellular response is influenced by the combination of signals a cell receives.
- Termination of the signal, or negative regulation of signalling, ensures that a response of the right magnitude and duration is generated.

RECAP 4.7

1 Create a timeline of events to recount how steroid hormones stimulate a response in cells.
2 Make a chart that identifies up to four cell responses and the effector proteins involved in coordinating these responses.
3 Discuss why only a small amount of hormone is required to initiate a response in a cell.

ACTIVITY 4.1

SONIC HEDGEHOG, THE CYCLOPS LAMB AND CANCER: A CHEMICAL SIGNALLING RESEARCH DETECTIVE STORY

In the 1950s, a small group of Idaho sheep farmers noticed that lambs were being born with strange defects. Scientific studies revealed that during drought years, sheep grazed on an alpine plant called the corn lily. Pregnant ewes eating these plants during early gestation gave birth to lambs with one eye, a condition known as cyclopia. Something in the plant was interfering with the development of the foetus.

In the 1970s, scientists conducted experiments to discover how a fruit fly embryo, a ball of cells, knows where to grow a leg, a wing or a head. Investigating the effect of more than 1000 mutations revealed 50 genes essential for development: the genetic toolkit for building a fruit fly. When one particular gene was mutated, the fruit flies grew spines on their underbellies, so they called this the hedgehog gene.

Scientists started a search for homologous genes in mammals. A few hedgehog genes were found; the most important was named sonic hedgehog (Shh). Mutations in this gene resulted in deformities in mammals, including cyclopia.

Aim

To construct a stimulus–response model demonstrating how cells respond to Shh and to explain how an endocrine disruptor is showing promise as a treatment for cancer

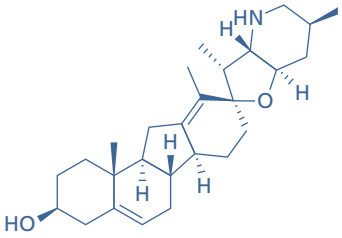
What to do

1 Use the following passage to construct a stimulus–response model that shows how Shh exerts an effect on cells.

When the Shh gene is expressed, Shh signalling protein is produced that diffuses out of the cell to affect nearby cells. These target cells have a receptor called Patched. Binding of the Shh signal activates Patched receptors to release a membrane protein called Smoothened into the cell.

Figure 4.23 ▶
(**a**) Cyclopamine is a molecule found in the corn lily plant. It interferes with a protein in the hedgehog developmental pathway, causing lambs to be born with fatal birth defects such as cyclopia. (**b**) The corn lily plant.

a



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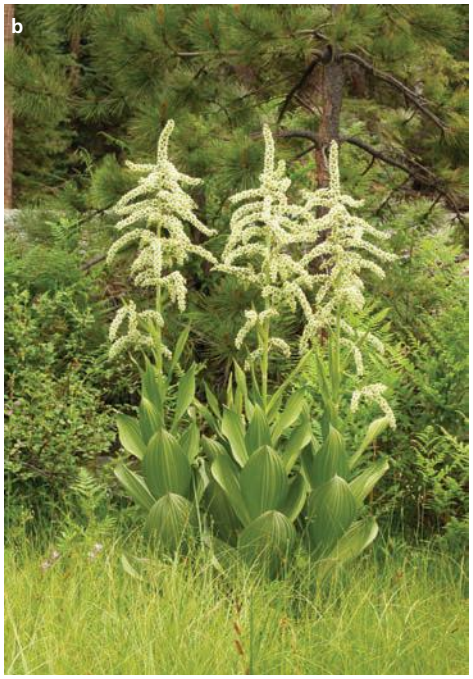


Alamy/Robert Shantz

Smoothened activates a signal transduction pathway that leads to transcription of a set of proteins. These proteins drive the cell response to divide. In this way, Shh is a growth factor, stimulating growth through cell division.

Shh signals cells in a developing limb bud to switch on one set of genes, while it signals for a different set of genes to be switched on in cells involved in nervous system development. Shh being produced in the wrong place at the wrong time can lead to extra digits being formed in the hand. If Shh is not present when nerve buds for the eyes are developing, then only one eye develops, leading to cyclopia.

This hedgehog signalling pathway is integral to embryonic development, but it is also important for regenerating short-lived tissues such as skin cells and blood, stimulating cells to divide to replace dying cells. If this pathway is not regulated then cancers may develop as cells divide uncontrollably.

A connection was made back to the Idaho sheep. It was found that the corn lily produces cyclopamine, which binds to Smoothened protein c and renders it inactive. It is an endocrine disruptor that interferes with normal signal transduction.

2 Draw and annotate a diagram of four large skin cells that reveals the following information.

- **a** Sonic hedgehog (Shh) is a peptide growth hormone. Show the location of the Patched receptor in the cell and defend your decision.
- **b** Indicate whether Shh is an endocrine, autocrine, paracrine or contact-dependent hormone by showing how it is secreted and travels to target cells.
- **c** Indicate how signal transduction of the Shh message elicits a response in target cells.

3 On your diagram indicate how the hedgehog development pathway might be disrupted, resulting in a loss of regulation of cell division in skin cells and leading to the development of basal cell carcinoma.

4 Cyclopamine shows great promise in the treatment of basal-cell carcinoma tumours caused by inappropriate hedgehog pathway activation. Add to your diagram to show how cyclopamine may be used as a drug to treat basal cell carcinoma.

5 Construct a timeline of scientific discoveries that led to the development of cyclopamine as a promising cancer treatment.

6 What sort of warnings would be important to include with this drug if it were to be marketed?

When cell communication goes wrong

Cells are constantly communicating with each other using a variety of carefully regulated mechanisms. What happens if this tightly regulated communications system malfunctions? This can result in disease. Many diseases involve breakdowns in cell communication, and most disease treatments address the breakdown in communication in some way.

In type 1 diabetes, the beta cells of the pancreas are destroyed by the immune system and no longer secrete insulin. Insulin is a signal to cells to take in glucose from the bloodstream and this loss of signal leads to high blood glucose levels. Patients can inject themselves with synthetically produced insulin to overcome this problem. In type 2 diabetes, the insulin receptor in cells no longer responds to the insulin signal and the patient has become 'insulin resistant'. This is much harder to treat and patients have to manage their diet very carefully to make sure their blood glucose levels do not spike.

Parkinson's disease occurs when a set of dopamine-producing nerve cells deep in the brain become impaired. Dopamine is a neurotransmitter required for controlled movement of muscles. When levels of dopamine become too low, the effects of another neurotransmitter, acetylcholine, are enhanced, creating a resultant tremor in parts of the body. The adult CNS has neural stem cells with the capacity to produce the major cell types of the brain. Scientists have stimulated these stem cells to produce neurons *in vitro*. Continued research may result in a treatment where stem cells in the brain can be stimulated to become dopamine-producing cells.

Multiple sclerosis is a disease that results when cells of the immune system destroy the protective myelin sheath of nerve cells. This slows down transmission of nerve impulses and disrupts signals. This nerve damage can lead to uncoordinated movements, blurred vision and depression.

Sometimes multiple breakdowns in cell communication need to occur for a disease to manifest. Cancer is one example. Cancer generally starts when the signals that control cell division are disrupted, so cells start to divide uncontrollably. This unregulated cell division should stimulate a signalling pathway in the cell to target it for apoptosis. If the cell no longer responds to death signals then a tumour will develop. These tumour cells are greedy. They need huge supplies of oxygen and nutrients, so chemical signalling pathways are switched on to promote the growth of blood vessels throughout the tumour. Many treatments for cancer target malfunctions in chemical signalling by blocking or inducing proteins in signalling pathways.

RECALL

- Cell signalling systems can malfunction, resulting in disease.
- Diseases involving disruption in chemical signalling pathways include type 1 and type 2 diabetes. Parkinson's disease and multiple sclerosis involve disruptions in electrical signalling pathways.
- In cancer, signalling pathways that are involved in cell division are altered and cells divide uncontrollably.

RECAP 4.8

1 Compare and contrast the disease mechanisms of type 1 and type 2 diabetes.

2 Explain why the loss of myelin can cause impaired neurotransmission in multiple sclerosis.

Cell death: an important outcome of cell signalling

An essential possible outcome of cell signalling is cell death by the process of apoptosis. Having this option means that cells that have become unnecessary, damaged or diseased can be removed, assisting normal development and preventing disease. For a bacterium or paramecium, cell death means the end, whereas in multicellular organisms cells die all the time and many cells are in fact fated to die. Consider a tadpole, which emerges from an egg with a tail made up of muscle cells, epidermal cells, notochord cells and pigment cells. Over a short period of time, each of these tail cells disappears and the tadpole becomes a frog. The death of these cells does not leave a hole or a wound, activate an immune response or cause any loss of function for the developing frog. Instead, the cells die by apoptosis, a highly controlled natural process that breaks cells down into fragments that are easily cleared away.

The removal of cells by apoptosis allows organisms to produce more cells and tissues than are needed, which provides some flexibility during development and later in life. For example, in humans, the female uterus and oviduct arise from a duct that is present in both male and female embryos. As the male embryonic testes develop, they secrete a factor that induces apoptosis of the cells that make up this duct, preventing the development of female reproductive organs in male embryos. Apoptosis is an essential mechanism for shaping developing embryos, and insurmountable problems with apoptosis usually cause early embryonic death.

The process of apoptosis

Apoptosis occurs in invertebrates, vertebrates and plants, and the proteins and fundamental processes involved are remarkably similar across diverse organisms. When coral cells are treated with a human protein that causes apoptosis, TNFα, apoptosis is up-regulated and the coral shows signs of bleaching. Bleaching in corals occurs when the coral is under stress and evicts the symbiotic algae that give it colour, and is usually associated with apoptosis of the coral cells. The ability of the human protein to activate the apoptotic program in coral cells indicates that this apoptotic pathway evolved very early and remained essentially the same throughout evolution.

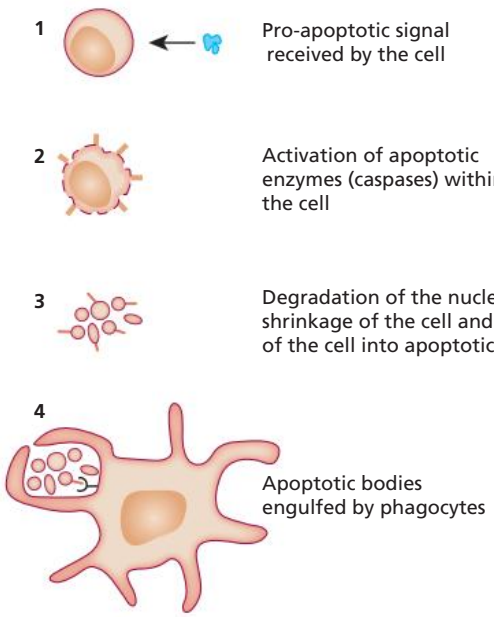


Figure 4.24▲
An overview of the apoptotic program

Because of the fairly uniform and highly coordinated way apoptosis proceeds once the process has begun, apoptosis is often called programmed cell death. The program begins when a cell receives a pro-apoptotic signal (Figure 4.24). Through a signal transduction cascade, this then activates the proteins that are involved in apoptosis. The activated apoptotic machinery breaks down the nucleus and causes the cell to shrink and form apoptotic bodies, or blebs. The apoptotic debris is engulfed by phagocytes, specialised cells that take up the blebs and degrade them, recycling components for later use.

Activating apoptosis

Pro-apoptotic signals fall into two main categories. The first is internal signals or signs of cellular damage that activate the **intrinsic pathway**. The second is external cues from signalling molecules that bind to **death receptors** on target cells to activate the **extrinsic pathway**. A proper apoptotic response to each of these signals is essential for the continuing homeostasis of the organism.

Internal cues: the intrinsic pathway

A cell is constantly receiving signals from its environment: contact-dependent signals from neighbouring cells, growth factors, hormones, and many other signalling molecules. The ability of a cell to detect and respond properly to this suite of signals is such an important part of its function that the absence of external signals can lead to death of the cell. This is because cells are usually easily replaced, but if a cell develops the ability to grow independently of external signals, it becomes a likely risk for cancer. Because of this, apoptosis is almost a default pathway that occurs when a cell does not receive adequate regulatory signals from its environment. Detecting growth factors, activation cues, or contact-dependent signals from neighbouring cells can cause expression or activation of **anti-apoptotic** proteins, which suppress apoptosis and allow the cell to continue to survive and carry out its functions.

Normal cell activity and homeostasis rely on cells functioning at their best. Ensuring that cells are turned over or removed when they acquire damage ensures that organs and tissues function normally and lowers the risk of cancer developing. For cancer to arise, multiple mutations in DNA must occur to allow abnormal expression of genes that promote survival, migration, establishment at new sites and support the formation of new blood vessels to feed the tumour. To prevent damaged DNA from persisting in cells, if mutations in the DNA are unable to be repaired, apoptosis is initiated and the cell is destroyed, removing any potentially harmful mutations.

Damage to cellular organelles, including the endoplasmic reticulum, lysosomes and particularly mitochondria, can also be a strong pro-apoptotic signal; leakage of the mitochondrial membrane allows release of some of the proteins that are responsible for apoptosis. On the other hand, many of the anti-apoptotic proteins act to stabilise the mitochondrial membrane and prevent release of these pro-apoptotic proteins. Cellular stress, for example from UV or heat exposure, can cause oxidative stress, in which large amounts of reactive oxygen species (ROS) accumulate in cells. ROS are also a natural by-product of cellular metabolism using the electron transport system in the mitochondria. They are usually kept at low levels by cellular enzymes and anti-oxidants. This regulation is necessary for cell survival as ROS are very damaging to the mitochondrial membrane and their accumulation can trigger mitochondrial leakage and apoptosis.

Other organelles can also be involved in apoptosis. The lumen of the ER contains a store of calcium ions. Leakage of calcium and ER luminal proteins into the cytosol can occur with ER damage, which triggers the ER stress pathway, leading to apoptosis.

External cues: the extrinsic pathway

In particular cases, such as the highly coordinated processes of development and the dynamic cellular changes that occur during an immune response, apoptosis can be initiated in an active way by external signals that bind through death receptors. During a viral infection, some body cells may become infected and harbour the virus within their plasma membrane, hidden away from cells of the immune system. A specialised immune cell, the cytotoxic T lymphocyte (CTL), has the ability to detect virally infected cells or cells that have become cancerous. A protein on the surface of the CTL can deliver a pro-apoptotic signal by binding to a death receptor on the target cell. This destroys both the infected cell and the virus hiding within it, preventing further spread of the virus. Importantly, the virus remains contained within the apoptotic bodies, whose plasma membranes remain intact, until phagocytes engulf and destroy the apoptotic bodies through a process called **phagocytosis**.

Apoptosis also maintains homeostasis in organs and in the immune system. During an immune response, immune cells called lymphocytes can rapidly proliferate to form many copies of themselves. This allows the infection to be cleared quickly and efficiently. Once the infectious agent is removed, the majority of responding cells are no longer needed. They undergo apoptosis, and the remaining cells become memory cells as discussed in Chapter 6.

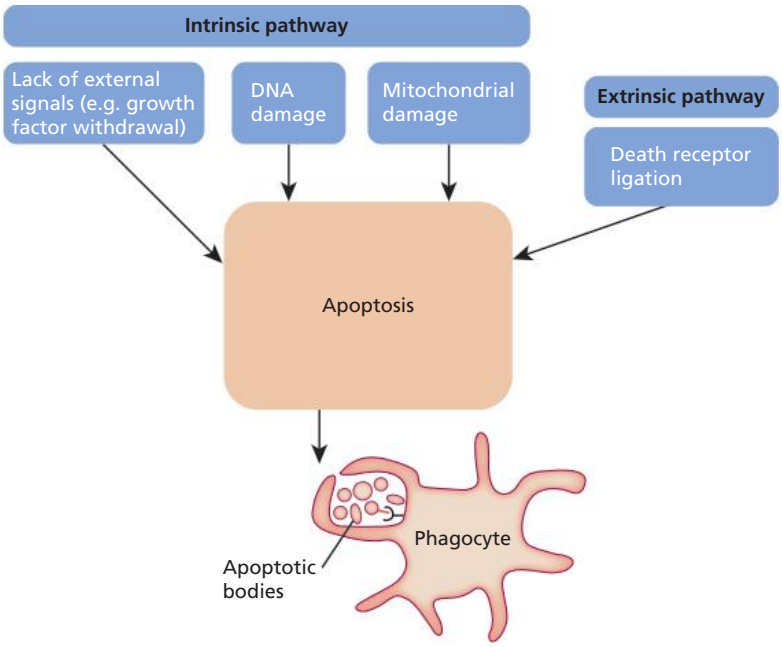


Figure 4.25 ▶ Factors that promote apoptosis. Following apoptosis of a cell, phagocytes clear away apoptotic bodies by phagocytosis.

RECALL

- Apoptosis is a carefully regulated, active process of programmed cell death that is essential for normal development and homeostasis of multicellular organisms.
- The intrinsic pathway of apoptosis is activated by irreparable mutations in DNA and damage to organelles, particularly the mitochondria, or by growth factor withdrawal.
- The extrinsic pathway of apoptosis is mediated by death receptor ligation and is a feature in development and during immune responses.

RECAP 4.9

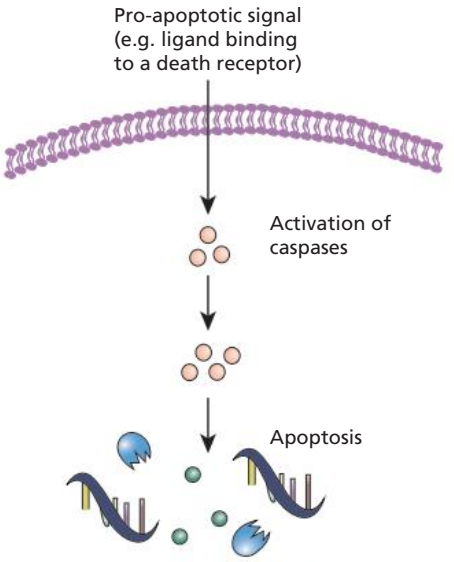
1 Outline the steps of apoptosis.
2 Explain why apoptosis is often referred to as programmed cell death.
3 Compare the intrinsic and extrinsic pathways of apoptosis and describe the ultimate outcomes of each pathway.

Intracellular pathways of apoptosis

Apoptosis is an active process. It involves signal transduction pathways and activation of several different types of proteins, many of which activate other proteins in a signal transduction cascade. Early studies of the apoptotic pathway involved the nematode *Caenorhabditis elegans*, whose development always proceeds in the same way. Of the 1090 somatic cells that are formed, 131 die by apoptosis, leaving 959 cells in the mature adult. Genetic screens of *C. elegans* identified a set of four genes that regulated apoptosis of the 131 cells. One of the genes was found to encode a protein that blocks apoptosis, showing it to be an anti-apoptotic gene. Three of these genes, however, were pro-apoptotic, as their loss prevented apoptosis and resulted in the survival of the 131 condemned cells. The pro-apoptotic proteins they encode belong to a family that is now known as **caspases**, and that is highly conserved (or similar) across multicellular organisms.

Caspases are enzymes (proteases) that cleave, or cut, intracellular proteins. Some caspases cleave inactive, immature forms of other caspases. Others are effector molecules that cleave cellular proteins to initiate the process of apoptosis. Substrates of caspases include cytoplasmic and nuclear proteins, including a caspase-activated DNA degrading enzyme (endonuclease) that is responsible for cutting DNA at specific linker regions between nucleosomes (approximately 200 base pairs apart). The DNA is then easily dispersed into small apoptotic bodies, ready for clearance by phagocytosis (Figure 4.26).

The signal transduction pathways that lead to caspase activation contain many different components, including signal transduction proteins, adaptor proteins, second messengers and ATP. Often there are multiple alternative proteins that can act at each step. This indicates how important apoptosis is: if the gene encoding one of the proteins becomes mutated and loses its function, the pathway can rely on another protein and apoptosis can proceed. Apoptosis does not always rely on caspases. The intrinsic pathway (involving damage to mitochondria, ER or other organelles) can occur independently of caspases, although the death receptor pathway generally relies on caspases for induction of apoptosis.



▲ **Figure 4.26**
Apoptosis often involves the actions of caspases.

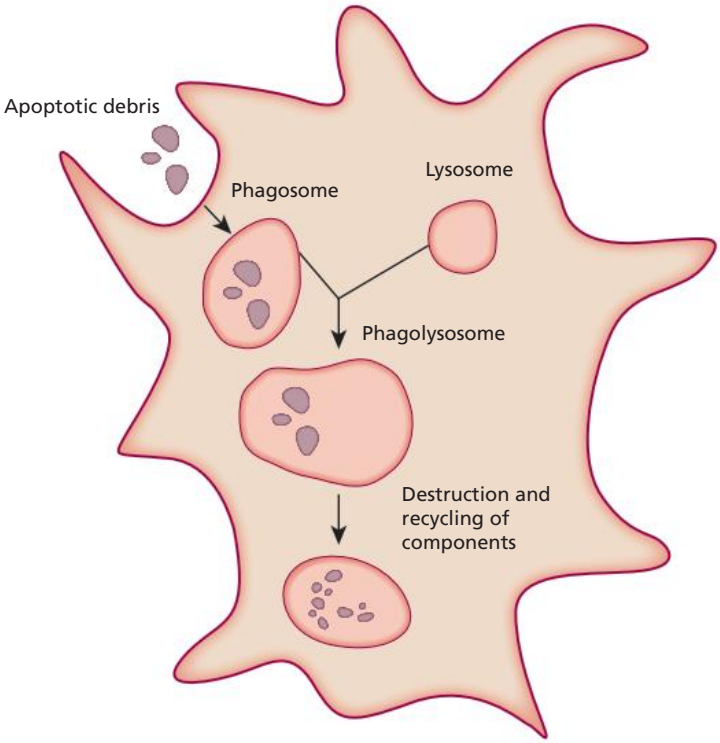
Cellular destruction and clearance

Once apoptosis is initiated, there is no turning back for a cell. Its nucleus shrinks and the DNA is degraded into fragments of multiples of 200 bp in length, thanks to the actions of endonucleases. The nuclear membrane breaks down and the cell fragments into small blebs. These contain DNA and organelles, which retain their function. The plasma membrane remains intact around the blebs and none of the cellular contents are released into the extracellular space.

Cells that are undergoing apoptosis release signals that attract phagocytes. During apoptosis, a lipid normally found in the cytosolic side of the plasma membrane flips to the extracellular side. An abundance of this lipid on the surface of a cell or apoptotic body is a signal to phagocytes to engulf the dead cell (see Figure 4.24). If apoptotic bodies are not cleared away quickly, they can burst and release their contents, a process that has been linked to the development of autoimmune diseases. The failure to clear away apoptotic debris contributes to several diseases including lupus, atherosclerosis, Alzheimer's disease and Parkinson's disease.

When a phagocyte engulfs apoptotic bodies, they enter a vacuole called the **phagosome** (Figure 4.27). This goes through several maturation steps including fusion with the lysosome, which is acidified and contains hydrolytic enzymes. The fused compartment is now called the **phagolysosome**, and now contains the perfect environment for the destruction of the apoptotic debris. Finally, after it has engulfed the debris, the phagocyte often releases anti-inflammatory signalling molecules. This is important because non-apoptotic cell death as a result of an injury or wound, called **necrosis**, is a strong stimulus for the immune system.

▼ **Figure 4.27**
Phagocytes degrade apoptotic debris by the process of phagocytosis.



When apoptosis malfunctions

The importance of apoptosis throughout the lifetime of an organism means that defects in apoptosis manifest not only in developmental abnormalities, but also in a variety of adult diseases. Cancer, diseases of the immune system and degenerative diseases are some of the better known cases in which apoptosis is defective (see Table 4.4).

Table 4.4 Examples of diseases in which apoptosis is involved

Disease	Involvement of apoptosis	Consequence of apoptosis malfunction
Cancer • Breast, lung, kidney, ovary, uterus, gastrointestinal tract, head and neck, melanoma, lymphoma, leukaemia	Maintains correct cell numbers in a tissue by removing excess cells	Prevents death of cells carrying a tumourigenic mutation Growth of large numbers of cells that have the opportunity to accumulate enough mutations to become tumourous
Neurological disorders • Alzheimer's disease (AD) • Parkinson's disease (PD) • Huntington's disease (HD) • Stroke	In AD, caspases cleave the amyloid precursor protein to form amyloid-β, which accumulates in plaques to cause neurodegeneration. In HD, caspases cleave the Huntington protein, which may affect neuronal degeneration. In stroke, apoptosis is associated with neurodegeneration.	In PD, selective death (possibly by apoptosis) of dopaminergic neurons leads to neurodegeneration.
Cardiovascular disorders • Heart failure • Ischaemia	Apoptosis of cardiac muscle cells leads to heart failure. Ischaemia occurs when blood flow is restricted to heart muscle cells, causing their apoptotic death.	Detecting ischaemia and preventing apoptosis soon afterwards may rescue heart muscle cells and prevent death of cardiac tissue.
Infection • Viral • Bacterial	Death receptor ligation of host cells infected with viruses or intracellular bacteria stops the infectious agents from spreading. Phagocytes can engulf extracellular bacteria and undergo apoptosis if they are unable to degrade them.	Viruses and bacteria have an impressive array of mechanisms to manipulate apoptosis in host cells to change the outcome of infection. For example, preventing apoptosis of phagocytes that have engulfed bacteria allows the bacteria to take up residence in the phagocyte.
Autoimmune diseases • Lupus • Rheumatoid arthritis • Multiple sclerosis	Cells of the immune system that detect a foreign infectious agent undergo apoptosis unless danger signals are also present; this prevents proliferation of cells that might attack normal components of the body.	Failure to clear apoptotic debris increases the concentration of potential autoantigens and inflammatory stimuli (see Chapter 6).

Cancer

For cancer to arise, a cell has to gain the ability to escape the normal mechanisms that limit its growth and survival. It does this is by acquiring mutations in its genome that change the expression or function of genes. Because apoptosis is the main way of removing new potentially cancer-causing mutations, the most important factor in the initiation of cancer is the ability of mutated cells to evade apoptosis and continue to survive and divide.

Many proteins exist to detect DNA damage, repair it, and halt the cell cycle while this repair occurs. These proteins are encoded by **tumour suppressor genes**, as collectively they prevent the cell from dividing to produce daughter cells that carry the mutation. Examples of this type of gene are the BRCA1 and BRCA2 genes; mutations in these are associated with the development of breast cancer. Another tumour suppressor gene is p53, which can activate apoptosis if DNA repair is not possible. If p53 is inactivated by mutation, a cell with mutated DNA can escape apoptosis and continue to proliferate. More mutations may accumulate until the daughter cells are able to form a tumour.

Because of the importance of apoptosis in preventing tumour formation, pro-apoptotic genes can also be considered tumour suppressor genes. On the other hand, genes that promote survival and proliferation in normal cells can become **oncogenes**, or cancer-promoting genes, if they become mutated. Generally oncogenes are genes whose protein products provide a strong activating signal to the cell, such as growth factors, receptors, signalling molecules or transcription factors. Normally, negative regulators of signalling would control the amount or duration of signalling by these factors, but a mutated form of the oncogene may cause the cell to receive growth signals of a much greater duration or intensity than normal.

Once a cluster of cancer cells has arisen that can survive, proliferate and evade apoptosis while DNA mutations accumulate, the scene is set for cancer development. Cells may acquire mutations that give them the ability to detach from their position and enter the circulation, then move through blood vessels and lodge in a new location. This process is called metastasis. Cancer cells may stimulate production of factors that promote their own survival, allow them to hide from immune detection, and stimulate growth of blood vessels to provide the developing tumour with oxygen and nutrients.

Treating cancer

Since they arise from normal body cells, with only a relatively small number of molecular changes needed to cause disease, cancers can be hard to treat specifically without inducing harmful side effects for other cells or tissues. Some small molecules can target specific gene mutations that commonly occur in cancers, or can reduce the activity or expression of signalling pathways that cancers rely on to grow and spread.

Monoclonal antibodies are another class of drug used to treat cancer and are discussed in more detail in Chapter 6.

RECALL

- The extrinsic pathway of apoptosis involves the activity of caspases, enzymes that cleave intracellular components to amplify and elicit the apoptotic response. The intrinsic pathway can induce apoptosis without caspase activation.
- Malfunctions in apoptosis are involved in several different types of diseases, including cancer, in which apoptosis is defective in cells carrying mutations in DNA.

RECAP 4.10

1 Outline the intracellular steps involved in apoptosis.
2 List three examples of diseases that involve apoptosis and describe the nature of the involvement.
3 Explain how cancer arises with respect to defects in apoptosis.

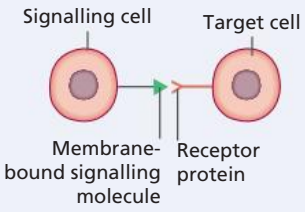
CONCEPT SUMMARY

Cell signalling

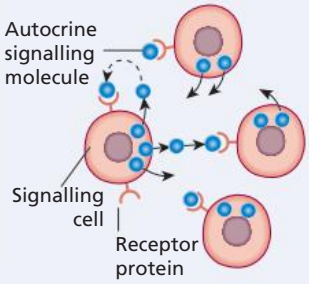
The cellular processes regulated by chemical signalling include gene expression, metabolism, apoptosis, reproduction, cell communication and molecule transport.

Types of signalling

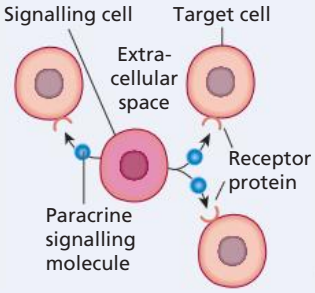
a contact-dependent



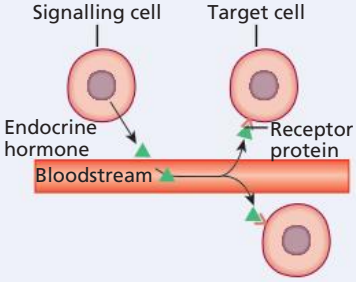
b autocrine



c paracrine



d endocrine



- Hydrophobic signalling molecules, such as steroid and thyroid hormones, bind to intracellular receptors.
- Hydrophilic signalling molecules, such as peptide and some amine hormones, bind to extracellular receptors.
- Cytokines control movement and behaviour of cells and tissues of the immune system. Signalling can be contact-dependent, autocrine or paracrine.
- Pheromones are secreted from the body and trigger a response in other members of a species.

Plant hormones

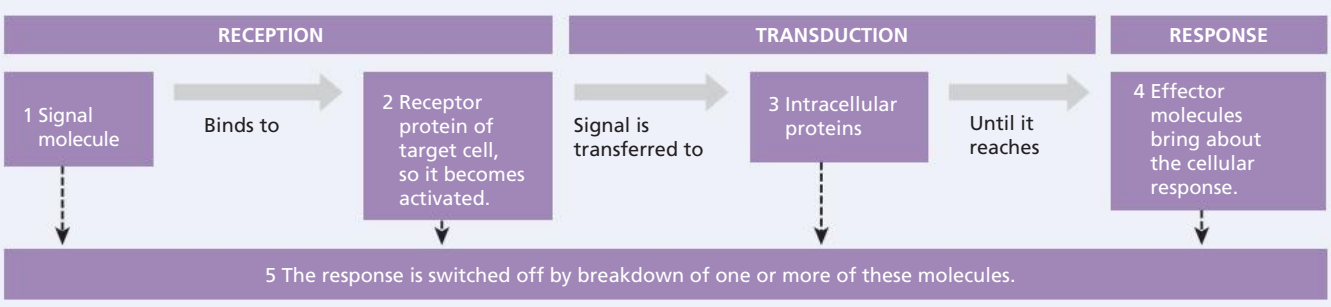
- Auxins mediate apical dominance, root growth and cell elongation. They are responsible for tropisms.
- Cytokinins promote cell division, chloroplast formation, and growth of lateral shoots and leaves.
- Gibberellins promote vertical growth are used widely for commercial purposes.
- Ethylene is a gas that stimulates ripening.
- Abscisic acid promotes a state of dormancy in seeds and buds. It also prevents water loss by causing stomata to close.

Neurotransmission

- Sensory neurons
- Interneurons
- Motor neurons

The resting potential of an axon is maintained by sodium-potassium ion pumps. An impulse is relayed along an axon of a neuron, causing sodium channels to open and resulting in depolarisation. The signal is transferred between neurons by release of neurotransmitters which cross the synapse.

Stimulus–response model



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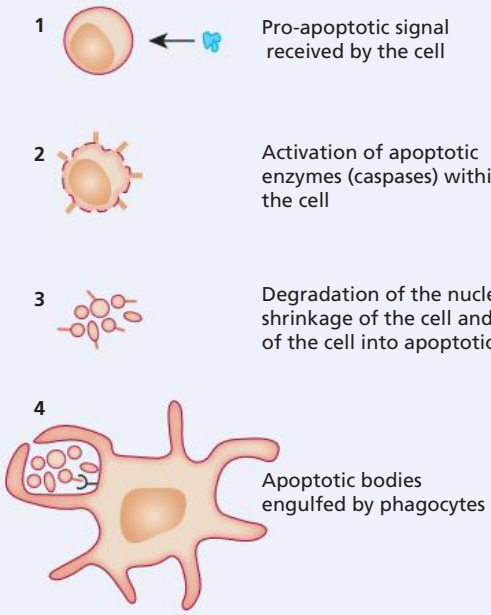
Apoptosis

Programmed cell death is important for the development and removal of unnecessary, damaged or diseased cells. A pro-apoptotic signal activates caspases, which break down the nucleus and cause the cell to shrink and form apoptotic bodies, or blebs. These are engulfed by phagocytes, which recycle components for later use.



Caspases

Caspases cleave other immature caspases (activating them), cellular proteins (degrading them) and DNA for dispersal into small apoptotic bodies for clearance by phagocytosis (Figure 4.26).

1-2 Binding of a signalling molecule to its receptor causes a change in conformation, triggering signal transduction.

3 Second messengers include ions and molecules derived from nucleotides and phospholipids. These relay the signal, and amplify the intensity of signal transduction.

4 Effectors proteins may be activated and gene expression can be altered as a result of signal transduction.

5 Negative feedback ensures the signalling is switched off as soon as the appropriate response is elicited.

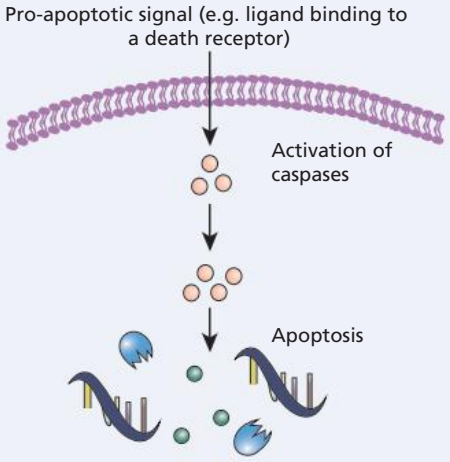
The intrinsic pathway of apoptosis

The intrinsic pathway of apoptosis is activated by internal signals or signs of cellular damage, including growth factor withdrawal, irreparable DNA damage and damage to organelles – especially mitochondria.

- Oxidative stress causes the accumulation of reactive oxygen species (ROS), which damage the mitochondrial membrane. This results in release of pro-apoptotic proteins into the cytoplasm.
- Many of the anti-apoptotic proteins act to stabilise the mitochondrial membrane and prevent release of these pro-apoptotic proteins.

The extrinsic pathway of apoptosis

The extrinsic pathway of apoptosis is activated by ligation of death receptors on the target cell. It is common in the immune system and in maintenance of tissue homeostasis.



Apoptosis in disease

Alterations in normal apoptosis are involved in cancer, neurological disorders, cardiovascular disorders infection and autoimmune diseases.

Cell signalling in disease

Malfunctions in chemical signalling pathways can result in multiple diseases including type 1 and type 2 diabetes. Parkinson's disease and multiple sclerosis involve disruptions in electrical signalling pathways.

CHAPTER GLOSSARY

abscisic acid a plant hormone that is involved in many plant developmental processes including seed and bud dormancy

acetylcholine a neurotransmitter in the human nervous system

action potential a brief change in the electrical potential on the surface of a nerve or muscle cell in response to stimulation, which results in the transmission of an electrical impulse

activator a regulatory protein that binds to an enzyme or DNA, causing a change of conformation so that enzymes become active, or activating gene expression

adhesion proteins proteins on the surface of cells that are involved in binding with other cells or to an extracellular matrix in a process called cell adhesion

amine hormone a hormone derived from amino acids; examples include epinephrine, dopamine and thyroxine

anti-apoptotic describes a gene or protein acting to prevent apoptosis and allow cell survival

apical dominance a growth pattern in which the central stem of the plant grows more strongly than (is dominant over) the side stems

apical meristem the tip of the shoot of a plant

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes, including caspases

auto-antigen (auto means 'self') a normal body component that activates an immune response in autoimmune disease

autocrine a type of signalling in which the signalling molecule binds to receptors on the cell type that produced it and affects the function of that cell type

auxins plant hormones that have three main effects: apical dominance, root growth and cell elongation

binding site a region on a protein, DNA or RNA molecule to which other specific molecules and ions bind through chemical interactions

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to form a final product

cancer a disease that arises when the signals that control apoptosis and cell division are disrupted, so cells survive and divide uncontrollably

caspases enzymes (proteases) that cleave, or cut, intracellular proteins and DNA in the process of apoptosis

cell differentiation the process by which a less specialised cell develops or matures to have more distinct characteristics and functions

cell signalling a complex system of signal transduction pathways that governs basic cellular processes and coordinates cell actions

cellular metabolism the sum of metabolic reactions in a cell

cellular process any process that is carried out at the cellular level but is not necessarily restricted to a single cell; for example, cell communication occurs among more than one cell but occurs at the cellular level

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) the chemical stimulus

contact-dependent describes signalling that requires cells to be in direct contact

contact inhibition the cessation of cell growth and division upon contact with other cells

cyclic AMP (cAMP) cyclic adenosine monophosphate; a nucleotide derivative that can act as a second messenger in signal transduction pathways

cytokines small signalling molecules that coordinate inflammation and immune responses, and that leukocytes use to communicate with one another; includes interleukins and interferons

cytokinin a plant hormone that promote cell division, chloroplast formation and growth of lateral shoots and leaves

death receptors cell surface receptors that transmit apoptotic signals

down-regulate decrease the quantity of a cellular component in response to an external variable

effector a muscle, gland, organ or protein that acts in response to a stimulus to bring about a particular outcome

endocrine the system comprising a collection of glands that secrete chemical signals directly into the bloodstream

ethylene a plant hormone involved in the ripening of fruit

extracellular occurring outside a cell or cells

extracellular receptor a receptor embedded in the plasma membrane that binds hydrophilic ligands

extrinsic pathway (of apoptosis) apoptosis activated by binding of a ligand to a death receptor on the target cell

feedback inhibition the cellular control mechanism in which an enzyme that catalyses the production of a particular product is inhibited by the product, therefore balancing supply and demand of a product for a cell

gibberellin a plant hormone that promotes vertical growth of the plant

hormone a chemical messenger secreted directly into the bloodstream, other body fluids, or into adjacent tissues, where they move to their target cells

hydrophilic hormone a hormone that is water soluble and binds to extracellular receptors to initiate a response in that cell; for example, peptide and some amine hormones

hydrophobic hormone a hormone that is water insoluble and binds to intracellular receptors; for example, steroid and thyroid hormones

inhibitor a substance that slows down or prevents a particular chemical reaction; by binding to proteins, inhibitors change the protein conformation so it no longer performs its job

intercellular occurring between cells

intracellular occurring within a cell or cells

intrinsic pathway (of apoptosis) apoptosis activated by growth factor withdrawal or damage to DNA or organelles

ion channel a protein or protein complex that spans the plasma membrane, forming a channel to facilitate the movement of ions across the membrane

ion gradient the concentration gradient of ions across a membrane; also referred to as an electrochemical potential

metastasis when cancer cells leave the tissue of origin and invade neighbouring tissues or travel to new sites around the body

myelin sheath the fatty layer surrounding and insulating the axons of many neurons; increases the speed at which electrical impulses travel along the nerve cell

necrosis cell death that results from tissue damage or infection; results in inflammation

neuron nerve cell

neurotransmitter a chemical substance that carries the action potential across a synaptic cleft

oncogenes cancer-promoting genes

paracrine a type of signalling in which the signalling molecule acts to induce changes in nearby cells of a different type from the cell that released the signal

peptide hormone a hydrophilic hormone composed of a chain of amino acids that can bind to extracellular receptors on target cells; for example, insulin and ADH

phagocytosis the bulk transport of solids into a cell, inside a vesicle

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

pheromones chemicals produced by animals that cause a change in the behaviour of another animal

phosphorylation the addition of a phosphate group to a protein or other organic molecule

phototropism directional growth of shoots towards a light source

pro-apoptopic signal a signal that results in apoptosis of the target cell

prostaglandins autocrine and paracrine hormones made from fatty acids

receptor a molecule on the surface or interior of the cell that binds specifically to a substance (ligand) to detect or receive a stimulus

resting potential the electrical potential difference between the two sides of an unstimulated nerve cell's plasma membrane; when this potential exists, the cell is ready for action

second messenger small molecules that relay a signal from receptors on the cell surface to target molecules inside a cell

signal tranduction the process by which a cell converts one kind of signal into another; occurs when an extracellular signal binds to and activates a receptor, which, in turn, alters intracellular molecules to bring about a cell response

sodium–potassium pump a membrane protein that moves potassium ions into, and sodium ions out of, a cell, using active transport

stem cell an undifferentiated cell that can divide indefinitely to give rise to more cells of the same type or specialised cells through the process of differentiation

steroid hormones hydrophobic signal molecules found in plants and animals; these are produced from cholesterol, giving them a common chemical structure; examples include oestrogen, testosterone and cortisone; these signalling molecules are lipophilic so they can pass through the plasma membrane and bind to intracellular receptors

synapse the point where an axon terminal meets another neuron, a muscle cell or a gland cell, separated by a synaptic cleft

synaptic cleft the space between the presynaptic cell and postsynaptic cell in a synapse, across which neurotransmitters diffuse to transmit a nerve impulse

target cell a cell that responds to a signalling molecule because it expresses specific receptors for that molecule

tropism directional growth or turning movement of a plant in response to an environmental stimulus

tumour suppressor genes genes that prevent a cell from dividing to produce daughter cells that carry the mutation that enables the cell to escape apoptosis

up-regulate increase the quantity of a cellular component, such as RNA or protein

CHAPTER REVIEW QUESTIONS

Remembering

1 Describe the cellular processes that might be induced by chemical signalling in the life of a liver cell.
2 Both testosterone and growth hormone are involved in increasing muscle mass. Identify where each hormone is produced, how it travels in the blood and how it exerts its effect at target cells.
3 Distinguish between the resting potential and the action potential of a neuron.
4 Discuss what happens to a nerve impulse when it arrives at a synaptic junction.
5 Give examples of how impaired apoptosis can contribute to disease in the case of infection, neurological disease and cancer.
6 What are the main mediators of apoptosis and what are their functions?

Understanding

7 Draw a diagram of a simple cell, illustrating the various types of chemical messages it may receive and listing potential cellular outcomes of the signal.
8 Draw a diagram to show how cell signalling can result in chemotaxis.
9 Are neurotransmitters hormones? Explain, using your understanding.
10 Protein hormones that use a second-messenger system often lead to a faster response than steroid hormones that bind with receptor proteins in the cytoplasm or nucleus of target cells. Explain why.
11 Construct a stimulus–response model to represent the chain of events that occur at the cellular level when ADH binds to receptors in a kidney cell.
12 Describe how you could design a drug to modify apoptosis and explain how this could be used to treat disease.
13 Draw a flow chart showing the steps involved in apoptosis.
14 Explain the terms tumour suppressor genes and oncogenes as if you were a doctor communicating to a cancer patient.

Applying

15 In the lab you are using yeast strains to study proteins that are similar (homologous) to those involved in cancer in humans.
   a Describe the results of mutating a tumour suppressor gene on yeast growth if the mutation stops the protein from functioning normally.
   b Describe the effect of mutating an oncogene.
   c For both of these types of genes, what effect would you see if the mutation results in enhanced protein function?
16 Use a stimulus–response model to explain how malfunctions in cell signalling can lead to disease.

Analysing

17 Refer to Figure 4.28 demonstrating the effect of the two hormones, adrenaline and oestrogen, on a human liver cell. Draw a table to compare the following for the signal transduction pathway triggered by each hormone.
   a Whether the hormone is hydrophobic or hydrophilic
   b Whether the receptor is intracellular or extracellular
   c A description of the signal transduction pathway
   d The effector molecule
   e The response

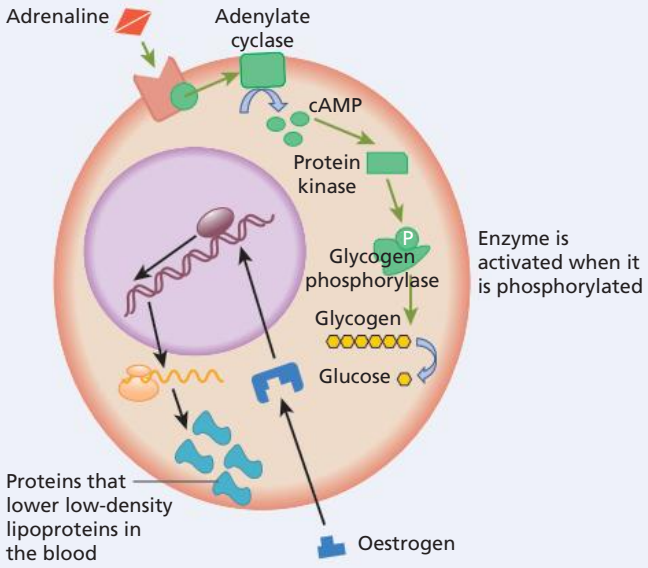


Figure 4.28 ▲
Adrenaline and oestrogen activate signal transduction pathways in a liver cell to elicit a response.

18 A toxic molecule from the venom of Chinese redheaded centipedes shows promise as a treatment for chronic pain that does not cause addiction. The molecule is selective for one of the nine different types of voltage-gated sodium (Nav) channels that humans have in their neurons.

- **a** Draw an annotated diagram that illustrates the role of Nav channels in transmission of nerve impulses.
- **b** Nav channels are important for pain perception, as well as heart and muscle function. Discuss why it is important that the toxic centipede molecule does not exert the same effect on all nine Nav channels.
- **c** People with a mutation that affects the gene for one of the Nav channels, known as Nav 1.7, are unable to experience pain. Discuss how this knowledge would help scientists to find an effective pain therapy.
- **d** The toxin from the Chinese redheaded centipede is a peptide called Ssm6a. Use an annotated diagram to propose how this toxin exerts its effect to inhibit pain transmission.
- **e** The toxin has been tested on mouse models that displayed minimal response to pain and had no side effects or change in blood pressure. Discuss if this is also guaranteed when human trials begin.
- **f** Most pain therapy drugs act by blocking pain receptors, making them addictive. Discuss why Ssm6a toxin should not be addictive.

Evaluating

19 Referring to chemical signalling, critique this comment made by Jacques Monod: 'What is true for *Escherichia coli* is also true for the elephant.'

20 Drugs that modify apoptosis are of great interest for the treatment of a number of diseases. Choose a disease from Table 4.4 and discuss how a drug that affects apoptosis might benefit patients with this disease.

Creating

21 Design an antibiotic that will disrupt intercellular signalling in bacteria. Use a diagram to illustrate how this antibiotic will work.

Reflecting

22 Consider the tools of summary tables, flow charts, timelines and annotated diagrams you have used in this chapter. Assess which are the most effective in enhancing your understanding.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CHAPTER 5

STRATEGIES OF DEFENCE AGAINST PATHOGENS

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

KEY KNOWLEDGE

Responding to antigens

- an antigen as a unique molecule or part of a molecule that initiates an immune response including the distinction between non-self antigens, self-antigens and allergens
- invading cellular and non-cellular pathogens as a source of non-self antigens, and preventative strategies including physical, chemical and microbiological barriers in animals and plants that keep them out
- the characteristics and roles of components (macrophages, neutrophils, mast cells, dendritic cells, complement proteins) of the innate (non-specific) immune response to an antigen including the steps in the inflammatory response

Biological knowledge and society

- strategies that deal with the emergence of new diseases in a globally connected world, including the distinction between epidemics and pandemics, the use of scientific knowledge to identify the pathogen, and the types of treatments
- use of chemical agents against pathogens including the distinction between antibiotics and antiviral drugs with reference to their mode of action and biological effectiveness

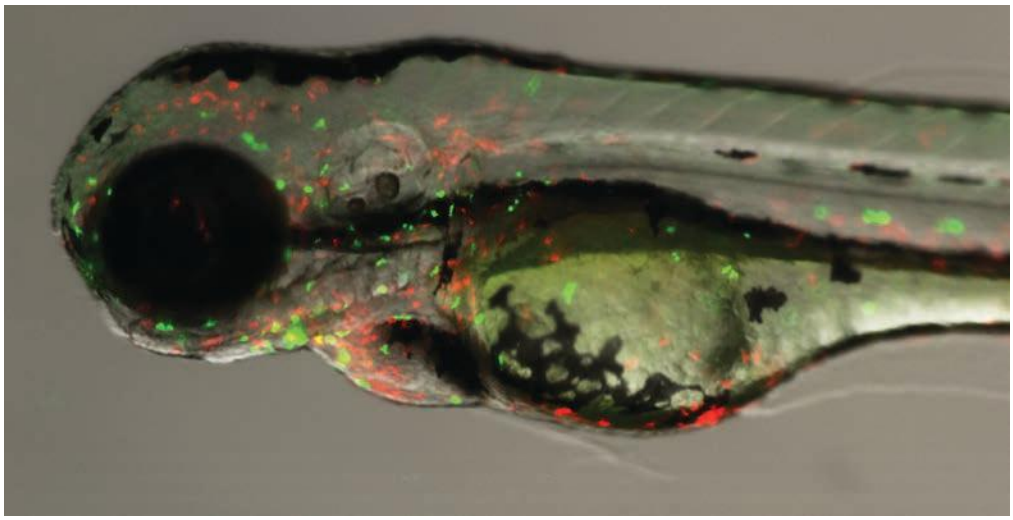
KEY SCIENCE SKILLS

Analyse and evaluate data, methods and scientific models

- organise, present and interpret data using schematic diagrams and flow charts, tables, bar charts, line graphs, ratios, percentages and calculations of mean

Communicate and explain scientific ideas

- use clear, coherent and concise expression



Prof. dr. Annemarie H. Meijer, Institute of Biology, Leiden University

◀ Figure 5.1
Fluorescently labelled cells of a zebrafish embryo's immune system patrolling its tissues for invaders

Disease is often described in terms of battles and wars: attacks on the body and invasions by pathogens. The **immune system** is commonly referred to as the defence system, fighting invaders using lines of defence, like a walled city under siege. If the first line of defence is breached and pathogens enter the body, they are attacked by second and third lines of defence. Hence, despite significant exposure to invading micro-organisms and parasites, in most cases we are able to resist infection. In what has been referred to as an evolutionary arms race between pathogens and their hosts, all organisms have evolved various types of defence mechanisms to inhibit the entry of pathogens and to deal with them should they gain a foothold. Even simple, single-celled organisms such as bacteria can defend themselves by producing enzymes to destroy invading viruses (bacteriophages).

In the immune system, the first line of defence is provided by physical and chemical barriers that prevent pathogens from entering, but sometimes the invaders succeed in gaining access to cells and tissues.

When that occurs, two other lines of defence come into play. The second line of defence is a **non-specific** response from the immune system in that it detects and responds to the pathogen regardless of its type. The next line of defence, the third, is a **specific response** that targets pathogens once they have been detected and identified by particular components of the immune system. Any substance that triggers an immune response is called an **antigen**. Some responses are specific to a particular antigen and others are more generalised, and responses can be localised or systemic – that is, working throughout the systems of the body, not just at the site of infection.

Some antigens are **allergens**, substances that trigger an allergic response. An **allergy** is a specific type of immune response that is activated in response to normally innocuous stimuli such as food, pollen or house dust mites. More harmful unwanted immune responses occur when the antigen is a **self-antigen**, that is, a substance that is normally present in the body. This can result in the development of an autoimmune disease, in which the body's own cells and tissues are mistakenly targeted for destruction by the immune system. Appropriate and effective immune responses depend on the ability of the immune system to distinguish **self** components from **non-self** antigens, derived from a foreign agent such as a pathogen.

Antigens are discussed in more detail on page 161 and in Chapter 6.

Pathogens

A disease is any condition that interferes with how an organism, or any part of it, functions. Diseases can be grouped according to their cause. Infectious diseases, such as TB, are caused by an agent that can be passed from one organism to another. The infected organism is the **host**. An infectious agent that causes disease is called a **pathogen**.

Pathogens are sources of non-self antigens, components of the pathogen against which an immune response will be launched with the aim of destroying or neutralising the pathogen. Pathogens include prions, viruses, bacteria, fungi, protists and **parasites**. Micro-organisms such as bacteria, viruses and fungi are responsible for familiar infections like sore throats, colds and tinea. Protists are the disease-causing agents in malaria, amoebic dysentery and giardiasis, while prions cause kuru and mad cow disease. A parasite is an organism that lives on or in its host for all or part of its life, causing harm and gaining nutrition from the host.

Viruses

A **virus** is a non-cellular agent composed of a protein coat and nucleic acid (Figure 5.2), either DNA or RNA, but never both. A virus is often referred to as an **obligate** parasite because it cannot function outside the host cell. When a virus infects an organism, it injects its nucleic acid into a host cell. Once inside the host cell, the viral nucleic acid takes over and directs the host cell to make multiple copies of the viral protein coat and nucleic acid. These then assemble into new viruses and are released when the host cell undergoes **lysis**, or splits open. This releases many more viral particles, which can infect other cells within the host. This life cycle is called a lytic cycle, and the viral genome stays separate from that of the host. Viral nucleic acids and proteins differ enough from those of the host that they can be identified as non-self components, stimulating an immune response to viral infection. Some viruses are able to incorporate their DNA into a host's chromosome and remain dormant, hiding from the immune system and being replicated along with the host's chromosome every time the cell divides.

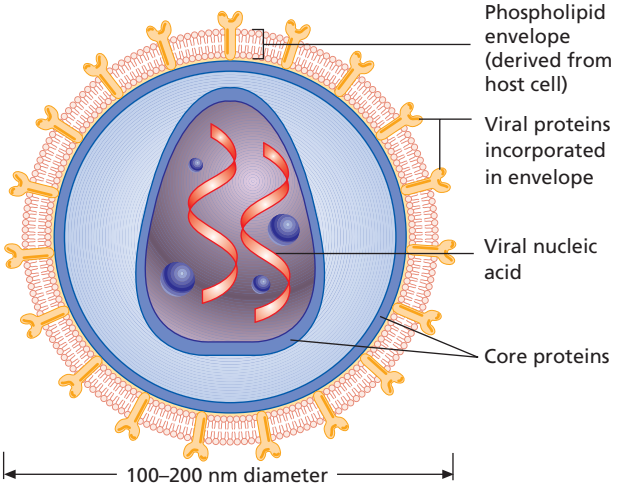


Figure 5.2 ▲
Viruses consist of a nucleic acid core surrounded by a protein coat.

Each virus is usually highly specific to the host cell or organism it can infect. For example, a rhinovirus specifically infects epithelial cells in the upper respiratory tract, causing the common cold (Table 5.1). This is because the virus is able to recognise and bind to receptors that are expressed only on respiratory tract epithelium.

Bacteria have their own group of viral pathogens, known as **bacteriophages** (Figure 5.3b).

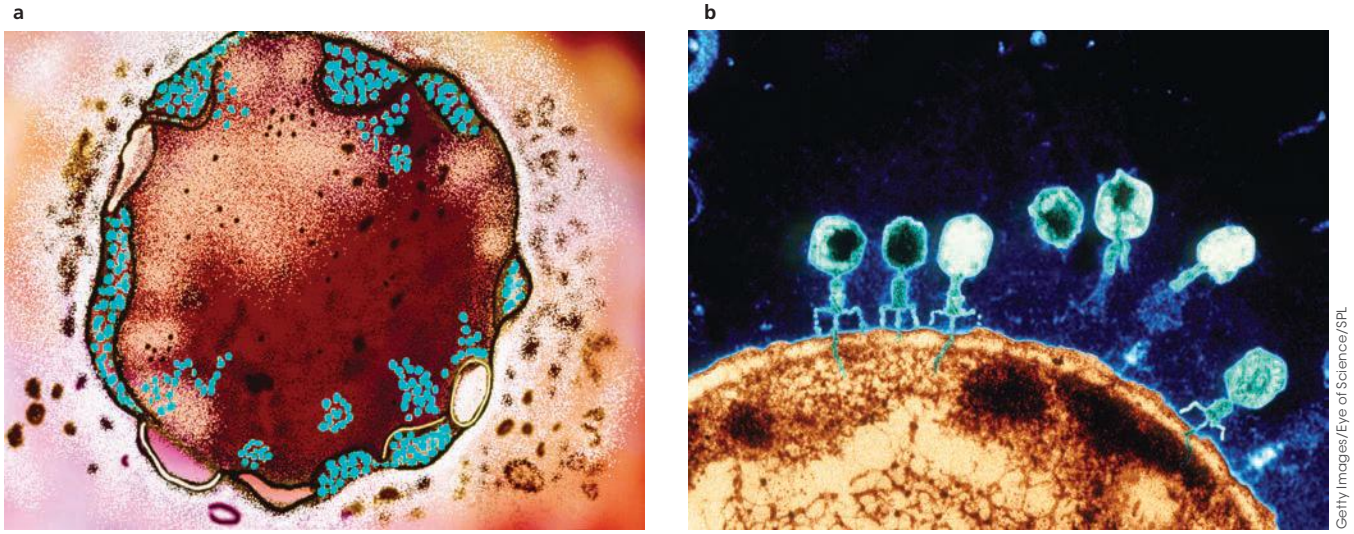


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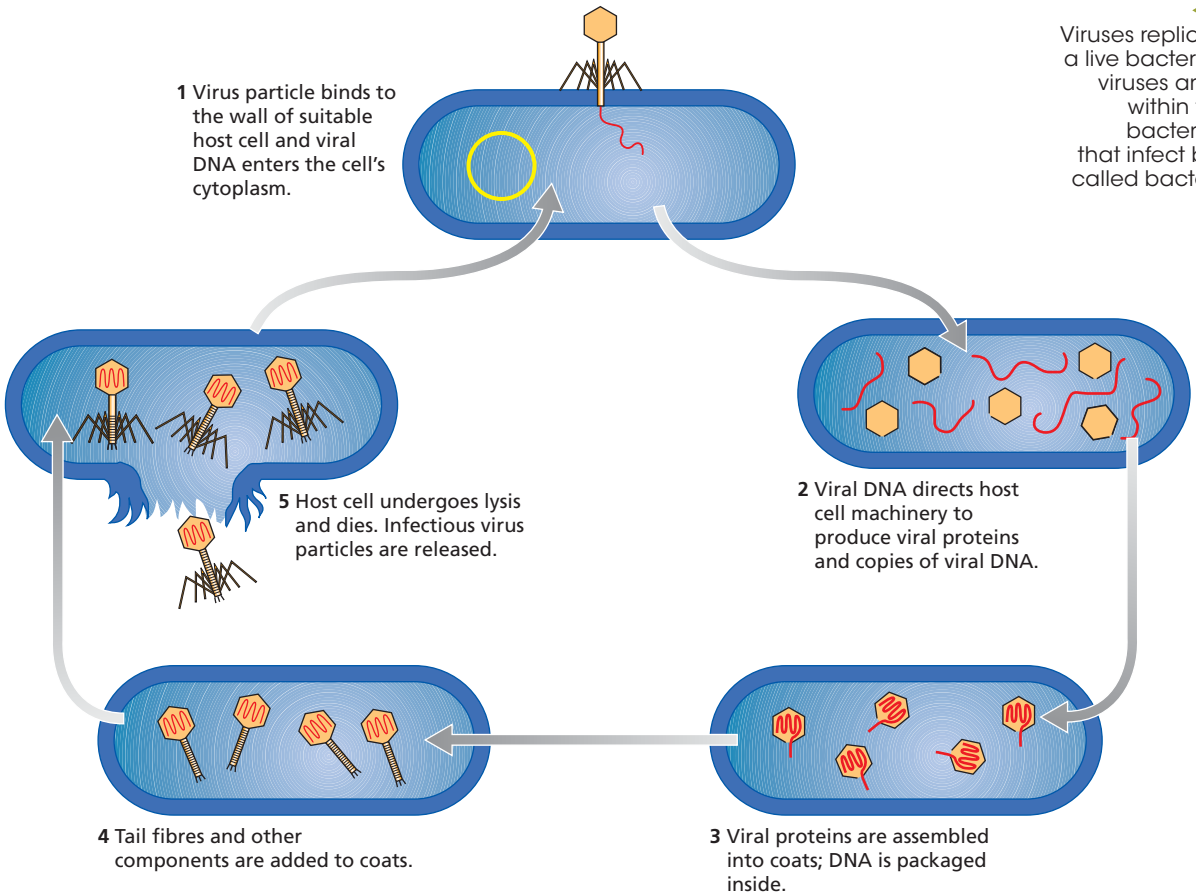
Figure 5.3 ▲
(**a**) The DNA virus that causes herpes in humans (**b**) A coloured TEM of T-bacteriophage viruses attacking a bacterial cell of *Escherichia coli*. Seven virus particles are seen (blue), each with a head and a tail. Small blue strands of genetic material (DNA) are being injected into the bacterium.

Table 5.1 Some diseases caused by viruses

Virus	Disease	Symptoms
Herpes simplex type I	Cold sores	Recurring blisters on skin, usually around mouth
Herpes simplex type II	Genital herpes	Recurring blisters in genital area; affects both males and females
Varicella zoster	Chickenpox	Fever, pink spots that blister and burst Can recur as the painful nerve inflammatory condition called shingles
Hepatitis A	Hepatitis A	Inflammation of liver, kidney, spleen; jaundice, fatigue, aching limbs, headache
HIV	AIDS	Fatigue, loss of appetite and weight, immune system impaired so the person becomes prone to many infections
Flaviviruses	Yellow fever, dengue fever	Fever, chills, jaundice, severe muscle pain
Morbillivirus	Measles	Fever, sore throat and eyes, rash; less commonly pneumonia and inflammation of the brain
Rubulavirus	Mumps	Fever, swollen salivary glands; possible inflammation of other organs including ovaries and testes, heart muscle and brain; can cause miscarriage and deafness
Rubivirus	Rubella	Usually mild illness with skin rash and joint pain, but can cause birth defects or death in unborn babies
Adenoviruses	Respiratory infections	Sore throat, coughing, sneezing
Rhinoviruses	Common cold	Sore throat, sneezing, coughing, headache



◀ **Figure 5.4**
Viruses replicating inside a live bacterial cell. New viruses are produced within the infected bacterium. Viruses that infect bacteria are called bacteriophages.

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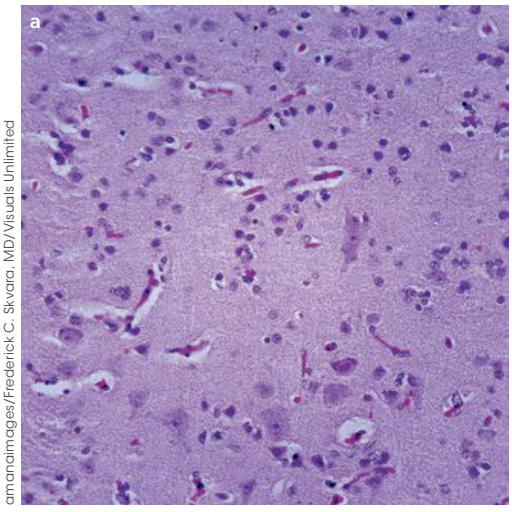
Figure 5.5 ▲
This cow is infected with mad cow disease, or BSE, which is caused by a prion.

Prions

In the United Kingdom in the late 1980s there was an outbreak of mad cow disease, which resulted in the slaughter of more than 4 million cattle. Mad cow disease is also known as bovine spongiform encephalopathy (BSE) and belongs to a group of diseases called transmissible spongiform encephalopathies (TSE). The name comes from 'encephalo' meaning brain, 'pathy' meaning disease and 'spongiform' meaning sponge-like, because the degeneration of brain tissue makes it look like a sponge. These diseases are caused by a small infectious protein called a **prion** (pronounced pree-on) that brings about degeneration of the nervous system with gradual loss of motor coordination, and dementia and paralysis and ultimately death. Other TSEs identified include Creutzfeld-Jakob disease in humans, scrapie in sheep and goats, BSE in cattle (Figure 5.5), and prion diseases in cats and mink.

Prion proteins actually exist in our bodies normally and play important roles in memory, learning and passing signals from cell to cell. They are often found at the surface of neurons. There are two forms: the normal prion protein cellular form, denoted PrP^{C}, and the disease-causing prion protein scrapie form, PrP^{Sc}. When a PrP^{Sc} protein molecule encounters a normal PrP^{C} form, it converts it to the harmful form, which in turn converts other normal forms to harmful forms and so on. When there are sufficient numbers of the pathogenic PrP^{Sc} form, they aggregate to form filaments. These fibres kill brain cells (Figure 5.6), leaving holes in the brain tissue and affecting muscle coordination and brain function as a consequence.

Figure 5.6 ▶
(**a**) Healthy brain tissue (**b**) Brain tissue from a victim of Creutzfeldt-Jakob disease. Note the plaques, and the holes that give the brain tissue a spongy appearance.



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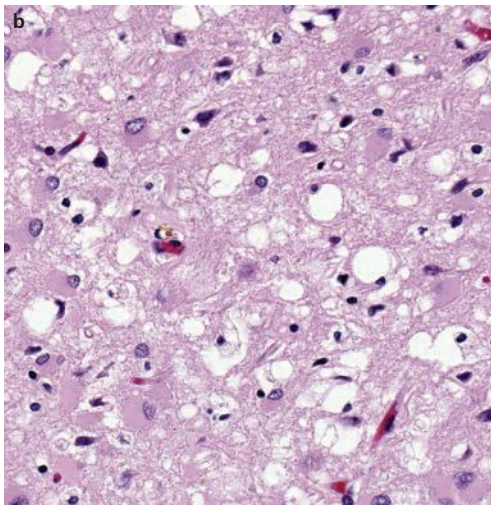

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RECALL

- Viruses are non-cellular pathogens, which are obligate parasites, as they must infect a host cell to reproduce.
- Prions are infectious non-cellular protein pathogens that cause transmissible spongiform encephalopathies.
- The pathogenic prion protein form, PrP^{Sc}, can convert the normal cellular form (PrP^{C}, found in healthy brain tissue) to the PrP^{Sc} form that causes neurodegenerative disease.

RECAP 5.1

1 Define 'obligate parasite'.
2 'All viruses are pathogens.' Justify this statement.
3 Viruses infect only specific host cells. Explain how this specificity comes about.
4 Outline the steps involved for a virus to reproduce.
5 List four diseases that are classified as TSEs.
6 Outline how altered prion proteins affect the brain.

Bacteria

Bacteria may have been the first life form on Earth, and today they are still the most abundant and most diverse group of organisms. Only a relatively small number of bacteria cause disease. There are billions of bacteria living on our skin and in our bodies that are not pathogenic and are often beneficial.

Typically bacteria are 1–10 μm (micrometres) in length and 0.20–2 μm in diameter. Like all cells, bacteria have a plasma membrane that encloses the cytoplasm (Figure 5.7). As they are prokaryotes they have no membrane-bound organelles or nucleus; however, bacteria do possess ribosomes and a single circular chromosome. Most bacteria have a cell wall outside their plasma membrane made of **peptidoglycan** (a protein–carbohydrate compound).

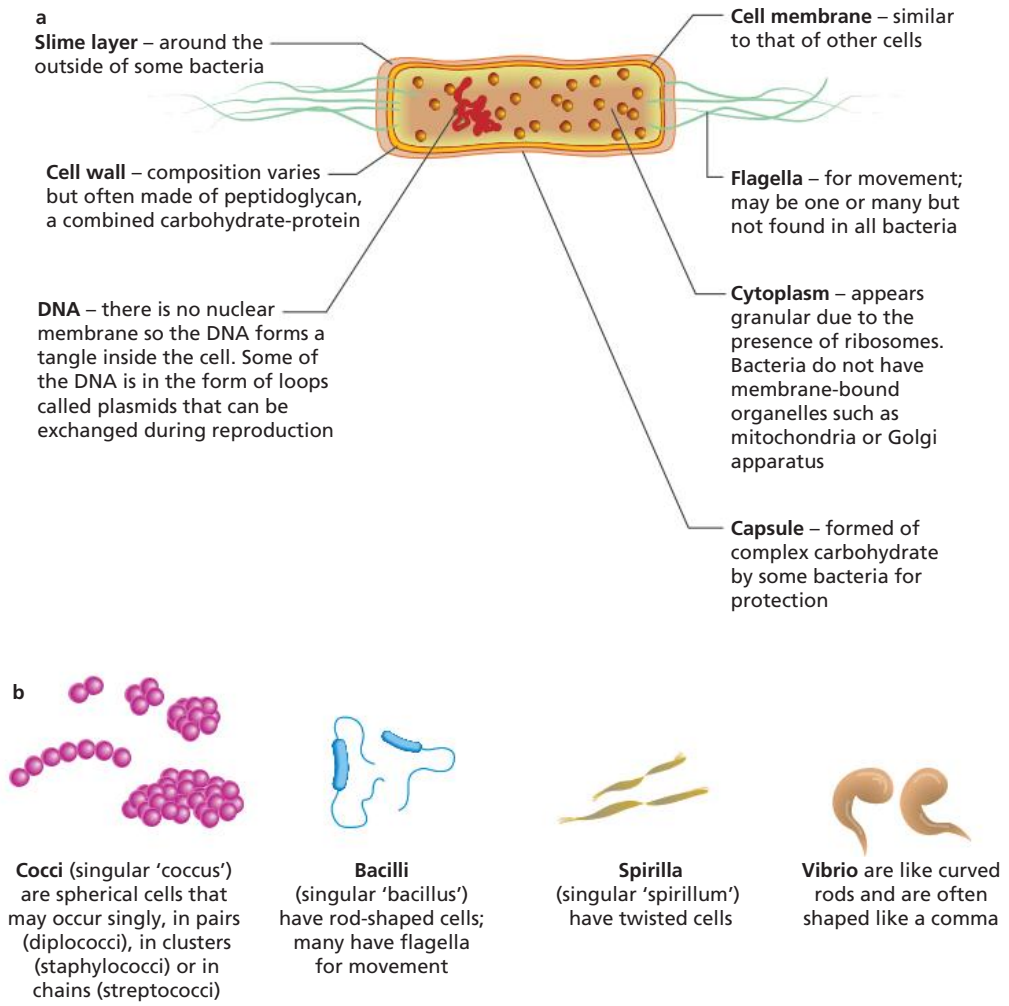

Figure 5.7
(**a**) The structure of a typical bacterial cell (**b**) Types of bacteria, classified according to the cell shape

Some bacteria possess a **flagellum** or multiple flagella, which help them to move about. Another adaptation found only in some species is a slimy **bacterial capsule**, which may be used to help the bacteria stick to surfaces such as teeth or mucous membranes. The capsule is a large, well-organised layer sitting outside the cell wall. It usually increases the virulence of a species (the degree to which it causes disease), as it makes it harder for the body's immune system or antibiotics to attack the inner bacterium.

Many bacteria are capable of forming tough, dormant structures called **endospores**, which are resistant to extreme temperatures, chemicals and drying out. This adaptation helps bacteria resist unfavourable conditions and facilitates dispersal to new hosts.

Some bacteria reproduce by **binary fission** (Figure 5.8), in which one cell splits into two. Others reproduce by budding off spores. These asexual forms of reproduction allow bacteria to reproduce very rapidly in favourable conditions and some species can reproduce every 20 minutes.

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Figure 5.8 ▶ Transmission electron micrograph of *E. coli* dividing into two by binary fission

Bacteria can be transmitted from one host to another in a number of ways: by direct contact, in food and water, and in droplets of moisture in the air. Biting insects, such as ticks and fleas, can also transfer bacteria on their biting parts.

Once inside a host, bacteria divide rapidly. Some damage host tissues directly, while others produce toxins (often their own metabolic wastes) that disrupt the functioning of cells nearby or even further away. For example, toxins produced by diphtheria bacteria in the throat affect tissues throughout the body. Many parts of the bacterial cell are highly pathogenic to the host. External molecules such as **lipopolysaccharides** (a lipid–carbohydrate compound) or peptidoglycans are such examples. These antigens can stimulate immune responses that are sometimes so strong that they damage host cells and tissues. Some bacterial strains interfere with the host's immune system, making the host susceptible to other pathogens.

RECALL

- Bacteria are cellular agents of many different species, some that are beneficial to their hosts and some that are pathogenic.
- Pathogenic bacteria exist in different forms and have adaptations that contribute to their virulence.
- Different species of bacteria have different features, such as toxins or structural features, that cause disease in different ways.

RECAP 5.2

1 State three ways that a bacterial pathogen can harm its host.
2 Define 'binary fission'.
3 Describe the advantages to a bacterium of:
   a having a capsule
   b forming endospores.

Fungi

The fungal world includes large organisms such as mushrooms and toadstools, as well as minute forms that were only revealed with the invention of the microscope. These microscopic fungi include unicellular yeasts and moulds. Fungi are eukaryotes that reproduce using spores and possess cell walls made of **chitin** rather than cellulose. Microscopic fungi are generally larger than bacteria. Some of them are pathogenic, causing disease in a wide range of organisms including plants and animals. As with bacteria, not all fungi cause disease.

Most fungal diseases in animals are external, where they irritate and inflame the skin. A common example is ringworm, a fungal skin infection of rabbits, dogs, cats, horses and humans. Tinea is another fungal skin disease of humans. Symptoms include a rash and itchy skin. Both diseases are easily transmitted from one individual to another. As they grow on the skin, fungi produce spores, and as the infected skin flakes off it carries these spores with them. If the spores come into contact with damaged or broken skin they may cause new fungal infections. Spores are very long lived, an adaptation that improves transmission rates as they can remain alive for years in bedding, furniture and grooming tools, germinating when conditions are suitable.

Protists

Protists are unicellular, eukaryotic organisms. They reproduce both sexually and asexually. Of the 65 000 known species of protists, fewer than 24 species cause diseases in humans, but these few infect hundreds of millions of people each year. To date, we still do not have effective preventatives against many of them and the treatment drugs we have are limited in their effectiveness.

Examples of pathogenic protists include *Giardia lamblia*, *Trypanosoma*, which causes African sleeping sickness, and the protists that cause chlamydia, cryptosporidiosis, amoebic dysentery and malaria.

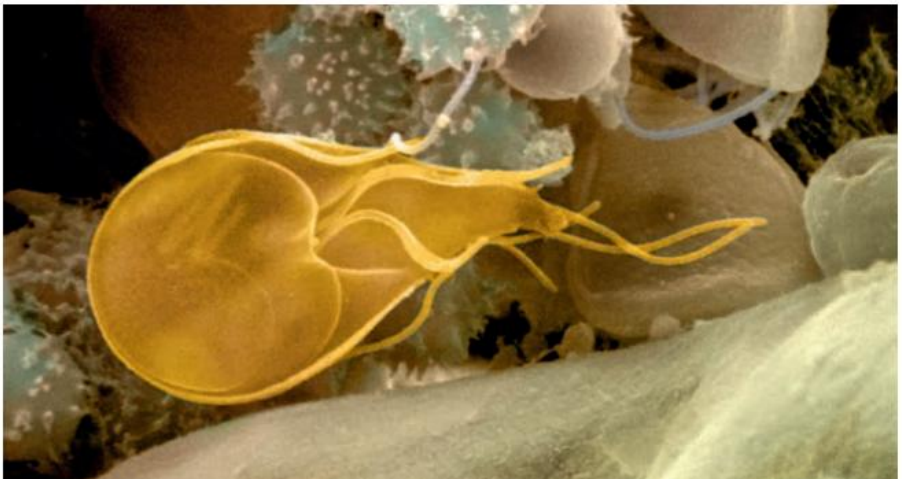

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Figure 5.9 Scanning electron micrograph of *Giardia lamblia* (yellow) in the human small intestine. This flagellated protist contaminates drinking water, causing intestinal upsets.

Malaria has been plaguing the human species for many thousands of years. It is caused by protists from the *Plasmodium* genus that are transmitted to the host by the bite of a female *Anopheles* mosquito. Sporozoite stages of the parasite are injected into the bloodstream as the mosquito feeds (Figure 5.10). After invading liver cells, sporozoites divide repeatedly to produce thousands of merozoites. These then leave the liver and enter the bloodstream, where they infect red blood cells and divide again. The life cycle of *Plasmodium* is completed when these merozoites form male and female gametocytes. At night, an *Anopheles* mosquito may bite an infected human, ingesting the gameocytes. Inside the mosquito, the gametocytes fuse to form zygotes that burrow through the wall of the mosquito stomach and form cysts. Sporozoites form within the cysts and migrate to the salivary glands of the mosquito, ready to infect a new host.

In the host, infected red blood cells eventually rupture, releasing merozoites and their metabolic wastes into the bloodstream. This toxic release induces the classic malarial headaches, chills and a burning fever. These symptoms eventually subside but can recur when more cells are lysed, releasing more merozoites. If left untreated, the host may develop enlargement of the liver and spleen or, in the case of cerebral malaria, brain injury that leads to death in severe cases.

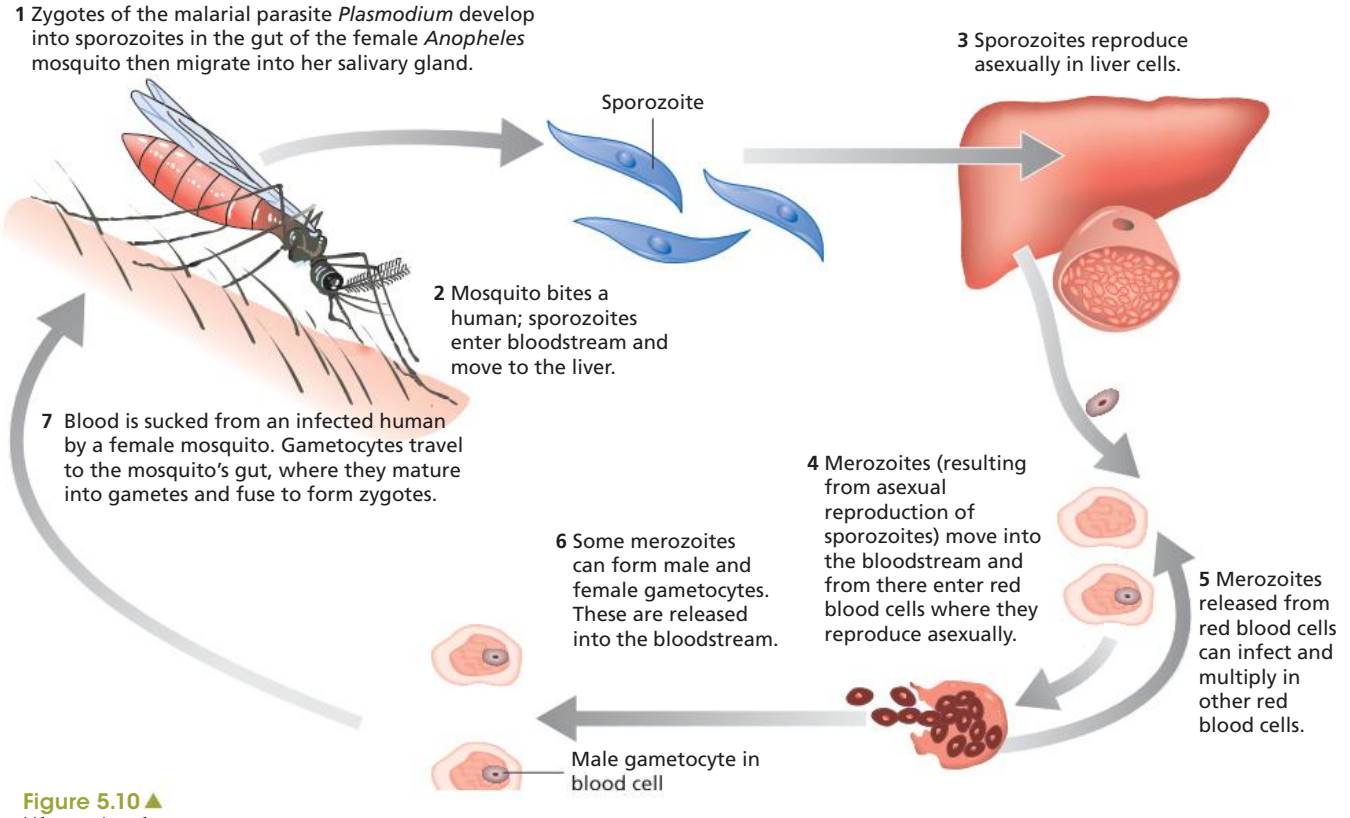

Figure 5.10 ▲ Life cycle of *Plasmodium*, the pathogen that causes malaria

RECALL

- Fungi are eukaryotic organisms that are usually external pathogens but can be internal, particularly in people with suppressed immune systems. Fungi are significant pathogens of plants.
- Fungi reproduce and spread via their spores, which are long lived and very resilient.
- Protists are unicellular eukaryotes, a small number of which are significant pathogens of humans. Examples of protist pathogens are *Giardia*, amoebas, *Trypanosoma* and *Plasmodium* species.
- Malaria is caused by the protist pathogen *Plasmodium*, which has a complex life cycle with multiple intracellular stages that makes it difficult to eradicate.

RECAP 5.3

1 Describe the way in which pathogenic fungi reproduce and spread.
2 Name and describe two fungal diseases.
3 Distinguish between the features of a fungal pathogen and a bacterial pathogen.
4 Distinguish between malaria and *Plasmodium*.

Preventing entry: keeping pathogens out

The most effective way of preventing the colonisation of an organism by pathogens is to keep them out of the body in the first place. This is often called the first line of defence, and comprises various physical, chemical and biological barriers designed to stop the entry of pathogens and other foreign substances. The scales of reptiles (Figure 5.11), the exoskeleton of arthropods such as insects and crustaceans, the shells of eggs and human skin are examples of physical barriers that protect the animal from invasion.

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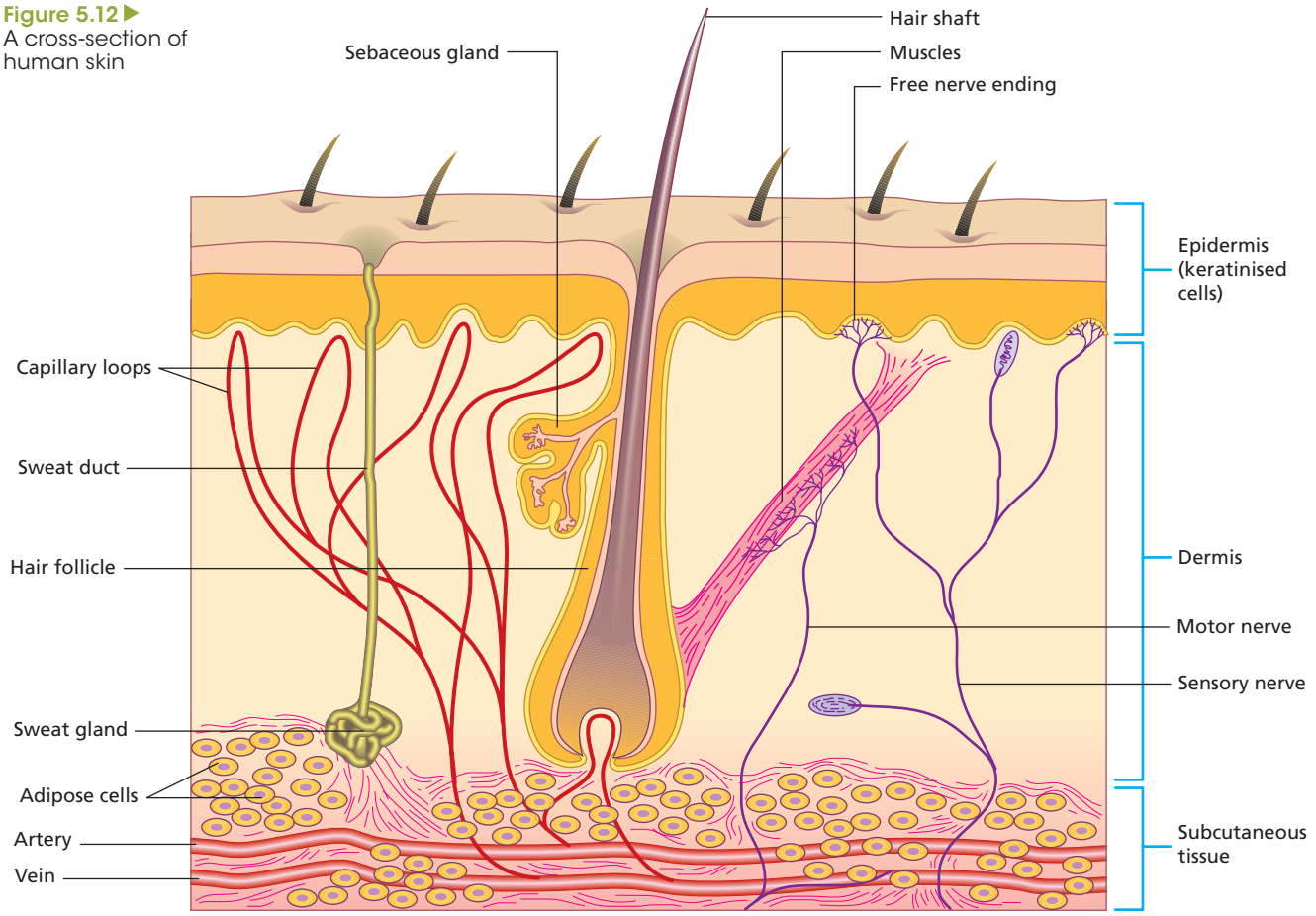
Figure 5.11
Reptiles such as this iguana have tough, scaly skin that is helpful for defence against some pathogens.

The skin: a tough physical barrier

As the largest organ in the human body, the skin acts as a tough physical barrier between the body and the outside world. Like all the inner and outer linings of the body, the skin is made from epithelial cells. After becoming keratinised, a process in which the structural protein keratin is deposited, the epithelial cells form a hard outer layer of the skin that is impervious to water and micro-organisms (Figure 5.12). The importance of the skin as a barrier can be seen in burns victims who lose a large proportion of their skin. If they survive the effects of heat and dehydration, they may still die as a result of multiple infections caused by invading micro-organisms that overwhelm the immune system.

Figure 5.12 ▶
A cross-section of human skin

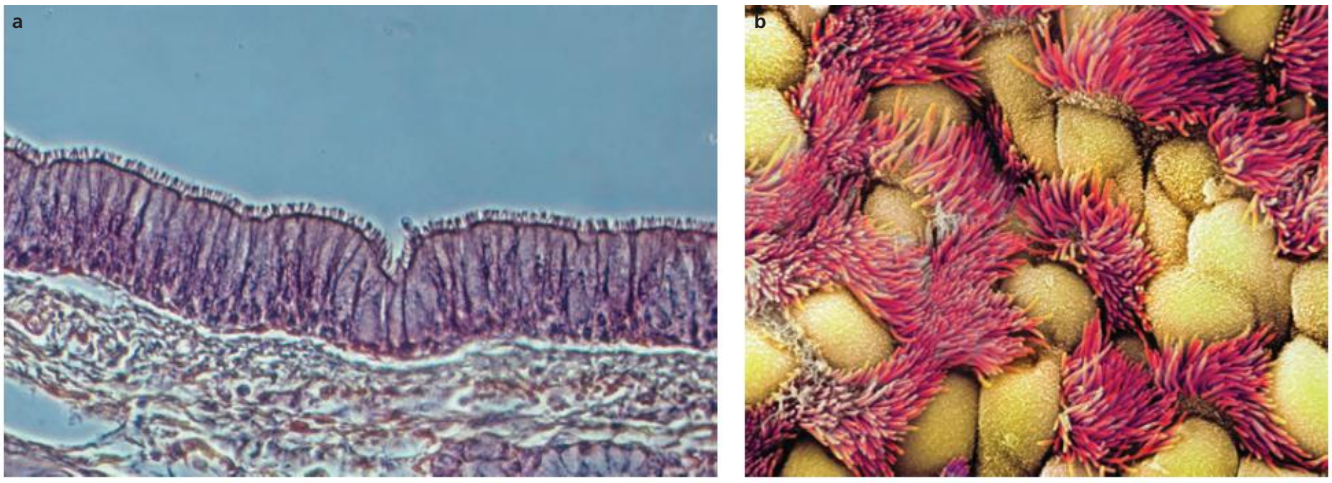


Damaged skin can become an ideal site for infection. In addition to burns, other injuries such as cuts and abrasions provide a potential site for the entry of pathogens. When the skin is cut and blood vessels are damaged, cell fragments in the blood, called **platelets**, are quickly attracted to the site of the wound. As they stick to the damaged tissue, they send out chemical messages. These messages trigger the formation of a web-like mesh of fibrin protein that stabilises the aggregation of platelets and traps red blood cells to form a clot. This plugs the break in the vessel wall, forming a scab that seals the wound and keeps out micro-organisms while the skin is healing.

Flushing out pathogens

As long as it remains unbroken, our tough waterproof skin is an effective barrier against invaders; however the external openings of the respiratory, digestive, excretory and reproductive systems provide ideal entry points into any organism. Various mechanisms exist to physically trap and expel invading micro-organisms and other foreign particles.

The human respiratory, gastrointestinal and reproductive tracts are lined with epithelial cells that secrete mucus, which traps invaders. For this reason they are called **mucous membranes**. Slender hair-like structures called **cilia** line the respiratory tract (Figure 5.13). Their beating pushes mucus up to the throat, where it can be coughed out or swallowed. The effectiveness of mucus flow in clearing infection is illustrated by people with defective mucus secretion or inhibition of ciliary movement. They frequently develop lung infections caused by bacteria colonising the epithelial surfaces.



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▲ **Figure 5.13**
(**a**) A light micrograph of a mammalian trachea, showing, in vertical section, the cilia lining the wall. Cilia help to trap pathogens and move them up and out of the body
(**b**) Scanning electron micrograph showing the cilia of cells lining the respiratory system; the structures between the cilia are mucus-secreting cells.

Coughing and sneezing can help to physically remove potentially harmful micro-organisms and foreign substances from the nasal passages and upper respiratory tract. Passing urine has a flushing effect on micro-organisms that are trying to enter the body via the urethra. Tears also help to flush out micro-organisms, preventing them from settling on the surface of the eyes. In the gut, peristalsis is an important mechanism for keeping both food and infectious agents moving through. Failure of peristalsis is typically accompanied by overgrowth of bacteria within the intestinal lumen.

RECALL

- The skin is a tough physical barrier made from keratinised epithelial cells that can prevent the entry of pathogens.
- When the barrier of the skin is broken, platelets quickly form a plug, or scab, that upholds the barrier until the skin is repaired.
- Mucus traps pathogens that invade mucous membranes, and cilia beat the mucus to a place where it can be expelled. Urine and tears flush out micro-organisms and peristalsis keeps them moving through the gut.

RECAP 5.4

1. Name the three types of barriers that form the first line of defence against disease.
2. List three openings in the skin that can allow the entry of pathogens.
3. Outline the role of mucous membranes.
4. Describe three ways in which the body is able to flush out micro-organisms.
5. Recount the role of platelets in blood clotting.

Chemical defences

The epithelial surfaces of skin and the respiratory, digestive, excretory and reproductive systems are more than just physical barriers to infection. They also produce chemical substances that destroy or inhibit the growth of micro-organisms (Table 5.2). This can be shown by spreading an equal number of typhoid bacteria on a person's skin and on a glass plate. Those on the skin die much more quickly than those on the plate. Skin secretions such as sweat and oil give the skin a pH ranging from 3 to 5, which is acidic enough to prevent colonisation by many pathogenic species. The low pH of the vagina also prevents the overgrowth of infectious agents.

The highly acidic environment of the stomach kills many micro-organisms contained in food and drinks, as do the digestive enzymes secreted by the stomach and small intestine. **Lysozyme**, which is an enzyme contained in tears, saliva and mucus, acts as an antimicrobial agent, breaking down the cell wall of certain types of bacteria and causing them to undergo lysis.

Table 5.2 Summary of human defence barriers

Point of entry for pathogen	Mode of transmission for pathogen	Barriers or mechanisms to prevent entry of pathogen
Skin	Direct contact	Keratinised skin cells, rapid blood clotting, rapid wound healing, **antiseptic** action of acidic secretions
Digestive system	Ingested food and drink	Lysozyme in saliva and mucus, enzymes and strong acids in stomach
Respiratory system	Water droplets in air	Mucus traps dirt and small pathogens; cilia lining trachea move this upwards
Reproductive tract	Sexual contact	Mucus contains acids; moving fluids flush out pathogens
Urinary tract	Bacterial entry into urethra	Urine flushes out pathogens and its acidity inhibits bacterial growth
Sense organs	Direct contact	Ear wax and hairs, eyelashes and nostril hairs trap pathogens; tears wash away pathogens and contain lysozyme
Bloodstream	Pathogens use a vector organism (e.g. a mosquito) to inject themselves directly into the bloodstream	As these pathogens avoid the first lines of defence, they are subject to the host's immune system internally

EXPERIMENT 5.1

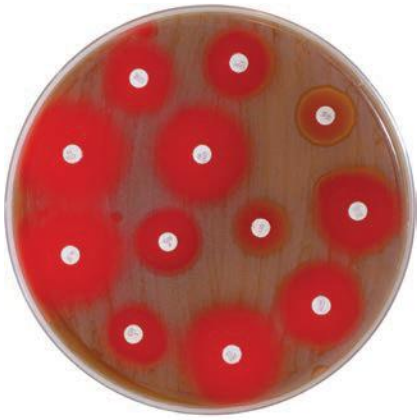
SECOND-HAND DATA ANALYSIS: IS LYSOZYME AN EFFECTIVE BARRIER AGAINST BACTERIA?

This experiment is designed to compare the antibacterial effectiveness of lysozyme from tears with an antiseptic and a disinfectant. The method and results from the experiment are presented for the students to interpret and discuss.

Introduction

With their warmth and moisture, the eyes are an ideal entry point for bacteria into the human body. Tears contain a powerful antibacterial enzyme called lysozyme that is able to destroy pathogens rapidly by lysing them. This experiment uses agar plates, spread with a culture of bacteria, to compare the bactericidal effectiveness of lysozyme with an antiseptic and a **disinfectant**. Bacteria can be grown on agar plates to produce a bacterial 'lawn', a cloudy film of millions of bacteria on the surface of the agar plate. If paper discs containing antibacterial substances are placed on the agar, they produce clear areas, known as the zone of inhibition, where bacteria cannot grow (see Figure 5.14).

▼ **Figure 5.14**
The size of the zones of inhibition indicates the sensitivity of the bacteria to the antibacterial substance on the discs.



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Aim

To compare the antibacterial effectiveness of lysozyme from tears with an antiseptic and a disinfectant

Materials

Class requires:

- broth culture of *Escherichia coli*
- incubator set to 25°C
- lab coats
- safety glasses
- gloves

Each group requires:

- three nutrient agar plates
- one filter paper
- one sterile 5 mL pipette
- forceps
- glass spreader
- onion
- 10 mL each of disinfectant, antiseptic and distilled water
- Bunsen burner
- sticky tape
- ruler
- dilute disinfectant solution, for example bleach

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
While lab strains are usually harmless, bacteria may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Onions contain substances that irritate the eyes and nose.	Ensure onion is held close to eyes, but does not actually come in contact with face or eyes.
Ethanol may be used to sterilise the bench top and is highly flammable.	Be careful to avoid ignition of ethanol liquid or fumes when using the Bunsen burner.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach, ensure the residue is wiped off; ensure lab coat sleeves are rolled down and gloves are worn.

Method

Before beginning this experiment make sure that you record your hypothesis.

Note: To minimise contamination, wipe the bench down with bleach or ethanol before you start.

1. Fold a piece of filter paper into quarters and, using a hole punch, make four filter-paper discs.
2. Label the base of the plate with date and name of group, and then divide the agar into four quarters. Near the edge of the plate, label each of the four quarters: water, lysozyme, antiseptic and disinfectant.
3. Remove 1 mL of *E. coli* culture with the pipette, lift the lid off the labelled plate and transfer the bacteria to the surface of the agar.
4. Either replace the lid quickly and spread the liquid evenly by swirling, or spread the liquid evenly with the sterilised glass spreader, then replace the lid. Leave the plate on the bench for 2 minutes to allow the bacteria to penetrate the agar.

5 Make your eyes water by holding a cut onion near them, and blink to release tears.
6 Sterilise the forceps in the Bunsen burner flame, allow them to cool, then pick up a filter-paper disc and carefully dip it into one of the tears. Quickly touch the edge of the disc to the remains of the folded filter paper to blot, then gently place the disc on the quarter of the agar plate labelled lysozyme.
7 Make small quantities (10 mL) of disinfectant and antiseptic solutions by diluting according to directions on the bottles.
8 Resterilise the forceps and moisten a disc by dipping it into antiseptic and blotting, then gently place the disc on the correctly labelled quarter of the agar plate.
9 Repeat step 8 for disinfectant and for distilled water.
10 Repeat steps 1 to 9 twice more to make a total of three replicates.
11 Seal the plates with sticky tape and incubate at 25°C for 24 hours.
12 Ensure the bench is wiped down with bleach and wash hands thoroughly.
13 The next day, observe the plates for the presence or absence of growth near the discs.
14 Measure the diameter of the zone of inhibition, which is the clear area around each disc. This shows the degree of sensitivity of the bacteria to each substance.

Results

The following table shows the data that one group of students obtained when following the above method. Calculate the mean values and draw a suitable graph to represent the data.

	Diameter of zone of inhibition (mm) for each substance			
Trial	**Tear**	**Antiseptic**	**Disinfectant**	**Water**
1	11	13	15	6
2	16	17	13	8
3	12	12	16	7
Mean				

Analysis of method

1 What steps were taken in the method to ensure there was no cross-contamination?
2 Explain the role of the disc dipped in water.
3 Explain the purpose of the three agar plates.
4 Identify one other risk and how you would manage it.

Analysis of results

Describe the results by stating the order of effectiveness of each of the solutions as bactericides.

Discussion

Compose a discussion of the findings (minimum of 300 words) as per the scientific method.

Biological barriers that prevent the entry of pathogens

During the birth process, a baby acquires various micro-organisms that will become permanently associated with it. The symbiotic micro-organisms that live on and in our bodies, but do not cause disease, are our normal **microflora**. By taking up space and using nutrients, our normal microflora prevent colonisation by other micro-organisms that may be pathogenic. When non-pathogenic bacteria are killed by **antibiotic** treatment, any pathogenic micro-organisms with antibiotic resistance may replace them and cause disease.

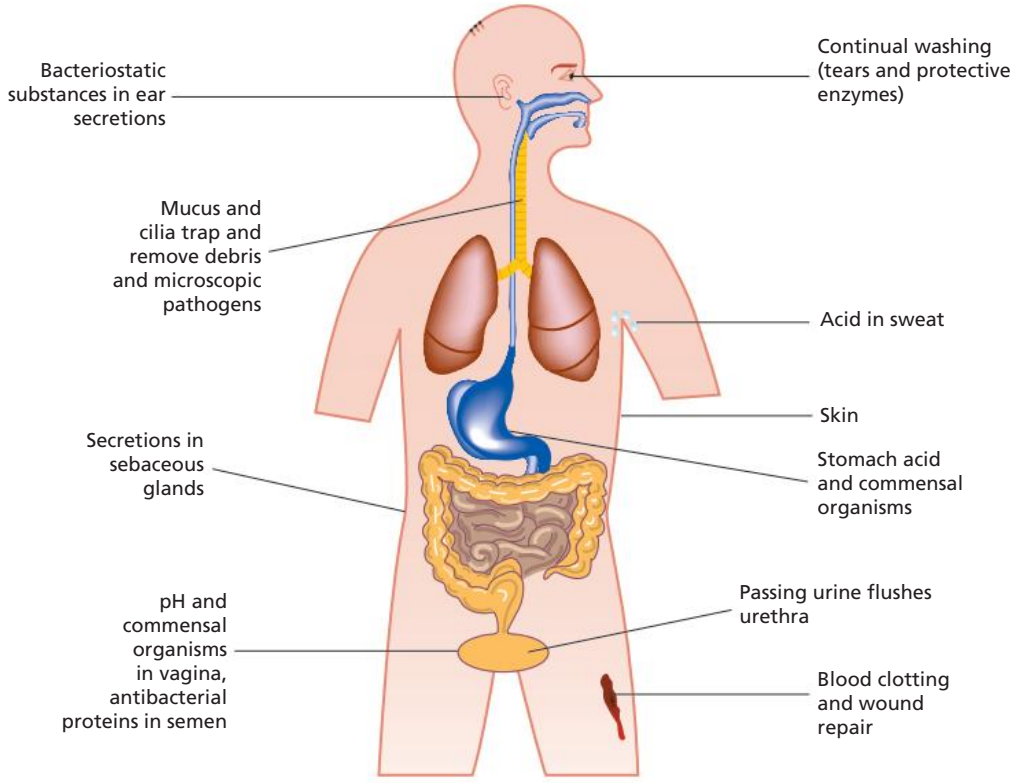


Figure 5.15
Summary of the physical and chemical barriers to pathogenic infections in a human

RECALL

- Structural, chemical and biological features can act as barriers to pathogens as a first line of defence.
- Chemical barriers that prevent pathogen colonisation include low (acidic) pH levels on skin and in the stomach, digestive enzymes produced in the gut, and lysozyme in tears, saliva and mucus.
- Symbiotic organisms form our microflora, which take up space and use nutrients. This prevents colonisation by pathogens.

RECAP 5.5

1. Outline three ways in which chemicals defend the body.
2. State three places in the body where low pH kills pathogens.
3. Outline the role of enzymes in defending the body from pathogens in food.
4. Describe two ways in which tears protect the body from disease.
5. Identify two advantages of hosting our own microflora on our skin.

The immune system

In all organisms, the first line of defence is provided by effective physical, chemical and biological barriers that reduce the chance of pathogens gaining entry. If a pathogen breaches the first line of defence, it will be detected and dealt with by the host's immune system. The immune system is often described as having two components. The initial response to a pathogen is rapid and general and occurs in the same way every time that particular pathogen invades the body. This response is called the **innate immune response**

The adaptive immune system is discussed in detail in Chapter 6.

and is sometimes described as being the second line of defence. The rest of this chapter will mainly discuss the innate immune response. The third line of defence is the **adaptive immune response**, which develops into a potent action against a pathogen and involves the activation of specific immune cells called **lymphocytes**. These cells have the capacity to 'remember' the pathogen and make a faster, stronger response to it the next time it appears.

Distinguishing self from non-self

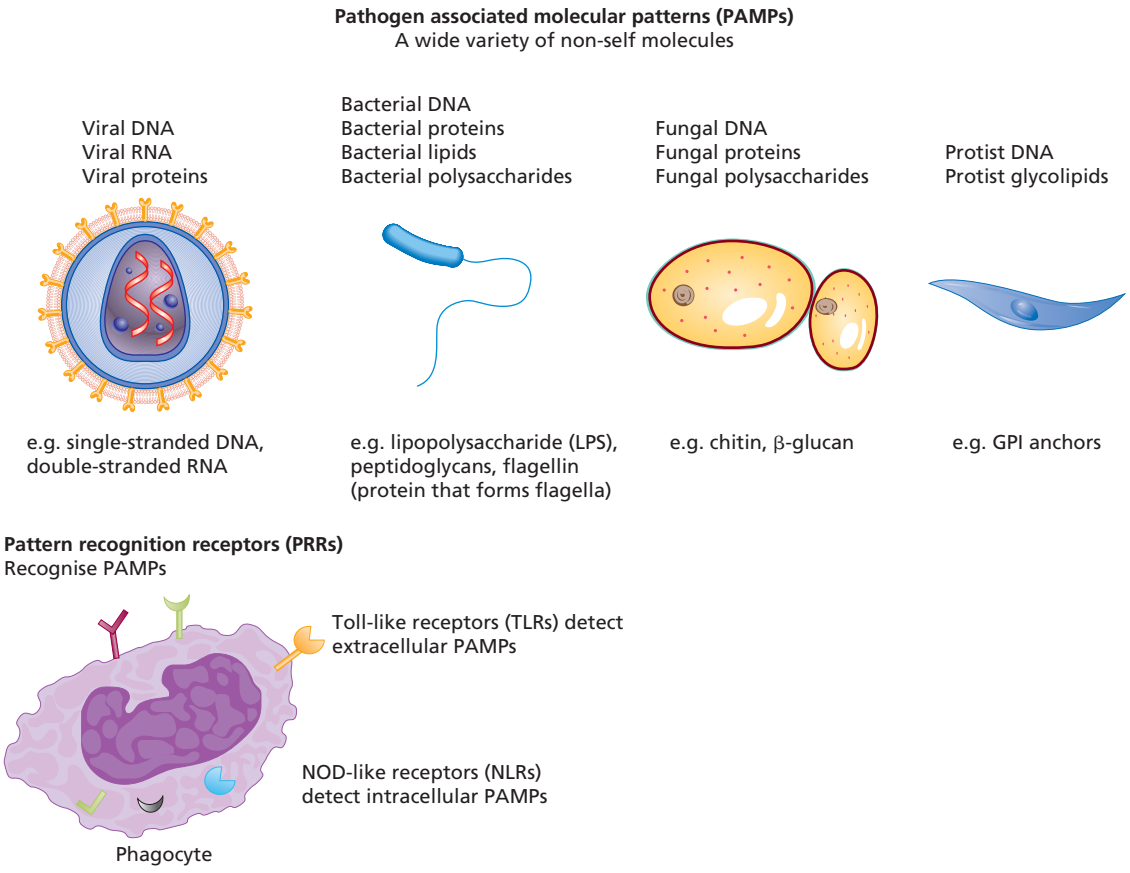
Autoimmune diseases are discussed in more detail in Chapter 6.

Despite effective barriers to infection, some pathogens are able to gain access to their host. Both plants and animals are alerted to this invasion by physical and chemical changes that occur in their cells or tissues, which enable them to distinguish self from non-self. The presence of foreign molecules, either on the outer surface of the invaders or in the toxins and enzymes they secrete, stimulates host immune responses that usually lead to the destruction and removal of the pathogen. It is essential for cells of the immune system to be able to distinguish self from non-self. If this function is compromised, immune responses can be directed against normal components of the body, and this is the basis of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

Cells of the immune system recognise as non-self molecular patterns that are characteristic of microbes but are not found on host cells. These molecules include lipopolysaccharides (LPS), peptidoglycans, chitin, some glycoproteins and particular protein sequences. Immune cells have evolved to recognise these molecules because they are unique to pathogens and have remained largely unchanged during evolution.

These molecules are called **pathogen-associated molecular patterns (PAMPs)**. The receptors that recognise PAMPs are called **pattern recognition receptors (PRRs)** and are found on the surface or in the cytoplasm of a variety of body cells, including white blood cells and epithelial cells. PRRs include **toll-like receptors (TLRs)** in membranes and **NOD-like receptors (NLRs)** in the cytoplasm (Figure 5.16).

Figure 5.16 ▶
PAMPs and PRRs



A particular PRR can recognise a variety of different pathogens if they all display the same molecular pattern (PAMP). For example, the material that makes up bacterial flagella, called flagellin, is a PAMP found in a wide variety of bacteria. This enables the PRR for flagellin to recognise many different types of bacteria as invaders. Similarly, double-stranded RNA is another PAMP that enables detection of a variety of viruses that have their genomes encoded in dsRNA, a form of RNA not naturally found in vertebrates. Although this system of recognition has the advantage of activating a rapid response to invaders, it lacks a high degree of specificity and some pathogens have evolved ways to evade detection by the system.

Other components of the immune system are able to recognise and react specifically to one particular pathogen. Antigens are usually proteins or polysaccharides and their name comes from their role as 'antibody generators'. PAMPs; toxins and enzymes secreted by bacteria and fungi; and substances produced by an organism, such as snake venom, can be antigenic. Recognition of an antigen as non-self occurs when antigens bind to receptors on the cell surface of various immune cells, especially the phagocyte cell type called **macrophages**. This is the first step in the initiation of an immune response.

Even a small section of a molecule, such as a toxin or PAMP, can generate an immune response. A part of an antigen that is recognised by receptors on cells of the immune system is called an **epitope**. As even a small peptide length may be potentially antigenic, most protein antigens have several epitopes, each of which is recognised by a different lymphocyte and induces the production of a different antibody. Each different epitope is a specific chemical group or structure.

The details of the roles of epitopes, antibodies and antigens in the adaptive immune response are discussed in Chapter 6.

RECALL

- The innate and adaptive immune responses rely on being able to tell self from non-self.
- Pathogen-associated molecular patterns are antigens that are recognised by pattern recognition receptors. This can initiate innate and adaptive immune responses.
- Examples of PAMPs include flagellin and double-stranded RNA, which are not usually found in eukaryotic hosts.
- Immune cells such as macrophages begin the immune response after their receptor sites bind with foreign antigens.

RECAP 5.6

1 Give the full name of PAMPs and describe their role in defence.
2 Provide three examples of the types of molecules that act as PAMPs.
3 Outline the role of PRRs and give two examples of these receptors.
4 Describe the importance of an organism being able to distinguish between self and non-self.

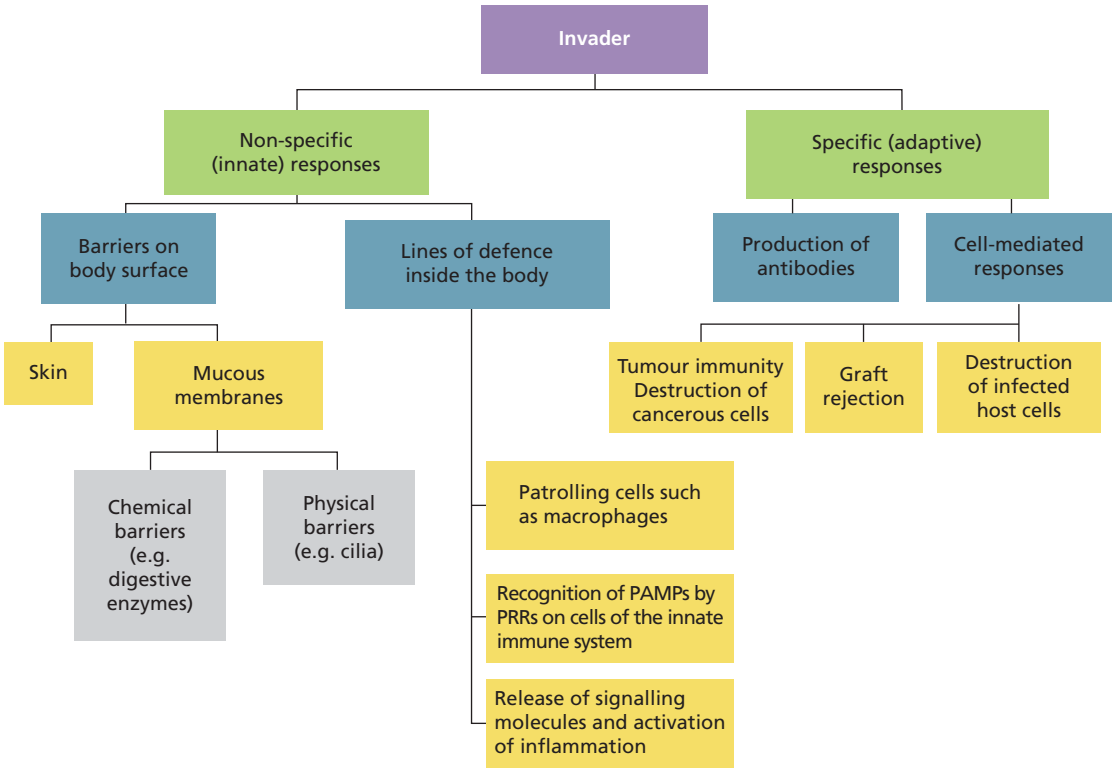
Host immune responses to the presence of invaders

When the immune system detects a pathogen it responds in a variety of ways. Innate immune responses are non-specific and are inborn features of the way the body works. Adaptive immune responses are specific and retain memory acquired from previous experience of that specific pathogen. For example, a person exposed to the rubella virus (Rubivirus) a second time will usually not develop the same symptoms of rubella as they developed the first time they contracted the virus, because of the

immunological memory generated by the adaptive immune response during the first infection.

The innate and adaptive immune responses are closely linked: the detection of danger signals such as PAMPs and subsequent initiation of an innate immune response is required for an adaptive immune response to occur, and molecules produced in an adaptive response can further stimulate an ongoing innate response.

Figure 5.17 ▶
The human body's defence system



Chapter 6 covers the adaptive immune responses of vertebrates in detail.

Adaptive responses

Adaptive responses, which exist only in vertebrates, target pathogens only after they have been specifically identified by particular components of the immune system. They are termed 'adaptive' because they are capable of change in response to the experience of an antigen. These responses are highly specific, as they attack only the organism that stimulated the response. Because of this specificity, the body requires some time to tailor its custom-made response, meaning that adaptive responses are not as rapid as innate responses. Adaptive responses occur in specialised structures in specific tissues and organs of the **lymphatic system**, which is discussed in Chapter 6.

People surviving diseases such as smallpox and the bubonic plague seldom contract the disease again. This feature is called immunological memory, and we say the person has become **immune** to the effects of that pathogen.

Innate responses

Innate immune responses are non-specific. This means that the immune system responds to any invader rapidly and regardless of its type. The responses are not learned and are not influenced by our own past experience, although they have been shaped by evolution, following past experiences of our ancestors. This type of immunity is the natural resistance with which any organism is born. Since the genes and proteins involved in innate immunity are genetically determined, there is individual variation in the effectiveness of

the innate immune responses. This explains the variation in natural resistance, and hence disease susceptibility, between individuals, whether they are people, plants, insects or other organisms.

The innate responses of plants, invertebrates and mammals are remarkably similar. It seems likely that this similarity reflects a common ancestry. In fact, scientists believe that the mechanisms evolved hundreds of millions of years ago in ancient eukaryotes and remain in the same defensive role in their modern descendants. Although the innate responses to infection have ancient origins, they are still highly effective at preventing an infection from being established. It is difficult to know how many infections are repelled in this way because the body defeats the invader before there are any symptoms of disease.

RECALL

- All plants and animals mount innate immune responses to pathogens, but only vertebrates have evolved adaptive immune responses.
- Innate immune responses are non-specific and are inborn features of the body.
- Adaptive immune responses are specific and can retain a 'memory' of previous pathogens.

RECAP 5.7

1 List the major types of organisms that show innate responses to infection.
2 Define 'adaptive responses to infection' and state the type of organism that can generate these responses.
3 Describe one similarity and one difference between innate and adaptive immune responses.

Inflammation

Inflammation is a complex process involving the activities of many different cell types and signalling molecules, which act together to initiate and maintain an immune response until the danger is cleared. The first signs of infection in a cut finger are usually swelling, redness, heat and pain. These are the four physical signs of inflammation. This process occurs in tissues where cells are killed or damaged by physical injury or invading pathogens, and is the key weapon of the innate immune response, although it is also essential for an effective adaptive response.

Initiation of inflammation

Inflammation can be triggered in two main ways. If pathogens enter the body, they are likely to be encountered by white blood cells (macrophages and **dendritic cells**). Small numbers of these cells are strategically distributed in all body tissues, especially in the skin, liver, lungs, kidneys, spleen and lymph nodes, where they act as resident sentinels. They are large cells with a non-granular cytoplasm, and engulf and destroy invaders. Macrophages tend to survive for a long period of time, sometimes for months. They develop from **monocytes** that have left the blood vessels and entered the tissues in response to signs of infection. Body cells at the site of infection can release signalling molecules that attract macrophages, helping to initiate the process.

The role of macrophages as phagocytes of apoptotic debris is discussed in Chapter 4.

As potent activators of other immune cells, macrophages are specialised to switch on inflammation. Macrophages have PRRs that recognise PAMPs on invaders. Macrophages activated in this way produce a number of cell signalling molecules called **cytokines**. Cytokines are important for the orchestration of an appropriate inflammatory response, and include **interferons** and various **interleukins**. Macrophages also double up as cleaners for the body, destroying pathogens and clearing apoptotic cells and damaged tissue.

Dendritic cells have similar functions to macrophages, including **phagocytosis** of antigens and secretion of cytokines that bring about an immune response. However, dendritic cells differ in that they are highly specialised to take up the pathogen at the site of the infection and transfer it to organs of the lymphatic system, where the dendritic cells can present the antigen to lymphocytes, cells of the adaptive immune system. Dendritic cells are therefore important in linking innate immune responses with the adaptive immune system.

Infection by a pathogen is not essential for inflammation. Intracellular molecules, which are usually hidden from the immune system, can be released through injury or tissue damage and can be detected as **damage- or danger-associated molecular patterns (DAMPs)**. These molecules, including adenosine triphosphate (ATP), DNA and some intracellular proteins, can also be detected by PRRs and may trigger **sterile inflammation** (inflammation arising in the absence of infection).

Inflammatory effector cells

Physical damage that ruptures body cells can stimulate **mast cells**, a member of a group of white blood cells called **granulocytes**. Granulocytes are so-called because their cytoplasm is packed with intracellular granules containing powerful defensive chemicals. Mast cells are located in the tissues. When activated, they release their granules, which are loaded with **histamine**, a major stimulus for the inflammatory response. Closely related cells called **basophils** circulate in the blood and also secrete histamines when damaged. Mast cells also secrete heparin, which prevents blood clotting in the injury site, although a clot forms around the outside of the injury site to prevent the spread of the pathogen.

Histamine, together with cytokines released by macrophages, promotes **vasodilation** (widening of blood vessels, especially arterioles) in the damaged region. With increased blood flow comes a battalion of cells and chemicals to fight off an infection. This increased blood flow is the cause of the redness and swelling. As blood also transfers heat, swollen areas often become very warm. Histamine changes the permeability of capillaries in the inflamed area, making it easier for **leukocytes**, blood plasma and blood proteins to squeeze out through the walls and into affected tissue.

Other leukocytes with a role in innate defence are **eosinophils** and **natural killer (NK) cells**, both granulocytes. Eosinophils secrete powerful enzymes that are capable of forming destructive holes in the cells of multicellular pathogens such as blood flukes and parasitic worms. NK cells circulate around the body acting like security guards. They check the credentials of the cells they encounter by looking for suitable surface markers that identify the cell as self. Any suspicious cells, such as those infected with virus or transformed by cancer, are destroyed by an attack on their plasma membranes. This leads to **apoptosis**, ensuring the destruction of both the cell and the virus inside. The importance of NK cells in the initial response to infection by a virus is shown by patients deficient in NK cells being highly susceptible to the early phases of *Herpes* infection. Table 5.3 and Figure 5.18 summarise the actions of cells of the immune system including eosinophils and NK cells. Cells involved in the adaptive immune system, including subsets of lymphocytes, are discussed in more detail in Chapter 6.

Table 5.3 The cellular components of our immune system

Cell	Function
Leukocytes	General term describing white blood cells. Includes all of the cells listed below.
Phagocytes	General term describing white blood cells that engulf and digest foreign pathogens in a process called phagocytosis. Neutrophils, macrophages and dendritic cells are phagocytes.
Granulocytes	General term describing white blood cells that are granulated (neutrophils, basophils and eosinophils). They have a granular cytoplasm due to the presence of secretory vesicles that contain powerful chemicals.
Macrophages	Macrophages are found in the body tissues. They are large phagocytes that become powerful stimulators of an immune response when they engulf a pathogen.
Monocytes	Precursors to macrophages that circulate in the blood. They grow in the bone marrow and are released into the bloodstream. They move through the walls of the capillaries in response to chemical mediators of inflammation released at the site of tissue damage, where they become macrophages.
Neutrophils	Classed as granulocytes and phagocytes and found in the blood and tissues. They rapidly enter sites of inflammation, engulfing the pathogen and then dying in large numbers. Pus contains the debris of dead neutrophils.
Eosinophils	Granulocytes with secretory vesicles that contain powerful enzymes that rupture (lyse) cell walls of pathogens. They are important in combating parasites such as worms and flukes. Their chemicals are toxic to the tissues of both parasites and host.
Mast cells	Granulocytes that release histamines; also involved in healing wounds. Mast cells are concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin.
Basophils	Granulocytes with granulated cytoplasm that secrete chemicals, including histamines. These cells circulate in the blood and play a role in inflammatory and allergic reactions.
Platelets	Cell fragments that assist blood clotting and wound repair, preventing the entry of micro-organisms into the body.
Dendritic cells	Phagocytes with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system.
Lymphocytes	This is a general term for a range of specialised white blood cells that respond to specific antigens in the process of adaptive immunity.
• B lymphocytes	White blood cells that are produced and mature in bone marrow and travel to the spleen and lymph nodes. They produce specialised proteins called antibodies, which bind to specific foreign material, thereby labelling it for engulfment and destruction by other white blood cells such as macrophages.
• Plasma cells	B lymphocytes that are differentiated to secrete very large amounts of specific antibodies. Can live in the bone marrow for many years.
• T lymphocytes	White blood cells that originate in the bone marrow, then travel to the thymus where they mature. T lymphocytes contribute to the immune system in a variety of ways.
– Cytotoxic T cells	T cells that contain lethal chemicals that destroy foreign, infected and altered cells
– Helper T cells	T cells that help or activate other cells of the immune system
– Regulatory T cells	T cells that suppress or turn off the activity of other cells once the threat has passed
• NK cells	Granulated lymphocytes that secrete chemicals that lyse cancer cells and cells that are infected with viruses. They attach to the glycoproteins on the surface of infected cells, and kill them.

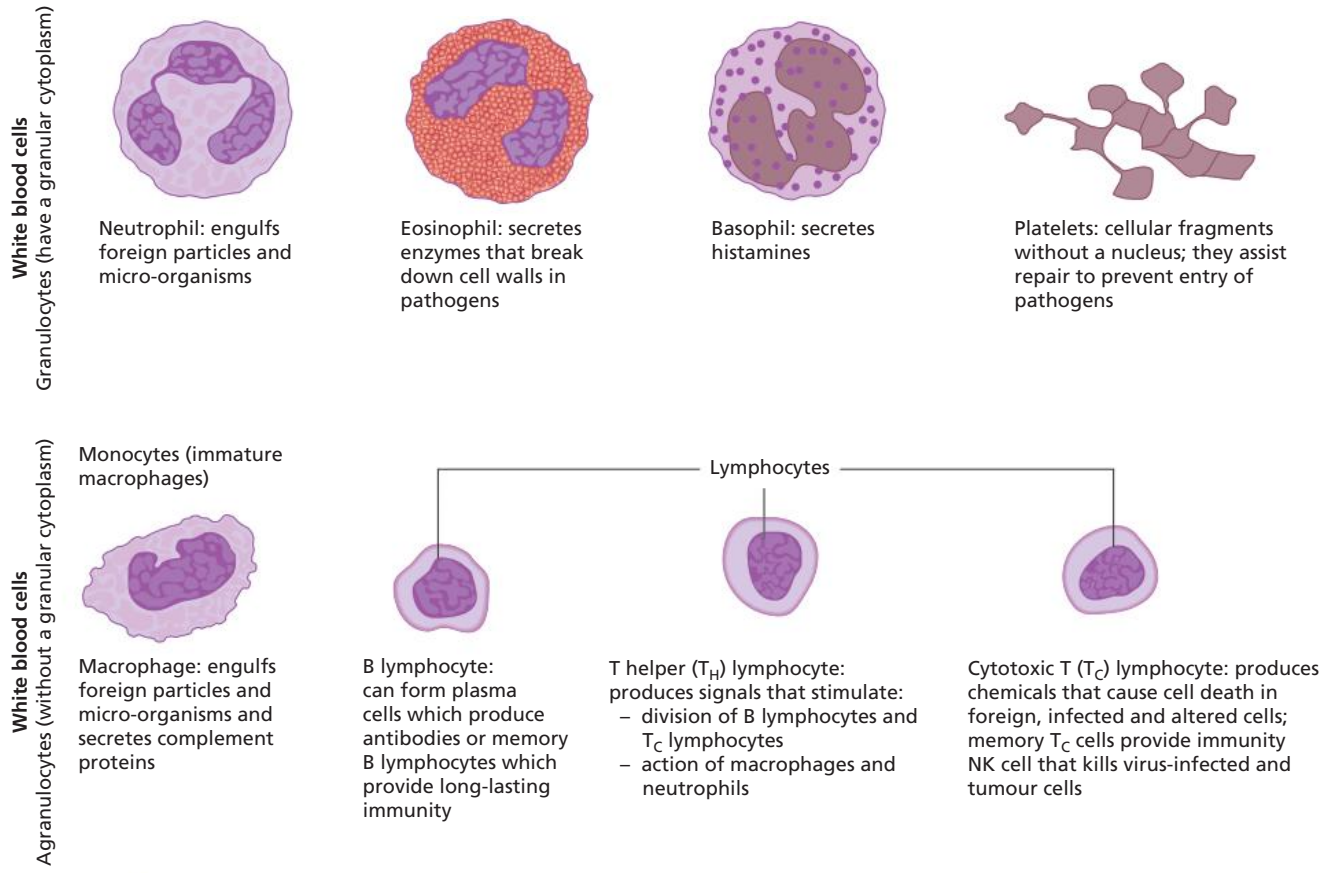


Figure 5.18 ▲
Different types of white blood cells (leukocytes)

RECALL

- Inflammation – characterised by swelling, redness, heat and pain – is the key weapon of the innate immune response, destroying invading pathogens before they can establish an infection.
- Inflammation is initiated when PRRs on macrophages, dendritic cells or other cells detect PAMPs and begin to produce cytokines that bring in and activate other leukocytes.
- Leukocyte recruitment to sites of inflammation is assisted by histamine, which is secreted by mast cells and basophils and increases the permeability of capillaries.

RECAP 5.8

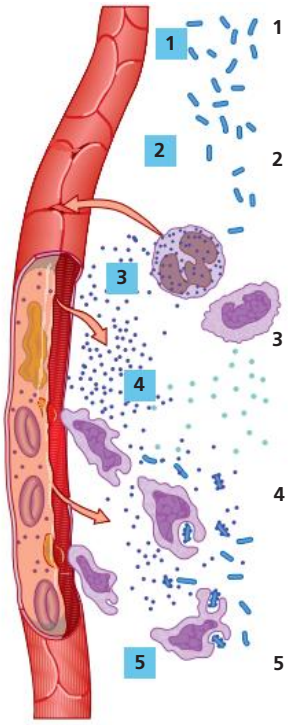
1. List the four physical signs of inflammation.
2. List five examples of molecules that may be ligands for PRRs.
3. Explain the steps that cause your ankle to swell if you sprain it.
4. Describe the mechanism by which macrophages, having detected a danger signal (i.e. a PAMP or DAMP), alert other cells of the immune system of the danger.

Inflammation involves cellular migration

Cells of the immune system travel in a process of directed migration called **chemotaxis**. This is an important way in which cells involved in the inflammatory response are recruited from the blood to sites of infection or tissue damage. During

chemotaxis, white blood cells move towards increasing concentrations of cytokines called **chemokines**, which are any molecules that induce chemotaxis. Chemokines include molecules released by micro-organisms, activated macrophages and other cells. There are many types of chemokines, each with specific receptors expressed by particular target cells. Only cells expressing a particular chemokine receptor will undergo chemotaxis in response to that chemokine.

In response to chemokines, two types of leukocytes, monocytes and **neutrophils**, squeeze out through the capillary walls into the tissues. After monocytes enter the tissues they mature into macrophages. A neutrophil is a type of granulocyte abundant in blood, and has irregular, multi-lobed nuclei and a granular cytoplasm. As neutrophils rarely survive longer than a few days, reinforcements from the blood are constantly required. At least 80 million of these cells are produced by our bone marrow every minute. Like macrophages, neutrophils carry out phagocytosis (and so these cells are sometimes collectively called **phagocytes**). They produce a wide range of cytokines that are capable of inducing chemotaxis, and can trigger further release of histamine by mast cells (Figure 5.19). Neutrophils also produce **defensins**, peptides that act as powerful natural antibiotics with wide antimicrobial activity.



1 Bacteria can invade cells or tissues where they can either kill the cells or release harmful by-products of metabolism.

2 Upon detection of bacterial PAMPs, granulocytes in infected tissues secret histamines, and phagocytes and other body cells secrete cytokines to recruit other immune cells.

3 Histamines can affect the permeability of small blood vessels so that plasma fluid and proteins can seep into surrounding tissue.

4 Cytokines cause phagocytes to move through the leaky blood vessel walls and into tissue where they are activated by detection of PAMPs.

5 Bacteria are engulfed by phagocytic white blood cells.

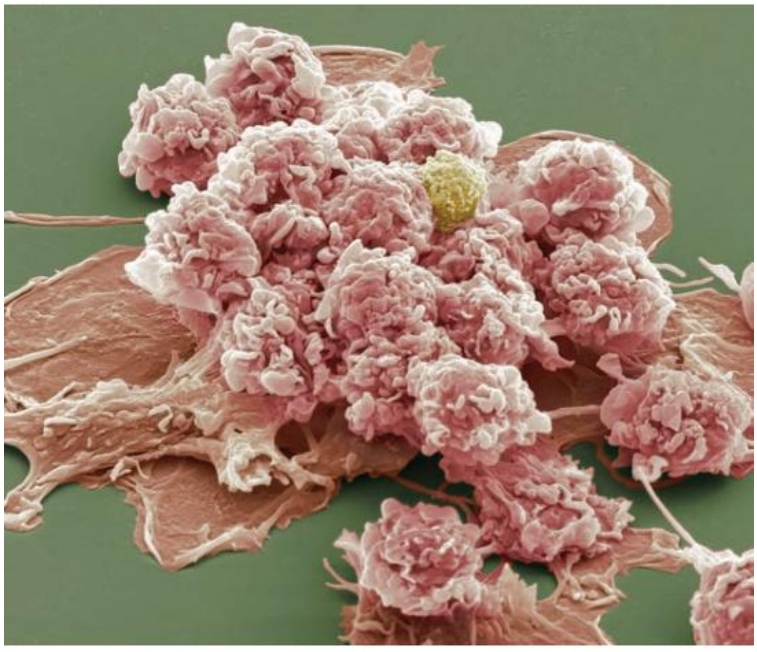
▲ **Figure 5.19** The steps that occur in acute inflammation after invasion by a bacterial pathogen

Phagocytosis

One of the key actions of inflammation is to destroy invading pathogens before they can establish an infection. Macrophages and neutrophils carry out phagocytosis in the same way that an amoeba engulfs food particles (Figure 5.20).

During phagocytosis, the pathogen is engulfed and destroyed within a membrane-bound vesicle called a **phagosome**. A lysosome fuses with the phagosome to form a **phagolysosome**, which becomes increasingly acidic. An array of digestive enzymes and antimicrobial compounds, often including a burst of highly reactive oxygen molecules, helps to destroy the invader.

An important behaviour of neutrophils is their death; as well as having a very short lifespan, they die rapidly after they have phagocytosed a pathogen. This adaptation ensures that pathogens cannot propagate in neutrophils and spread through the body. Pus contains the cellular debris of large numbers of neutrophils that have died in this way. Some pathogens are able to evade death during phagocytosis. In as little as 30 seconds after ingestion, *Rickettsia*, an intracellular parasite of phagocytes, uses an enzyme to free itself into the cytoplasm. *Legionella* bacteria survive by preventing lysosomes from fusing with the phagosome, and pathogenic *Streptococci* cause lysosomal granules to explode and release their lethal contents into the cell, thus killing the phagocyte and releasing the pathogen.



Getty Images/Steve Gschmeissner

▲ **Figure 5.20** Scanning electron micrograph of a macrophage with cytoplasmic extensions. It uses these to engulf the foreign particle (yellow) that it encounters.

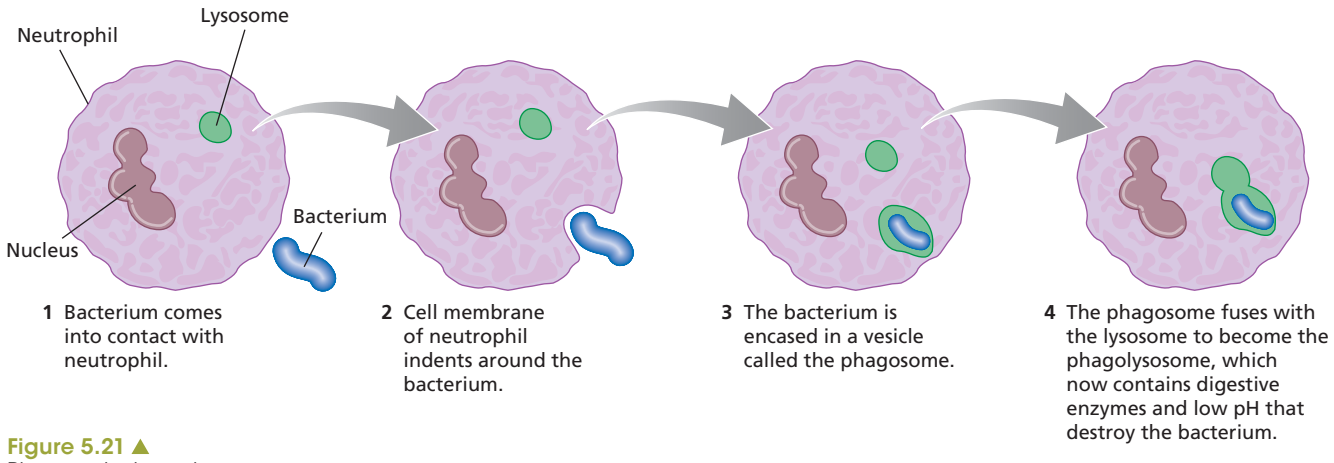


Figure 5.21 ▲
Phagocytosis and lysosomal degradation of a bacterium within a neutrophil

Fever and pain

Fever is another symptom we commonly associate with an infection. What is its role in defending our body against infection? As macrophages attack an invader, the interleukins they secrete send a message to the hypothalamus, the region of the brain that controls body temperature. As a result, the body's temperature is set at a higher point, about 39°C (Figure 5.22). The higher temperature can restrict the functioning and reproduction of many pathogens, allowing other components of our immune system to catch up in the fight against the infection. In addition, some cytokines also cause drowsiness, thus lowering general body activity and allowing more energy to be used for destroying the pathogen and repairing damaged tissue.

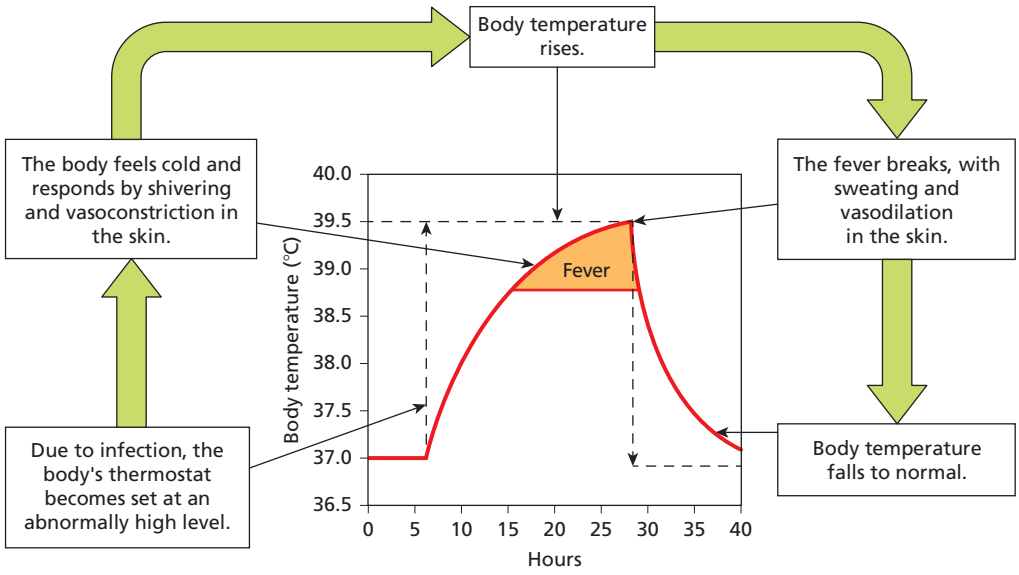


Figure 5.22 ▶
Body temperature during fever

The swelling caused by the action of histamine, which increases leakage of blood plasma and leukocytes into the inflamed area, also causes some localised pain. Feeling pain is an important process, as it reduces voluntary movement in that area, thus limiting further tissue damage and speeding up the repair process.

RECALL

- Chemotaxis is the process of cellular migration towards regions of higher concentrations of chemokines. Each cytokine has a specific receptor which is expressed by particular leukocyte types.

- Neutrophils are a type of phagocyte that is abundant in blood. They readily migrate from capillaries to sites of infection, where they phagocytose pathogens then self-destruct to stop pathogen spread.
- Phagocytosis (internalisation) of a pathogen is followed by fusion of the phagosome to the lysosome. The resulting phagolysosome contains digestive enzymes and an acidic environment to break down the pathogen.
- Fever raises the body temperature to a higher point that restricts pathogen functioning and reproduction. Pain reduces voluntary movement to assist the repair process.

RECAP 5.9

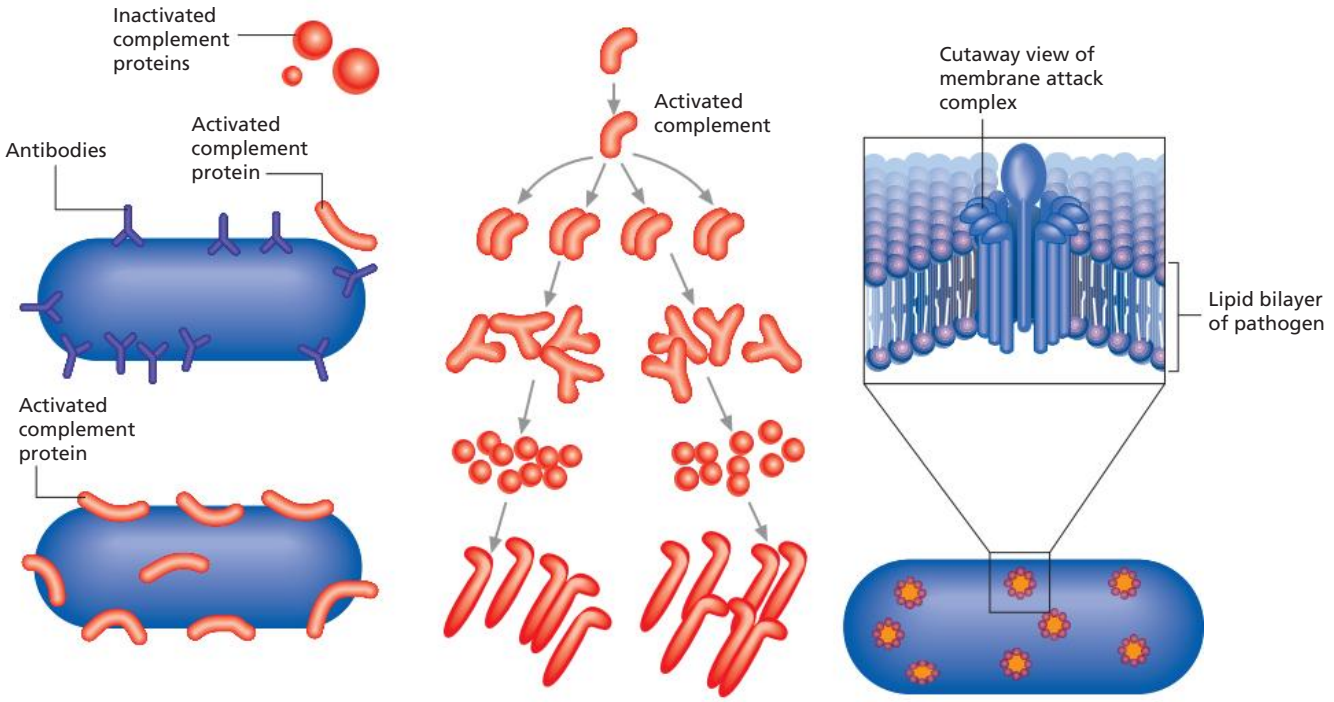
1 Describe the role of phagocytic leukocytes in the immune system.
2 List one similarity and one difference between:
   a macrophages and eosinophils
   b neutrophils and basophils.
3 Some white blood cells are called granulocytes. Describe:
   a one feature that distinguishes them from other white blood cells
   b their function.
4 Suggest why phagocytes, such as macrophages, typically contain large numbers of ribosomes and lysosomes.
5 Describe the role of fever in defending the body against pathogens.
6 Arrange the following points in order, to illustrate the sequence of events that would occur when a macrophage encounters a bacterium.
   - Lysosome fuses with vacuole.
   - Macrophage recognises bacterial surface molecules as non-self.
   - Powerful enzymes digest bacterium.
   - Vacuole forms around bacterium.
   - Macrophage envelops bacterium with its cell membrane.

The role of the complement system

The **complement** system consists of a number of small proteins with an important role in inflammation. Approximately 20 different kinds of complement proteins circulate in the blood as inactive precursors. They are secreted principally by the liver, but also by macrophages, monocytes and other body cells. The inactive precursor proteins become activated when they encounter a foreign body, such as an invading bacterium. Activation of one complement protein has a cascading effect, stimulating the activation of other complement proteins, which then activate other proteins in turn. These proteins produce a range of effects for defending the body (Figure 5.23).

In a process called **opsonisation**, complement proteins bind to the surface of pathogens, in particular yeasts and bacteria, acting as a tag to facilitate their detection and uptake by phagocytes, which have complement receptors on their surface. They induce chemotaxis by creating concentration gradients that attract phagocytes and other white blood cells to the damaged or infected site. Complement activates these phagocytes by increasing their ability to ingest and destroy pathogens. Complement also stimulates mast cells to release mediators such as histamine. An important product of the complement cascade is the membrane attack complex (MAC). The MAC forms pores in the membranes of target cells, disrupting the phospholipid bilayer. With membrane integrity destroyed, osmotic cell lysis and death follows.

With its powerful and potentially dangerous effects, the complement system must be subject to tight regulation. One important safeguard is that activated complement proteins are rapidly inactivated unless they bind to the surface of a pathogen. In addition, MAC-inhibitory proteins exist on all body cells. This protects the body's own cells by inhibiting MAC formation in their membranes. People lacking the gene for the MAC-inhibitory protein suffer episodes of intravascular red blood cell lysis caused by activated complement.



a Activation of complement proteins occurs when they bind to antibodies attached to a pathogen or to the pathogen directly.

b Alternatively, complement proteins can become activated in a cascade of reactions. This contributes to inflammation at the affected site.

c Membrane attack complexes become inserted into the plasma membrane of the pathogen and form large pores, which induce lysis so the pathogen dies.

Figure 5.23 ▲
Activation of complement proteins can (**a**) encourage phagocytosis, (**b**) enhance inflammation, and (**c**) cause pathogens to lyse.

Cytokines such as interferons also play an important role in the adaptive immune response. This will be discussed in Chapter 6.

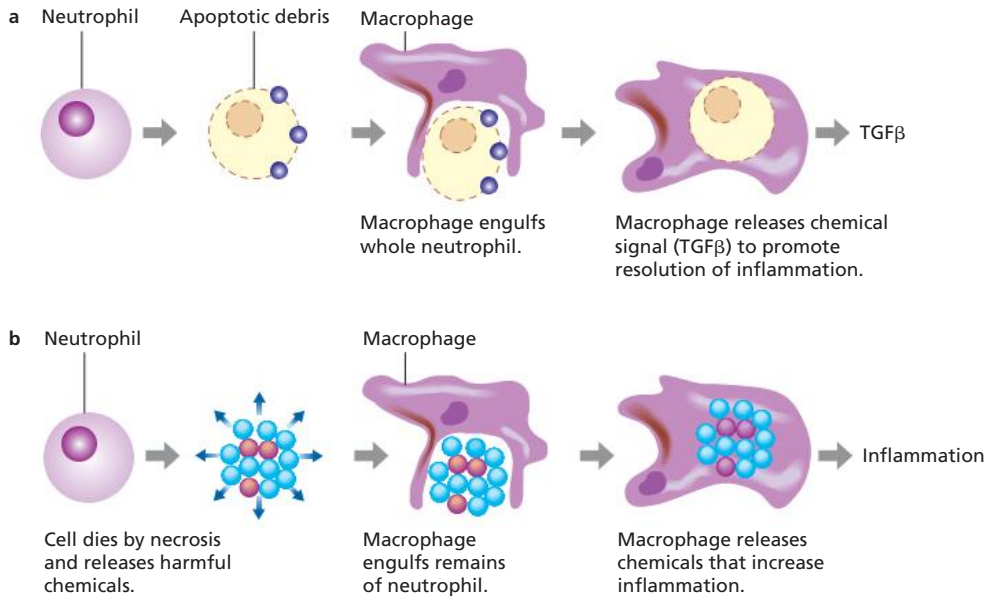
Interferons

Complement proteins and lysozymes are extremely effective against bacteria, but cannot destroy viruses. Instead, some virus-infected cells secrete cytokines called interferons, which induce resistance to viral infection in the surrounding cells. Interferons act as warning signals from the doomed cell and cause changes in the surfaces of the surrounding cells, making it more difficult for a virus to infect them. By targeting multiple points in the viral life cycle, interferons also prevent replication of virus particles inside the host cell.

Because of their particular effects on cells, individual interferons have proved useful in the treatment of a number of diseases including multiple sclerosis, rheumatoid arthritis, hepatitis C and some cancers. Like complement proteins, interferons are non-specific in their effects, being secreted in response to the detection of any viral invader.

Resolving inflammation

It used to be thought that inflammation passively resolves after an infection has died down. That is far from the case. Resolution of inflammation is a highly coordinated, active process that is controlled by several factors, including cytokines and other signalling molecules. These switch off movement of leukocytes to the site of inflammation, reverse vasodilation, and reduce the permeability of fine blood vessels to the level before the inflammation. They also stimulate macrophages to safely dispose of material that has accumulated at the site of infection. This includes neutrophils that have done their job and died, fibrin, and exudate, the fluid that leaks out of blood vessels at the site of inflammation. During phagocytosis, macrophages detect and recognise a molecule on the surface of neutrophils that have died by apoptosis. This triggers the release of cytokines including those that promote the resolution of inflammation. Sometimes the signal for apoptosis fails and the neutrophils die by **necrosis**, unprogrammed cell death that occurs as a result of injury or infection (Figure 5.24). Whereas apoptosis is an immunologically 'silent' form of cell death that does not normally activate the immune system, necrosis stimulates inflammation, and macrophages release cytokines that further enhance inflammation rather than suppressing it.



◀ Figure 5.24
Different signals, from (**a**) apoptosis and (**b**) necrosis, can resolve or prolong inflammation.

Successful resolution of inflammation limits excessive tissue injury and reduces the opportunity for chronic, or long-term, inflammation. Defects in these clearance mechanisms appear to be associated with persistent tissue inflammation and autoimmune responses directed against cellular contents. Repeated bouts of inflammation, as happens with Crohn's disease and rheumatoid arthritis, result in ongoing tissue damage.

Table 5.4 The role of chemicals in the innate immune response

Chemical	Source	Role
Cytokines	Macrophages, neutrophils, other cells of the immune system and body cells	Cell signalling molecules with diverse roles
• Interferons (a type of cytokine)	Virus-infected cells	Induce resistance to viral infection in surrounding cells, enhance phagocytosis of apoptotic cells
• Interleukins (a type of cytokine)	Macrophages, neutrophils and other leukocytes	Many different functions, usually with pro-inflammatory effects Some trigger further release of histamine by mast cells and reset body thermostat to a higher temperature
• Chemokines	Micro-organisms, activated macrophages and other immune cells	Induce chemotaxis to recruit other immune cells from the blood to sites of infection or tissue damage
Complement proteins	Principally liver cells, also macrophages, monocytes and other body cells	Facilitate uptake and destruction of pathogens by phagocytes, attract phagocytes, and form pores in pathogen membranes, leading to lysis
Defensins	Epithelial cells and neutrophils	Powerful natural antibiotics with wide antimicrobial activity
Enzymes (e.g. eosinophil peroxidase and major basic protein)	Eosinophils	Destruction of multicellular pathogens
Histamine	Basophils and mast cells	Dilates local blood vessels, changes permeability of capillaries in inflamed area
Intracellular enzymes and reactive oxygen molecules	Lysosomes	Destroy pathogens after engulfment by phagocytes

RECALL

- The complement system consists of a number of small molecules in the blood that have important roles in inflammation, including opsonisation and deposition of the membrane attack complex (MAC).
- Interferons are produced by virus-infected cells and alert neighbouring cells to the danger, causing changes in gene expression that make them more resistant to viral infection and replication.
- The resolution of inflammation is a highly active process that is necessary to limit and repair tissue damage caused by the inflammatory response.

RECAP 5.10

1. Complement proteins are found in the blood in an inactive form. Identify what activates them.
2. Describe three effects that follow activation of complement.
3. Identify a source of interferon.
4. Describe two beneficial effects of interferons.
5. Discuss what is involved in the resolution of inflammation and why this is important for limiting tissue damage at sites of infection.

Plant defence strategies

Plants are prone to the ravages of parasites, pests and disease. They are subject to attack by a huge array of mites, insects, roundworms, fungi, bacteria and viruses (Figure 5.25), yet the plants usually survive. They too have mechanisms of defence. An understanding of plant defences may help scientists to reduce crop losses caused by plant disease. This research is critical to the wellbeing of all people, because plants are a vital component of our ecosystems. We depend on plants for food, as well as valuable materials including wood and a range of plastics, textiles, medicines, dyes, inks and industrial chemicals.

Figure 5.25 Leaves of (**a**) a healthy tobacco plant, *Nicotiana sylvestris*, and (**b**) a plant infected with tobacco mosaic virus

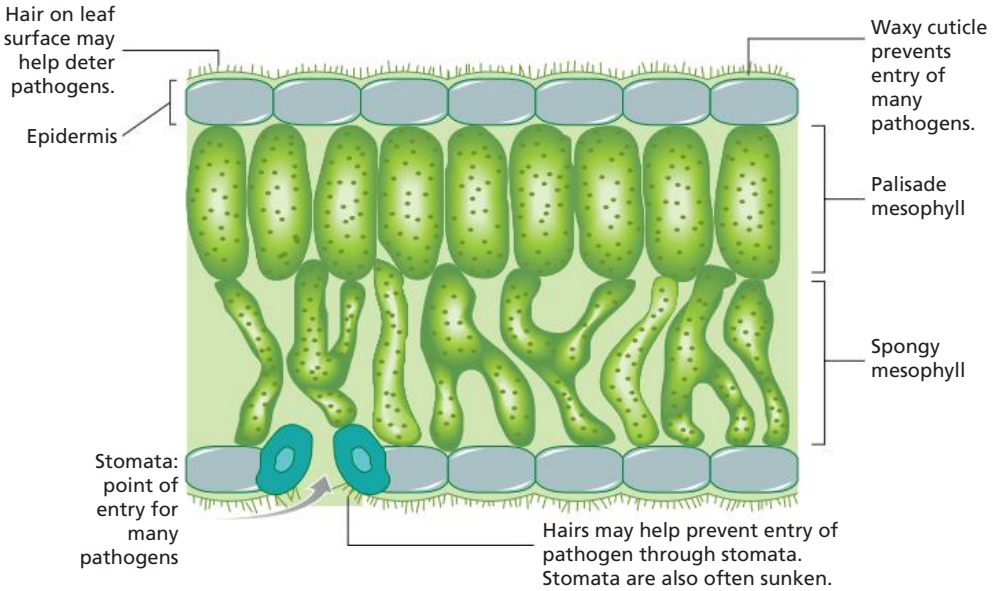
Barriers preventing entry of pathogens

Plants possess physical and chemical barriers to infection that form their first line of defence against invaders. As these are present before contact with the pathogen, they are termed passive defences.

Physical barriers

Physical barriers in plants that prevent invasion by pathogens include the thick bark of stems and a thick and waxy cuticle (leaf surface) (Figure 5.26). Waxy cuticles and

vertically hanging leaves may also prevent the formation of moisture films on leaves. This inhibits bacteria and roundworms that require water for motility, and fungal spores that germinate only in water. Hairs and thorns may also deter vectors of particular pathogens. Stomatal openings are weak spots, as they offer an entry point. Many plants have hairs that guard these openings, or have sunken stomata in the leaf that make access difficult.



◀ **Figure 5.26**
A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

Chemical barriers

The first line of defence in plants includes chemicals that inhibit the growth and development of pathogens. Some of these substances are released into the environment; for example, by asparagus plants and marigolds. The chemicals they secrete into the soil are toxic to nematodes, making them good **companion plants** for tomatoes, which are commonly attacked by these parasitic roundworms. Other substances remain in the plant, ready to stop invaders. These include wetting agents that destroy fungal cell membranes, and phenols and other compounds on leaf surfaces that discourage herbivore feeding and inhibit many potential pathogens.

Humans have come to appreciate and rely on some of the substances that plants produce to defend themselves from attack. Many of our spices, seasonings, condiments and perfumes are made using plant compounds that function as insect toxins and protection against fungal or bacterial attack. The bitter-tasting tannins that give tea its characteristic colour and taste are widespread throughout the plant kingdom and are toxic to insects. Caffeine, an alkaloid found in plants such as coffee, tea and cocoa, is toxic to both insects and fungi, while pyrethrins are esters produced by chrysanthemum plants that act as insect neurotoxins.

As another part of the first line of defence, plants contain small, stable peptides that are able to inhibit the development of fungi, as well as bacteria, viruses and insects. These have been termed plant defensins, and more than 300 defensin-like genes have been identified in plants. Defensins may constitute up to 10% of the total proteins in some types of seeds, and they are also present in the cells of flowers, leaves, fruit, bark and tubers. Their antimicrobial action comes from their ability to reduce membrane permeability and inhibit the action of enzymes and ribosomes. Because of their anti-feeding activity against insects, defensins can also provide a defence against insect-transmitted viruses.

As well as protecting plants from pathogens, defensins appear to be involved in cellular signalling, regulation of growth and heavy metal tolerance. While many defensins accumulate during normal plant development, some are produced in response to attack by pathogens while others are induced by environmental stress such as drought,

salt and cold. As they have been shown to inhibit the human cancer cell cycle, plant defensins may potentially be used to treat human diseases.

Reactions to invasion

Despite the many barriers, pathogens still enter plants. When they do, plants mount a strong defence. These innate immune responses may be very rapid, with host gene expression beginning minutes or even seconds after exposure to pathogens. Unlike animals, plants do not possess a circulatory system that can efficiently transport their defence mechanism. Instead, their responses tend to be more localised, with most cell types retaining the capacity to express a broad range of antimicrobial defences.

Stimulation of plant immune responses by pathogens

Plants recognise invaders in much the same way as the cells of animals. The broad molecular patterns commonly shared by pathogens (such as flagellin, glycoproteins, lipopolysaccharides and chitin) are recognised as PAMPs. These molecules activate PRRs, which in turn trigger a number of actions that attempt to destroy the invaders. DAMPs, such as breakdown products of plant cell walls, can also stimulate the innate defence responses of plants.

Plant responses to the detection of invaders

Once invaders have been detected, part of the chemical attack involves the synthesis of a toxic cocktail of antimicrobial compounds that includes defensins and **phytoalexins**. Since their discovery, more than 350 phytoalexins have been found in more than 100 plant species from 30 families of plants. They are low molecular weight antimicrobial compounds that can puncture cell walls, delay maturation, disrupt metabolism, or prevent reproduction of the pathogen. The effectiveness of these defences is shown by plants in which phytoalexin biosynthesis is inhibited. Such plants show an increased susceptibility to infection and are extensively colonised by pathogens.

Another chemical response to invasion is the production of a burst of highly reactive oxygen molecules, like that produced by neutrophils as discussed earlier. These substances have a direct antimicrobial action, and are also highly toxic to plant cells, causing rapid and localised programmed cell death at the site of pathogen invasion. This has the effect of producing a physical barrier around the area of infection, which acts to keep the pathogen isolated from the rest of the plant. Similarities between programmed cell death in plants and apoptosis in animal cells suggest that cell suicide is an ancient defence response conserved through many millions of years of evolution.

Several other mechanisms serve to help stop the spread of infection through the plant. In an option not open to animals, parts of some plants are treated as disposable, with the plant shedding infected leaves and branches. Wounds caused by a pathogen can be quickly plugged by resin, and cells can thicken and fortify their walls, thereby preventing the spread of pathogens into nearby cells.

After the initial reaction to invasion, plant tissues may become highly resistant to a broad range of pathogens for an extended period of time. This is called **systemic acquired resistance** (SAR). It occurs because a signal travels through the vascular system to activate synthesis of antimicrobial proteins in distant tissues when a pathogen attacks a plant. This brings about a heightened state of readiness in which the whole plant, not just the part initially attacked, is prepared in case of further invasion. SAR is effective against a broad spectrum of plant pathogens, making it fundamentally different from the adaptive immune response of mammals.

Despite these many plant defences, pathogens frequently reduce plant growth, reducing productivity and causing poor yields in crops. Crop breeding programs often select plants with strong innate defences that will provide the individual plant with resistance to one or more important diseases.

RECALL

- Plants have physical and chemical barriers to prevent invasion by pathogens. These include thick bark, waxy cuticles, and production of molecules that are toxic to pathogens and small antimicrobial peptides called defensins.
- Like animals, plants use PRRs to detect PAMPs on invading pathogens. They mount a rapid innate immune response to invasion by pathogens.
- Following invasion by a pathogen, plants attain a state of systemic acquired resistance (SAR) that makes them more prepared to fight invasion by a broad range of pathogens.

RECAP 5.11

1. Provide three reasons that explain why it is important for us to understand plant disease and defence.
2. Describe five physical adaptations that prevent the entry of pathogens into plants.
3. Outline the effects of phytoalexins on invaders.
4. Summarise the chemical defences of plants in a suitable table, including their names and the ways in which they act.
5. Describe the interactions of a companion plant with a crop plant.
6. Describe the mechanism by which defensins kill invading micro-organisms.
7. Draw a flow chart to summarise plant responses to invasion by a pathogen.

Biological knowledge and society: The war against bacterial infections

The discovery of antibiotics

In the 1920s, after witnessing many deaths caused by bacterial infections in the First World War, Alexander Fleming was trying to discover an effective treatment. His discovery of the first antibiotic in 1928 was a lucky accident. He was not very tidy in his lab, and on returning from a holiday he realised he had left a stack of plates containing *Staphylococcus* bacteria in a corner of his lab. While throwing these plates away he noticed something interesting. One plate was contaminated with a fungus. Bacteria were also growing on the plate, except in zones immediately surrounding the fungus. He wondered if the fungus was producing a substance that was killing the bacteria. He tested his theory on a number of bacterial species and found this to be true. He named this substance penicillin.

An Australian scientist named Howard Florey led a scientific team to develop a method for extracting penicillin from cultures of *Penicillium* fungus. Florey's group of scientists tested the effects of penicillin first on saving mice from a lethal dose of streptococci bacteria, then their first human patient was Albert Alexander, who had cut himself on a rose bush. His wound had become infected with bacteria and he was suffering with sepsis. His face was so swollen that his eye was popping out. Florey administered doses of penicillin and the infection started to clear. Sadly, Florey ran out of penicillin. His team collected urine from Albert to extract any penicillin present. It was not enough. Albert's infection returned and he died. After this, Florey successfully treated children requiring smaller penicillin doses. Soon American factories were cultivating *Penicillium* fungus and extracting penicillin. The first antibiotic was now available on the market. It made a huge difference in treating Allies for sepsis in the Second World War. Some historians believe it was pivotal in the Allies winning this war.

How do antibiotics work?

Penicillin is a chemical that blocks the action of an enzyme called glycopeptide transpeptidase. Many bacteria need this enzyme to build cell walls. Penicillin irreversibly binds to the active site of this enzyme so it cannot function and cell walls can no longer be built. Bacteria require a cell wall to stop them from bursting. Without the ability to build a cell wall the bacteria stop dividing and the infection is eradicated. Any

bacteria that use the enzyme glycopeptide transpeptidase to build cell walls will be sensitive to the action of penicillin. Not all bacteria use this enzyme to build their cell walls, and those that do not are not susceptible to penicillin. Scientists began to search for novel antibiotics to treat infections caused by these non-susceptible strains. There are now hundreds of antibiotics available to treat infections by different bacteria. Table 5.5 outlines different classes of antibiotics, the infections they treat and their mechanism of action.

Table 5.5 Some of the classes of antibiotics and their mechanism of action to treat infections

Class of antibiotics	Common infection they treat	Mechanism of action
Aminoglycosides	Tuberculosis, typhus and pneumonia	Bind to ribosome subunits, blocking translation and, hence, protein synthesis
Penicillins	Respiratory, ear, skin, dental and urinary tract infections. Also treat gonorrhoea and syphilis	Stop synthesis of bacterial cell walls
Cephalosporins (5th generation)	Methicillin-resistant *Staphylococcus aureus*, and penicillin-resistant *Streptococcus pneumoniae* infections including bronchitis, tonsillitis, and skin infections	Stop synthesis of bacterial cell walls
Lincosamides	Serious staphylococcal, pneumococcal, and streptococcal infections in penicillin-allergic patients	Bind to 50 S ribosomal subunit, blocking translation and, hence, protein synthesis
Polypeptides	Ear, eye and bladder infections	Interact with membranes, changing permeability (like detergent)
Quinolones	Urinary tract infections, pneumonia, diarrhoea, gonorrhoea	Inhibit enzymes required for DNA replication and transcription
Sulfonamides	Kidney and urinary tract infections, burn infections	Inhibit enzyme required to synthesise folate. Folate is required to synthesise nucleotides.

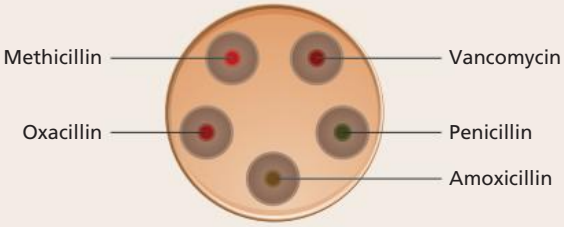
The emerging issue of antibiotic resistance

Bacteria use asexual reproduction to replicate by binary fission. If conditions are right, one bacterium can give rise to a billion cloned bacteria in just ten hours. All essential genes coding for the proteins required for survival are found on a large circular chromosome. Any mutations arising during chromosome replication are passed on to all clones. Other non-essential genes are carried on small circular pieces of DNA called plasmids. These non-essential genes often confer extra, very useful, functions, including antibiotic resistance. Sometimes bacteria from different species swap plasmids through the process of conjugation. One bacterium builds a tube-like structure called a pilus that is used to transfer plasmids to a second bacterium. Through mutations and plasmid transfer, bacteria are constantly evolving, acquiring new traits so they can survive in new environments. The use of antibiotics applies an environmental pressure to bacteria populations. Those that can survive because they contain a gene for antibiotic resistance pass on the gene to their clones when they divide, and their clones are then also resistant. Several mechanisms have evolved in bacteria to provide antibiotic resistance. Some render the antibiotic inactive by changing its shape or properties; others can remove it from the cell, while others change the target site so the antibiotic can no longer bind. This evolutionary process has resulted in many bacteria developing resistance to the action of antibiotics.

Developing recommendations for antibiotic use and discovery

Staphylococcus aureus, commonly known as golden staph, is a species of bacterium that lives on our skin and in our nose. It is generally harmless. However, if it enters our body through punctures in the skin, it can cause a range of infections. Some are mild infections such as boils and school sores. Others are

severe, including toxic shock syndrome, meningitis (brain), osteomyelitis (bone), pneumonia (lungs), septic phlebitis (veins) and endocarditis (heart valves). If the infection gets into the bloodstream it can spread to other organs, causing severe infections known as sepsis. Sepsis can lead to multiple organ failure and death. Figure 5.27 shows the antibiotic sensitivity test for *Staphylococcus aureus*.



Hans Newman, www.microbiologyinpictures.com

▲ **Figure 5.27**
Staphylococcus aureus sensitivity test. The white background of the plate is a 'lawn' of densely growing bacterial cells. Discs soaked in antibiotics are placed on the lawn and rings of bacterial inhibition or death around the discs can give a measure of the susceptibility or resistance of the bacterial strain to the antibiotics.

The hunt for new antibiotics

Why be concerned about the growing threat of antibiotic resistance when all we need to do is discover new antibiotics to take their place? At the peak of antibiotic discovery and development, around 15–20 new antibiotics were released onto the market every ten years. Between 2000 and 2010, only six new antibiotics were released. Currently only a few are in the early stages of development and the success rate at these early stages is very low. Why are companies turning away from the development of new antibiotics? A number of factors influence this, including the cost of developing and testing a new drug, limited lifetime as the drug may only be effective for a limited number of years if resistance develops, doctors are starting to prescribe antibiotics more sparingly, and limited sales because antibiotics are only prescribed for a short duration to overcome the bacterial infection.

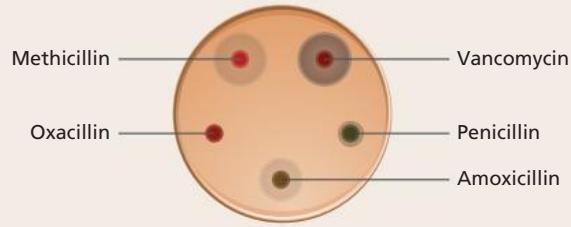
Over to you

1 What is the issue?

2 **a** Use Figure 5.27 to decide which antibiotic(s) would be effective against *Staphylococcus aureus*.

b Case study: Patient X

A patient in a hospital, Patient X, had a catheter inserted into his arm to deliver intravenous fluids. A golden staph infection emerged at the puncture site and the patient was given methicillin to treat the infection. Over time the infection became worse and Patient X's doctor realised she was dealing with a methicillin-resistant strain of *Staphylococcus aureus* (MRSA). She ordered an antibiotic sensitivity test to determine the best antibiotic to treat this MRSA infection. The results of this test are shown in Figure 5.28.



Hans Newman, www.microbiologyinpictures.com

▲ **Figure 5.28**
Staphylococcus aureus sensitivity test for Patient X

Use Figure 5.28 to decide which antibiotic would be most effective against MRSA for treatment of Patient X.

3 Because of the fundamental differences between bacteria and viruses, and the fact that antibiotics target proteins or molecules that are present in bacteria and not viruses, the use of antibiotics for treatment of viral infections is completely ineffective. Yet, patients with cold- or flu-like symptoms, which arise as a result of viral and not bacterial infections, are often keen to visit the doctor for a course of antibiotics. Write a 500 word letter to the editor of a general circulation newspaper explaining how the antibiotic penicillin works, explaining why resistance to antibiotics is occurring, and discussing how indiscriminate prescription of antibiotics to patients without a confirmed bacterial infection affects the patient, the wider community and the health system.

4 Suggest some approaches that governments could take to ensure drug companies increase investment in antibiotics research.

CONCEPT SUMMARY

Pathogens

Pathogen	Description	Diseases
Virus	Non-cellular particle containing nucleic acid surrounded by protein coat. Obligate parasite.	Many including Herpes, HIV, adenovirus, varicella (chickenpox)
Prion	Infectious proteins that convert the normal protein PrP^{C} to the pathogenic form PrP^{Sc}	Transmissible spongiform encephalopathies such as Creutzfeldt-Jakob Disease in humans.
Bacteria	A large group of prokaryotes with many different features that contribute to their pathogenicity	Bacteria cause many different diseases but most bacteria are not pathogenic.
Fungi	A diverse group of organisms including unicellular and multicellular examples	Usually external pathogens of humans. Examples include ringworm and tinea
Protists	Unicellular eukaryotes that are significant parasites of humans	Trypanosomiasis, Chlamydia, amoebic dysentery and malaria

Human defences against infection

Physical barriers

- Skin
- Mucous
- Ear wax
- Tears
- Basement CT
- Blood clotting (platelets)
- Tight junctions

Fever

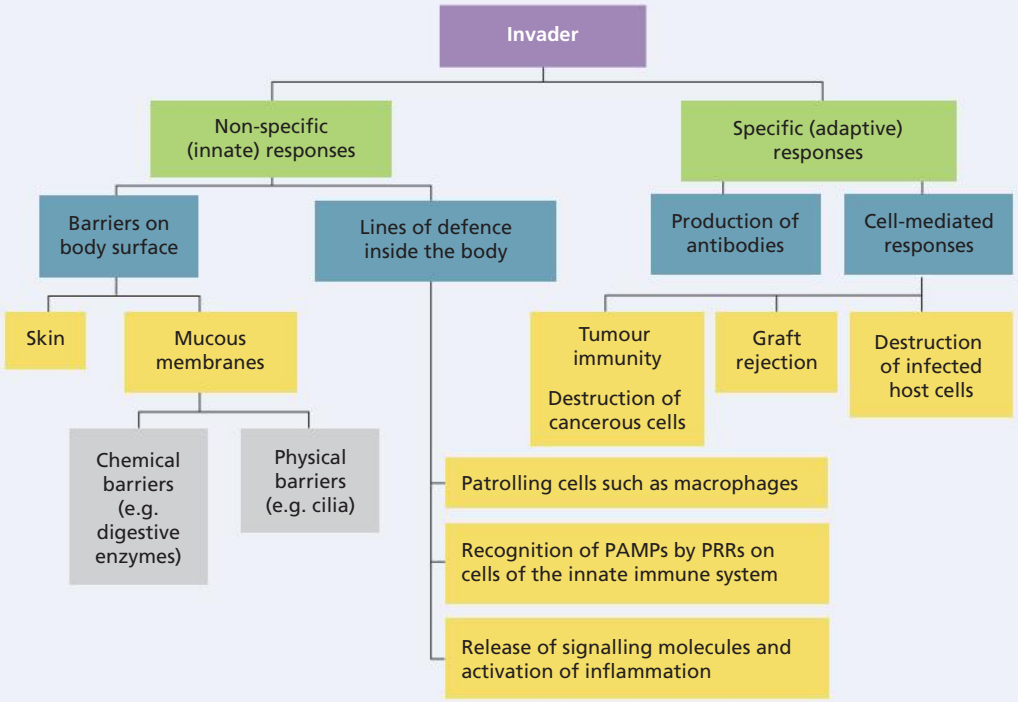
Protective movements

- Fluid flow (tears/urine)
- Blinking
- Peristalsis
- Cilia

Chemical defences

- Low pH of skin, stomach and vagina
- Lysozyme in tears, saliva and mucus
- Antibacterial proteins secreted over mucus membranes

Key in the immune system is distinguishing *self* from *non-self* antigens.



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Steps in the inflammatory response

- Recognition of pattern- or danger-associated molecular patterns (PAMPs or DAMPs) by pattern recognition receptors (e.g. TLRs and NLRs) on tissue-resident dendritic cells or macrophages
- Activated dendritic cells and macrophages release cytokines including interferons, interleukins and chemokines
- Basophils and mast cells in affected tissues release histamine, which promotes vasodilation of local blood vessels.
- Cells of the immune system (particularly neutrophils and monocytes) migrate to the affected tissue, travelling by chemotaxis towards increasing concentrations of chemokines at the site, and leaving the circulation through the leaky blood vessels.
- Monocytes mature to become macrophages, which continue to produce cytokines and chemokines until the infection is cleared, when they then inhibit further inflammation and stimulate repair at the site.
- Neutrophils and macrophages engulf pathogens and apoptotic debris by phagocytosis. These phagocytes can recycle the components or undergo apoptosis themselves as a way to contain the infection. Neutrophils also secrete chemokines and stimulate continued histamine release.
- Dendritic cells carry the antigen to lymph nodes draining the affected tissue, where they can activate lymphocytes to initiate an adaptive response
- Lymphocytes can enter the tissues in an adaptive response, which is specifically targeted to the particular infectious agent, but is a slower response than the innate response.

Additional protection

- In viral infections, interferons are produced by infected cells which act on neighbouring cells to make them more resistant to viral infection and replication.
- Defensins are powerful natural antibiotics with wide antimicrobial activity
- Complement proteins opsonise pathogens to facilitate uptake and destruction by phagocytes, attract phagocytes and form pores (MAC) in pathogen membranes, leading to lysis
- Interleukins have many different functions, usually with pro-inflammatory effects. Some trigger further release of histamine by mast cells and reset the body thermostat to a higher temperature

Plant defences

- Thick bark, waxy cuticles, production of protective molecules including defensins

Plant non-self recognition

- Plants use PRRs to detect PAMPs on pathogens and DAMPs

Plant immune responses

- Plants mount strong, rapid innate responses to infection
- Following infection plants attain a state of systemic acquired resistance

CHAPTER GLOSSARY

adaptive immune response an immune response that is directed against a specific antigen and retains memory of that antigen, responding with a secondary response on subsequent exposure to the same antigen

allergen an antigen that is normally innocuous but in some circumstances causes allergy

allergy an immune response characterised by IgE production

antibiotic a naturally produced or synthetic compound that is toxic to bacteria

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antiseptic a substance that kills or inhibits the growth of micro-organisms on external surfaces of living things

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes, including caspases

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

bacteriophage a virus that infects bacteria

basophil a circulating leukocyte that secretes histamines

binary fission an asexual mode of reproduction in which a unicellular organism grows and then divides into two cells, forming two separate organisms

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) a chemokine

chitin a fibrous substance containing polysaccharides that forms the tough outer shell of insects and fungi

cilia slender hair-like structures projecting from the cell surface that beat against fluid outside the cell

companion plant a plant that is grown together with another plant because one species improves the growth of the other

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

cytokines signalling molecules that coordinate inflammation and immune responses, and that leukocytes use to communicate with one another; includes interleukins and interferons

damage- or danger-associated molecular pattern (DAMP) a body (or plant) component that is released during tissue damage, such as internal cellular components that stimulate innate immune responses

defensin a small antimicrobial peptide secreted by virtually all plants and animals

dendritic cells antigen-presenting cells that phagocytose and present antigens to cells of the adaptive immune system

disinfectant a substance that destroys micro-organisms and their spores but is too strong to be used directly on skin

endospore a tough, dormant structure for asexual reproduction formed inside some bacterial cells; it is resistant to extreme temperatures, chemicals and drying out

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular pathogens

epitope a small part of an antigen that is specifically bound by antigen receptors such as B cell receptors and T cell receptors

fever increased body temperature

flagellum a helical filament that rotates to give bacteria locomotion

granulocyte a leukocyte containing intracellular granules

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

host the organism in which a parasite lives

immune having resistance to infection by a specific pathogen

immune system a complex network of cells, tissues and organs in the body that detects differences between self molecules and foreign organisms, and mounts an immune response that results in formation of memory lymphocytes

inflammation an innate response to infection or damage that causes swelling, pain, heat and redness

innate immune response a response to a pathogen that is not specific and does not generate antibodies or memory lymphocytes

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

interleukin a subset of cytokines that assist with coordination of cells involved in immune responses

leukocyte the general term for white blood cell

lipopolysaccharide a complex bacterially derived molecule containing lipid and polysaccharide components; a PAMP that strongly activates inflammation

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and in removing foreign matter

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting (verb: to lyse)

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

macrophage a large white blood cell in tissues that phagocytoses pathogens; originate as monocytes in circulation

mast cell located in the tissues; when activated, releases granules containing histamine

microflora a community of micro-organisms, including fungi and bacteria, that live in or on another living organism

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

natural killer (NK) cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

necrosis cell death that results from tissue damage or infection; results in inflammation

neutrophil a phagocytic leukocyte found in the blood and tissues

NOD-like receptor (NLR) a type of pattern recognition receptor (PRR); intracellular sensors of PAMPs and DAMPs

non-self a molecule that is not recognised by the immune system as being part of the organism

non-specific describes a response that is the same regardless of the type of pathogen

obligate restricted to a particular way of life

opsonisation a process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

parasite an organism that lives in or on a host organism and derives nutrients from the host, at the host's expense

pathogen an organism foreign to the body that can cause disease

pathogen-associated molecular pattern (PAMP) a broad molecular pattern commonly shared by a number of pathogens and not normally present in the host

pattern recognition receptor (PRR) a receptor that recognises molecular patterns commonly shared by a number of pathogens; includes NOD-like receptors and toll-like receptors

peptidoglycan a polymer in prokaryotic cell walls consisting of interlinked peptide chains and polysaccharides

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis the bulk transport of solids into a cell inside a vesicle

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

phytoalexin a chemical produced by plants under attack

platelet a cell fragment found in the blood involved in blood clotting

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

self describes agents (e.g. cells, organisms, substances) that are recognised by the immune system of an organism as being part of that organism; the immune system tolerates all cells in the body without attacking them because cells carry marker molecules that identify them as self

self-antigen an antigen or molecule that is a normal body component

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

sterile inflammation inflammation resulting from detection of DAMPs released during tissue injury in the absence of infection

systemic acquired resistance a plant's reaction to invasion by a pathogen that leads to long-term resistance to a broad range of pathogens; 'systemic' refers to the whole body

toll-like receptor (TLR) a pattern recognition receptor in membranes that responds to PAMPs and DAMPs

vasodilation dilation (widening) of blood vessels, particularly arterioles

virus an obligate intracellular pathogen able to use the host cell's machinery to replicate itself; usually consists of a nucleic acid surrounded by a protein coat

CHAPTER REVIEW QUESTIONS

Remembering

1. State two important differences between a bacterium and a virus. Give two examples of diseases that are caused by each of these pathogens.
2. State two diseases caused by fungi, and two diseases caused by protists.
3. Identify the groups of organisms that have:
   a. innate immune responses
   b. adaptive immune responses.

4 Identify the type of change (physical or chemical) that PRRs detect in host tissues after a pathogen has entered.
5 Outline the role of the bone marrow in the defence system.
6 Outline the advantage of the keratinisation of skin cells.

Understanding

7 Describe the unique feature of a prion that distinguishes it from other non-cellular infectious agents.
8 Describe two changes to the structure of prion proteins that lead to Creutzfeldt-Jakob disease.
9 *Staphylococcus aureus* causes food poisoning by releasing a heat-stable toxin. Describe the effect of reheating food on the potential of this pathogen to cause food poisoning.
10 *Giardia lamblia* is a waterborne pathogen that can form resistant cysts. It is often found in the bodies of cattle or wild animals and usually leaves them in the form of a cyst in the faeces. Cysts have a tough resistant coat enabling them to survive for long periods in the environment under cool, moist conditions. People become infected if they drink water containing as few as 10 of these cysts. Explain how this adaptation aids the pathogen's:
   a survival
   b transmission
   c entry into a new host.
11 As defensins are peptides, their synthesis requires significant amounts of nitrogen compounds, which are generally a scarce resource for plants. Yet, up to one tenth of some seeds are defensins. Suggest an important advantage to a plant of putting very large quantities of such an 'expensive' chemical into their seeds.
12 Describe the events that follow the activation of complement.
13 Explain what is meant when we say the body can discriminate between 'self' and 'non-self'.

Applying

14 Eating diseased tissue that contains abnormal prion proteins can cause the brain to become infected. Predict a property you would expect prions to have, given that they manage to enter the bloodstream without being digested. Provide evidence to support your answer.
15 Figure 5.29 shows the life cycle of the liver fluke *Fasciola hepatica*.

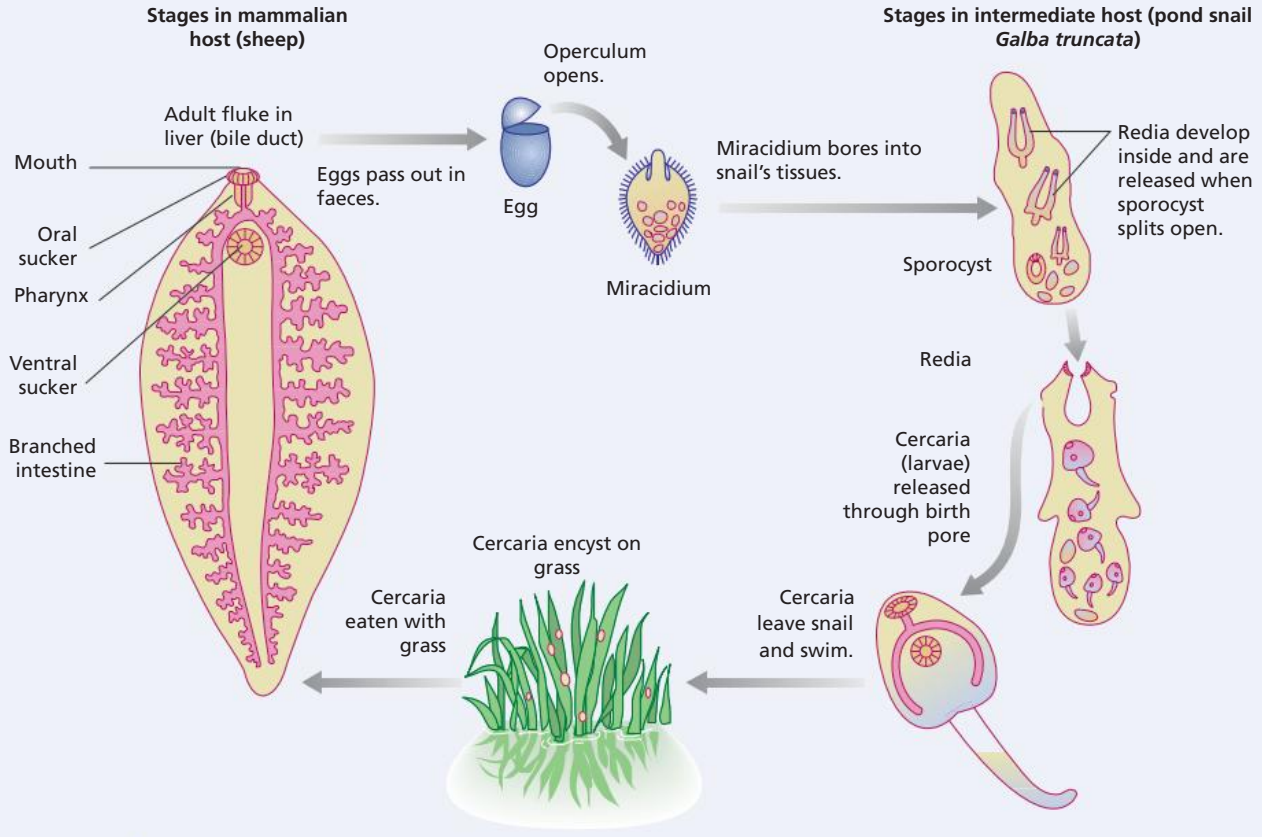


Figure 5.29 ▲
Life cycle of the liver fluke *Fasciola hepatica*

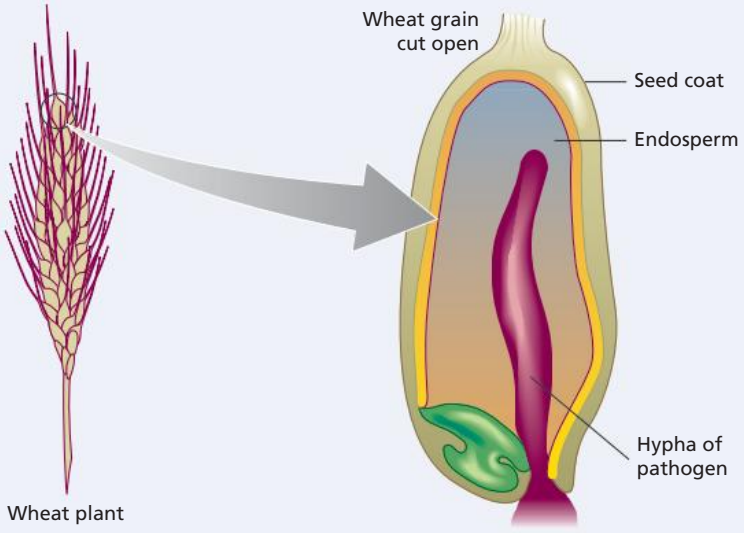
a In what group of organisms would you classify this pathogen?
b Look at the diagram of the adult fluke. Describe two adaptations of the fluke that suit its parasitic way of life.
c How does the liver fluke enter its mammalian host?
d Describe the advantage of the liver fluke in having a free-swimming stage.
e The adult liver fluke is hermaphroditic; that is, it possesses both male and female reproductive organs. Describe the advantage of this feature for the parasite.

16 Cigarette smoke has been shown to decrease ciliary beat frequency and reduce the number of ciliated cells in the airway epithelium. Predict the effect of smoking on the body's defences.

17 Both an infection in your foot and a sprained ankle will cause the local area to swell, become red and painful, throb and feel hot. Explain why these two different events lead to the same response by the body.

Analysing

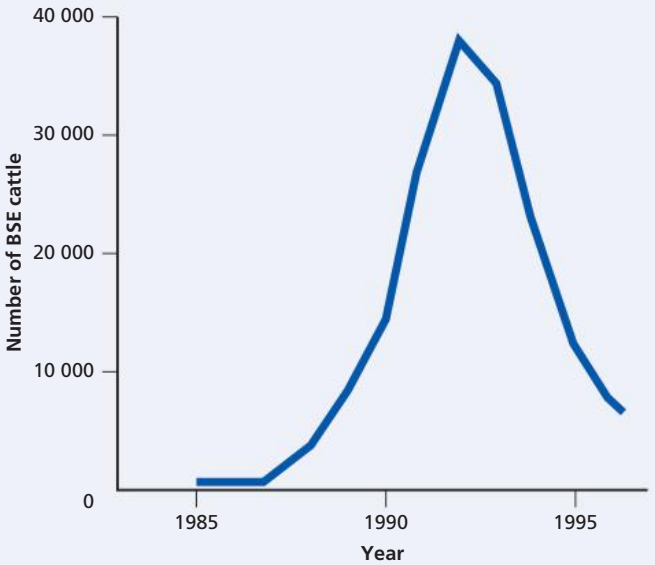
18 Figure 5.30 shows infection by a fungus responsible for rust in wheat and rye.
a Identify the part of the plant the fungus probably gains access through.
b Describe the damage it causes to its host.
c Predict, with reasons, whether antibiotics would be useful in controlling its spread. Design a controlled experiment to test your hypothesis.
d Describe two methods that could be employed to control the fungus.



▲ **Figure 5.30**
Wheat plant and magnified cut wheat grain, showing infection by a pathogen

19 Figure 5.31 shows the number of cattle infected with the prion causing BSE (also known as mad cow disease) in Britain for the years 1985–95. Since 1992, feedstuff containing animal neurological tissue, such as brain and spinal cord, has been banned.
a Describe the trend in numbers of BSE-infected cattle in Britain from 1985 to 1995.
b Describe the action of a prion when it causes disease.
c Suggest a reason for the decline in the incidence of BSE since 1992.
d There are fears that the infectious agent causing BSE is now infecting humans, causing Creutzfeld-Jakob disease. Describe measures that could reduce the transmission of this disease.

20 Consider the stages in the replication of a virus. Imagine you are a chemist trying to find antiviral medicines. Describe two points at which a virus would be susceptible to antiviral chemical therapies.



▲ **Figure 5.31**
Number of cattle infected with BSE from 1985 to 1995

Evaluating

21 Given the increase in antibiotic resistance in recent years, discuss whether we should restrict the use of antibiotics to only those people with a life-threatening illness.

Creating

22 It has been said that we underestimate the effectiveness of our innate immune system because we do not usually become aware of the potential infections that it prevents. Design an investigation using mice and an immunosuppressant to test this idea.

23 Your task is to design an experiment to distinguish between three samples of bacteria. You will do this by using fluorescent labelling techniques to observe macrophages engulfing the bacteria in a Petri dish.

When macrophages engulf bacteria, the phagosomes fuse with lysosomes to form phagolysosomes, where the bacteria are killed and broken down. One sample, the control, contains normal bacteria. The two other samples contain bacteria that can evade immune destruction, one by surviving inside the phagosome and the other by escaping into the cytoplasm from the phagosome.

Design an investigation to distinguish between these three samples, ensuring that you describe the results that you would expect from each sample of bacteria. If you use macrophages with red fluorescent labelled phagolysosomes and bacteria with green fluorescent proteins in their cytoplasm, you can see:

- disappearance of green bacteria as they are digested in the phagolysosome
- green bacteria in the cytoplasm when they escape the phagolysosome
- green bacteria remaining in the red fluorescently labelled phagolysosomes.

24 Prepare a flow chart to summarise the steps involved in inflammation.

Reflecting

25 Consider the observation that plant defensins have been shown to inhibit the growth of human cancer cells. Reflect on why this might be.

CHAPTER 6
IMMUNITY WITH MEMORY

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

KEY KNOWLEDGE

Responding to antigens

- an antigen as a unique molecule or part of a molecule that initiates an immune response including the distinction between non-self antigens, self-antigens and allergens
- the role of the lymphatic system in the immune response including the role of secondary lymphoid tissue (with specific reference to lymph nodes) as the site of antigen recognition by lymphocytes, and as a transport system for antigen presenting cells including dendritic cells
- the characteristics and roles of components of the adaptive (specific) immune response including the actions of B lymphocytes and their antibodies (including antibody structure) in humoral immunity, and the actions of T helper and T cytotoxic cells in cell-mediated immunity.

Immunity

- the difference between natural and artificial immunity, and active and passive strategies for acquiring immunity
- vaccination programs and their role in maintaining herd immunity for a particular disease in the human population
- the deficiencies and malfunctions of the immune system as a cause of human diseases including autoimmune diseases (illustrated by multiple sclerosis), immune deficiency diseases (illustrated by HIV) and allergic reactions (illustrated by reactions to pollen)
- the use of monoclonal antibodies in treating cancer.

Biological knowledge and society

- strategies that deal with the emergence of new diseases in a globally connected world, including the distinction between epidemics and pandemics, the use of scientific knowledge to identify the pathogen, and the types of treatments
- the concept of rational drug design in terms of the complementary nature (shape and charge) of small molecules that are designed to bind tightly to target biomolecules (limited to enzymes) resulting in the enzyme's inhibition and giving rise to a consequential therapeutic benefit, as illustrated by the Australian development of the antiviral drug Relenza as a neuraminidase inhibitor
- the use of chemical agents against pathogens including the distinction between antibiotics and antiviral drugs with reference to their mode of action and biological effectiveness.

KEY SCIENCE SKILLS

Communicate and explain scientific ideas

- use clear, coherent and concise expression

Courtesy of Baylor College of Medicine

Figure 6.1 ▶
David Vetter was raised from birth in a sterile isolator unit designed by NASA to protect him from pathogens.

Spacesuits protect astronauts from the extremes of outer space, allowing them to survive in an environment too hostile for human existence. Filled with potential pathogens, Earth's atmosphere is also hostile, but the constant efforts of our immune system allow us to survive.

David Vetter was born in 1971 without an adaptive immune system, affected by a condition known as severe combined immunodeficiency (SCID). Without an immune system, his risk of catching a fatal infection was so high that he was raised from birth in a sterile isolator unit, or bubble, designed by NASA to keep all pathogens out. Not even his family was allowed into the bubble. At 5 years of age, David was able to walk outside for the first time using a special suit, also designed by NASA and based on their spacesuits.

Today, medical knowledge about SCID has improved and children with the disorder no longer have to be raised in such isolation. This rare disease demonstrates the critical role that the cells of the adaptive immune system, B and T lymphocytes, play in fighting pathogens.

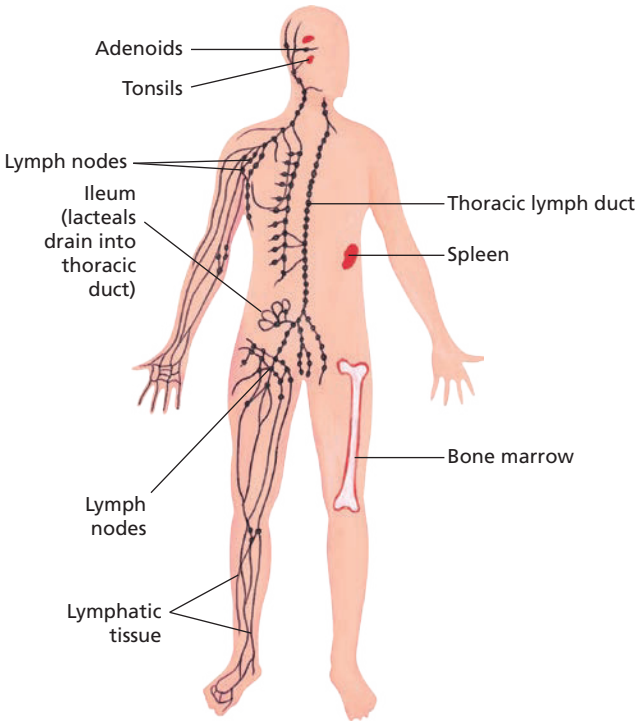
Cells, tissues and organs of the immune system

All cells of the immune system, including B and T lymphocytes of the adaptive immune system and the many cell types of the innate immune system, are produced in the bone marrow from blood stem cells. Collectively they are called white blood cells, or leukocytes. Some reside in the lymphoid organs while others circulate in the blood and lymph, acting like a mobile surveillance squad. As they move around the body, they detect invading pathogens and initiate an immune response to clear the infection. Inflammation at the site of the infection triggers the activation of an adaptive immune response, which occurs in specialised organs and tissues of the lymphatic system.

See Chapter 5 for more information about the innate immune system.

The lymphatic system

Under normal circumstances, when inflammation is not occurring, blood capillaries allow a small amount of plasma to leak out through their walls. This fluid that surrounds the body cells is called tissue fluid, or **interstitial fluid**. Although most tissue fluid returns to the capillaries, some, now called **lymph**, is drained away by lymph vessels of the **lymphatic system**. The lymphatic system (Figure 6.2) consists of lymphoid organs (see Table 6.1) and transport vessels that carry lymph between these organs and back to the blood. Blood plasma, tissue fluid and lymph are essentially the same fluid, named according to where it is found.

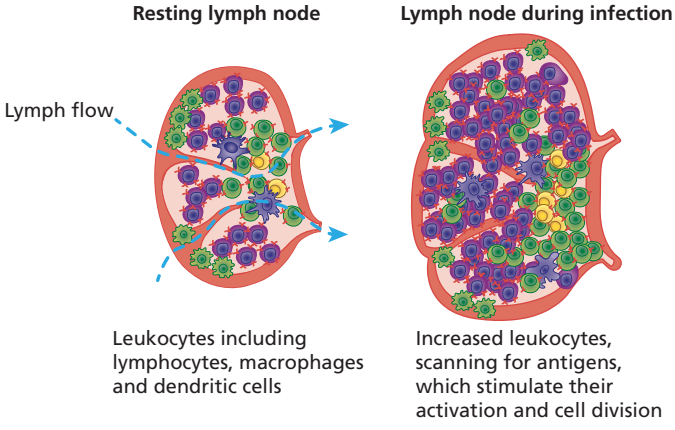


▲ **Figure 6.2**
The location of organs and tissues involved in the lymphatic system in the human body

Lymph nodes

Unlike the blood circulatory system, the lymphatic system has no pump. It relies on muscle contraction and one-way valves to move the lymph away from the tissues towards the heart. Lymph vessels coming from the tissues eventually join up with the circulatory system by draining into the bloodstream near the neck. Lying along the course of lymphatic vessels, sometimes in chains, are **lymph nodes**. Humans have approximately 500–600 lymph nodes distributed throughout the body, with clusters found in the underarms, groin, neck and chest, and abdomen. These collect and monitor material drained from the arms, legs, oral and nasal passages, and gut, respectively. They range in size from a few millimetres to about 1–2 cm in diameter and are tightly packed with white blood cells (Figure 6.3). As lymph moves along the lymph vessels, the lymph nodes act as filters or traps for foreign particles and invading pathogens. When an antigen is present in a lymph node, white blood cells become activated, causing an influx of more white blood cells and enlargement of the node as an immune response begins to occur within it.

The **primary lymphoid organs**, the bone marrow and thymus, are responsible for the production and development of the cells of the immune system. The **secondary lymphoid organs** harbour matured cells of the immune system and provide the environment for the initiation of the immune response. Table 6.1 provides a summary of the main organs of the immune system.



◀ **Figure 6.3**
Lymph nodes are sites where lymphocytes scan for antigens and can initiate responses when they come across a particular antigen.

Table 6.1 Organs and tissues of the human immune system

Organ or tissue	Role
Primary lymphoid organs	These organs are responsible for the production and maturation of the cells of the immune system.
Bone marrow	Bone marrow is found in the central shaft of most bones, with a substantial amount in the thigh and pelvic bones. All blood cells develop from bone marrow stem cells. These stem cells are multipotent because they can develop into all types of blood cells.
Thymus	The thymus sits inside the ribcage and is made up of two pinkish grey lobes. It is involved in the development of T cells (a type of lymphocyte) and shrinks (involutes) with age, beginning soon after birth.
Secondary lymphoid organs	Secondary lymphoid tissue provides the environment for the initiation and progression of the immune response.
Lymph nodes	These small, bean-shaped structures are found in specific locations throughout the body, including the throat, armpits, groin, abdomen and chest. They filter the extracellular fluid (lymph) that drains from limbs and mucosal tissues, trapping foreign material, and are sites where lymphocytes can come across antigens and begin to respond to them.
Spleen	The spleen is a large, dark red organ located just above the stomach. Functions include filtering the blood, recognising and destroying old and faulty red blood cells, detecting foreign invaders and producing antibodies. The spleen is generally the site for immune responses directed against blood-borne pathogens.
Mucosal-associated lymphoid tissue (**MALT**)	Clusters of immune cells including lymphocytes found in association with the wet mucosal surfaces of the body, such as those of the respiratory, digestive and female reproductive systems. Cells in these structures survey the mucosa for pathogens and protect the body from an enormous variety of invaders. Tonsils and adenoids are more complex examples of MALT. In the gastrointestinal tract, this is called gut-associated lymphoid tissue (GALT) and includes Peyer's patches, small clumps of white blood cells sitting in the wall of the intestine. Other examples include bronchus-associated lymphoid tissue (BALT) in the lungs and nasal-associated lymphoid tissue (NALT) in the nose.

The lymphoid system is a conduit for linking innate and adaptive responses

When an infection occurs, for example if a cut on the arm becomes infected, localised inflammation will occur at the cut site as part of an innate response. This inflammation causes chemotaxis of white blood cells into the area, including neutrophils, macrophages and **dendritic cells**. There, the phagocytes engulf foreign material, damaged cells and apoptotic debris, and secrete cytokines that stimulate further influx and activation of immune cells into the site.

Dendritic cells, upon taking up the foreign antigen, become activated and leave the inflamed site. They enter the lymphoid system and travel through lymphoid vessels to lymph nodes. Along the way they change their surface receptor expression, down-regulating receptors that help detect and engulf antigens and up-regulating receptors that help present these to B and T lymphocytes of the adaptive immune system. When dendritic cells carrying antigens arrive in lymph nodes, where lymphocytes are concentrated, they also spread out their membrane to ensure a large surface area on which lymphocytes can scan for antigens. Dendritic cells are named for their small finger-like projections, or dendrites, that are on the surface of the cell that help them to take up, process and present antigens to lymphocytes efficiently.

RECALL

- All blood cells are produced from stem cells in the bone marrow. Some leukocytes reside in lymphoid organs and others circulate in the blood and lymphatic system. Others are resident in the tissues.
- Primary lymphoid organs include the bone marrow and thymus, where cells of the immune system are produced and mature.
- Secondary lymphoid organs include lymph nodes, spleen and mucosal-associated lymphoid tissue (MALT), where immune responses are initiated and carried out.

RECAP 6.1

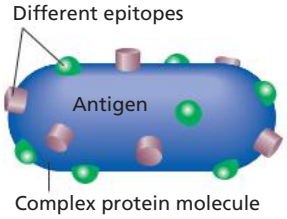
1 Describe the role of lymph nodes in the immune system.

2 Where is an immune response likely to be initiated when the pathogen:
- a enters through a cut in the hand?
- b is a gastrointestinal pathogen that causes 'food poisoning'?
- c enters and circulates in the bloodstream?

Immunity with memory

In Chapter 5 you learned about the cells and actions of the **immune system**. **Innate immune responses** are non-specific, meaning that they do not distinguish between one type of pathogen and another. These responses are also characterised by the fact that they are non-adaptive. That is, the innate immune system does not have 'memory' and responds in a similar fashion every time a particular pathogen invades the body's territory.

In addition to the protection provided by innate immunity, vertebrates have a further line of defence known as the **adaptive immune response**. The cells and processes of this response differ from those previously described because they are specific. This means that the cells can detect and distinguish between different types of invaders, attacking only those that contain the specific molecular pattern or **epitope** matching the receptors on their surface. A foreign **antigen** will usually have several epitopes, and each one will only be detected by the cells with a complementary receptor for that specific epitope (Figure 6.4). The adaptive immune response is also characterised by having memory; this allows the immune system to mount an enhanced defence against a pathogen that infects the host for a second time. This memory is the reason that people who suffer from chickenpox as a child do not usually catch the disease again. It is also the scientific basis for immunisation.



▲ Figure 6.4
The distinction between an antigen and an epitope. A large antigen, such as a bacterium or a large protein complex, may have several different antigenic determinants, called epitopes. The different epitopes are specific chemical groups or structures.

Telling friend from foe

Lymphocytes are the key cells of the adaptive immune system. There are two major types: B and T lymphocytes. B and T lymphocytes (or B and T cells) look so similar that scientists cannot tell them apart under the microscope; special tests that measure surface proteins are required distinguish between them. **B lymphocytes (B cells)** are responsible for the destruction of pathogens by producing proteins known as **antibodies** that bind to them. Destroying virally infected and cancerous cells is the major role of **cytotoxic T (T_C or killer T) lymphocytes (cytotoxic T cells)**. **Helper T (T_H) lymphocytes (helper T cells)** and **regulatory T (T_{reg}) lymphocytes (regulatory T cells)**

assist the other lymphocytes in performing their roles. We will explore how this occurs later in the chapter, but first it is important to understand how lymphocytes tell friend from foe.

For the immune system to function properly, it is important that cells of the immune system are able to distinguish between cells of the body and foreign antigens. Our body cells identify themselves to the immune system as 'self' by marker proteins on the surface of the plasma membrane. As is the case with all proteins, the markers are expressed according to the information coded in genes. The group of genes that determine these protein markers is called the **major histocompatibility complex (MHC)**. Because these MHC markers are determined by the genotype of an individual, they are unique to that individual. It is as if each cell of an individual's body is tagged with a message that is read as 'self'. Any cell not displaying that particular marker is 'non-self' and treated as an antigen.

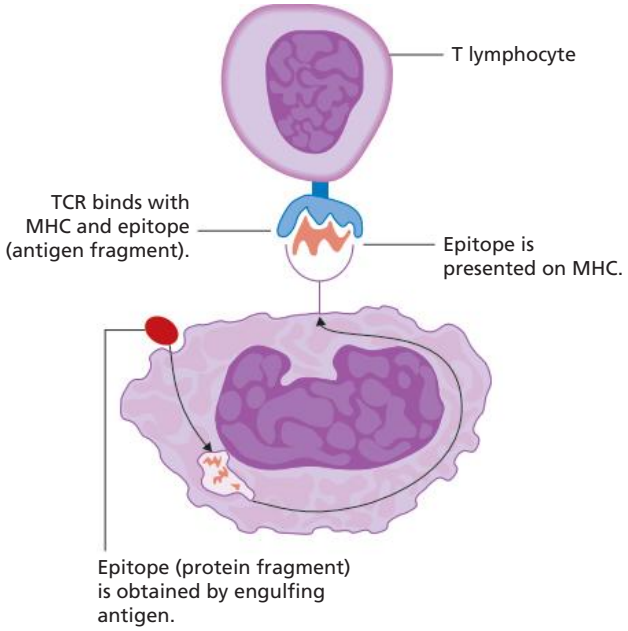


Figure 6.5▲
T cells must be activated to respond. TCRs will only recognise antigens presented by MHC markers.

Antigen receptors

Lymphocytes have surface receptors that are used to distinguish self from non-self. In this section we will explore how **B cell receptors (BCRs)** and **T cell receptors (TCRs)** allow lymphocytes to identify foreign antigens. B cell receptors and T cell receptors recognise and bind to specific epitopes of the antigen. The binding of an antigen to a lymphocyte receptor is similar to that of a substrate binding to an enzyme. Again, the emphasis is on the molecules having the correct shape (or conformation) and charge to be able to bind to each other.

Antibody molecules are glycoproteins whose function is to bind to antigens. When antibodies are bound to the surface of B lymphocytes they act as the B cell receptors. Antibodies also serve as effector molecules when secreted by B lymphocytes.

RECALL

- The adaptive immune response differs from the innate immune response because it has specific recognition of antigens and displays memory.
- B lymphocytes, T_H lymphocytes and T_C lymphocytes are the main players of the adaptive immune system. The adaptive immune response relies on these cells detecting foreign antigens and distinguishing them from self.
- Antigens are molecules that can generate an immune response. The particular molecular structures on antigens that are recognised by components of the immune system are called epitopes.

RECAP 6.2

1 List the different types of lymphocytes that make up the adaptive immune system.

2 Name the type of molecule that lymphocyte receptors are composed of.

MHC markers

T cell receptors are present on the surface of T cells. These receptors do not bind directly with the antigen but rather with epitopes derived from the antigen protein that are displayed on the end of MHC marker molecules. MHC markers are the only molecules that can present the antigen to a T cell. The fact that the T cell receptor will only recognise the antigen when in association with the MHC marker molecule is termed **MHC restriction**.

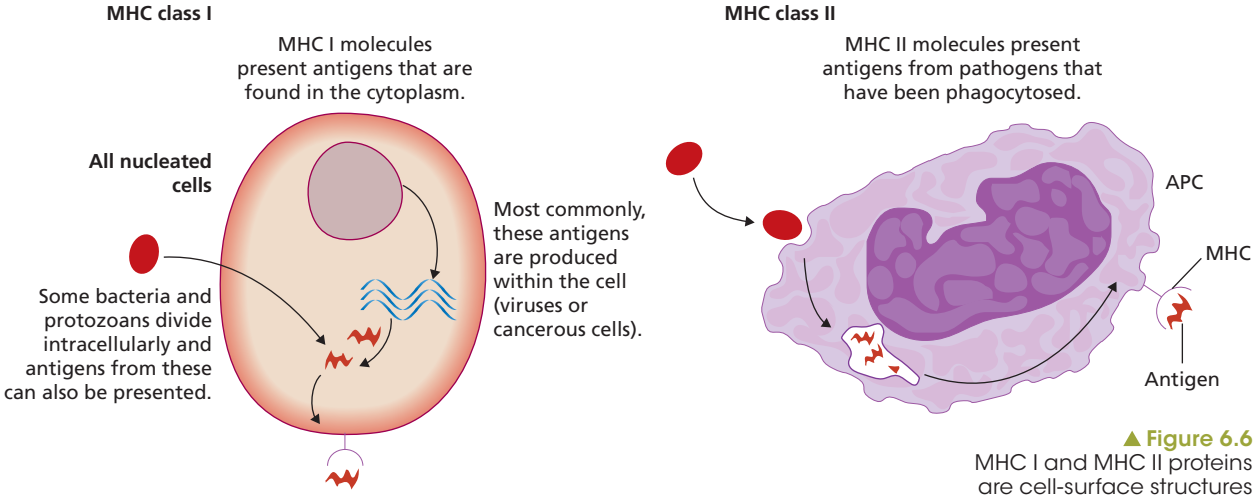
There are two types of MHC proteins: MHC class I and MHC class II. Both types of MHC proteins contribute to the specific identity of the cell. MHC class I markers are found on all body cells that have a nucleus. MHC class II markers are found only on **antigen presenting cells (APCs)**: macrophages, dendritic cells and B lymphocytes.

MHC proteins contain a deep groove that is capable of holding a short peptide. Within a cell, antigens are broken down into small peptides. MHC proteins are synthesised inside the cell and pick up the antigen peptide lengths that sit inside the groove. The MHC protein (bound to a peptide) travels to the cell surface where T cell receptors can then bind to the MHC–antigen complex (Figure 6.5).

MHC class I and MHC class II differ in the type of antigen that they can present (see Figure 6.6). MHC class I presents antigens that are found within the cell cytoplasm. These antigens are usually produced within the cell itself, and this method of antigen presentation allows the immune system to survey the intracellular activity of cells to detect virally infected or cancerous cells. Some pathogens are able to enter and divide within cells. Antigens from these pathogens are also presented this way. This process does not distinguish between antigens and normal proteins produced. Instead, a random sample of peptides from the breakdown of proteins within the cell is presented on the MHC. T cells can then bind to the MHC–antigen complex and trigger apoptosis of the cell if they recognise the presented peptide as non-self. As MHC class I proteins are found on all nucleated cells, this is how the immune system patrols the cells of the body to find any abnormal proteins within cells.

In Chapter 5 you learned about how cells of the innate immune system use pattern recognition receptors (PRRs) to recognise pathogens.

MHC class II molecules are only found on antigen presenting cells and are used to present extracellular antigens. Antigen presenting cells phagocytose pathogens following recognition by pattern recognition receptors, and then break them down in lysosomes. Antigen presenting cells then travel to the lymph nodes to present these antigens to T cells. Peptides that are derived from these pathogens are presented on MHC class II



▲ **Figure 6.6**
MHC I and MHC II proteins are cell-surface structures that present pieces of antigen to T lymphocytes.

proteins. Antigen presenting cells are usually macrophages and dendritic cells but B cells can also present antigens this way. Antigen presenting cells also express MHC class I molecules because they are nucleated cells.

Receptor diversity

Each B or T lymphocyte carries a large number of identical copies of a receptor protein that will bind to a single, specific antigen. There are so many different receptors that around 10 million different epitopes can be recognised by all the B cell clones combined. This diversity means that, by chance, there will be a B and T cell receptor that is able to bind to almost any antigen that the body could encounter.

How can the genome encode for this number of different receptors? It does not. The particular type of receptor carried by a lymphocyte is determined during early embryonic development by random genetic recombination of the antibody or receptor genes. As a result of this genetic rearrangement, each B or T cell and all of its descendants will produce a unique receptor. This genetic lottery accounts for the huge diversity of lymphocyte receptors that are able to respond to the millions of different antigens that we experience in our lifetime. Whatever the antigen, there is a strong chance that there will be a lymphocyte receptor that can bind to it.

Avoiding self-recognition

The random generation of receptors also results in some receptors that will bind to self molecules. Both T and B cells are produced from stem cells in the bone marrow. B lymphocytes remain within the bone marrow to mature, while T lymphocytes travel to the thymus to undergo further development. A group of cells in the thymus express a wide range of proteins that are usually found elsewhere in the body. These proteins are not expressed to perform their normal function, but rather so that T cells can develop self-tolerance. In effect, these cells serve as a 'showroom' of the proteins that the body is capable of producing. Any T cell bearing a T cell receptor that recognises a peptide presented in the thymus undergoes apoptosis and is deleted from the collection. A similar selection process may occur for B cells as they develop in the bone marrow and also as they mature in the spleen. This negative selection of self-reactive lymphocytes continues when they are mature, and those clones that carry receptors for molecules that already exist in the body are either inactivated or self-destruct by apoptosis. This process provides the adaptive immune system with the capacity to distinguish self from non-self. The result is **self-tolerance**, which means that ideally there are no mature lymphocytes that will react against self molecules. However, sometimes the clones that react against self molecules are not completely eliminated and the immune system will attack the body. This can result in autoimmune diseases, which are discussed further on page 210.

The interaction between antigen presenting cells and T cells is another mechanism for preventing responses against self antigens. T cells can only recognise an antigen if it is loaded on to an MHC protein, which means they must interact with an antigen presenting cell. A T cell that recognises a complementary antigen on a MHC protein must receive appropriate signals from the cell presenting that antigen to become activated. If that antigen presenting cell has recognised a pathogen-associated molecular pattern or a damage- or danger-associated molecular pattern, indicating infection or tissue damage, it will signal to the T cell that it should mount a response against the peptide presented on its MHC. This signal is usually in the form of cytokines, such as interleukins, and contact-dependent signals. Without this danger signal, a T cell recognising a peptide bound to an MHC protein will not mount a response against the peptide. This provides an additional safeguard that prevents T cells from mounting an immune response against the body's own cells and tissues.

RECALL

- Together with PRRs, the major histocompatibility complex (MHC) is an important way of distinguishing self from non-self.
- MHC restriction refers to the fact that T cells will only recognise epitopes when they are presented on an MHC molecule.
- MHC class I molecules present intracellular antigens and are present on all nucleated cells. MHC class II molecules present extracellular antigens derived from phagocytosis and are present on antigen presenting cells (macrophages, dendritic cells and B cells).

RECAP 6.3

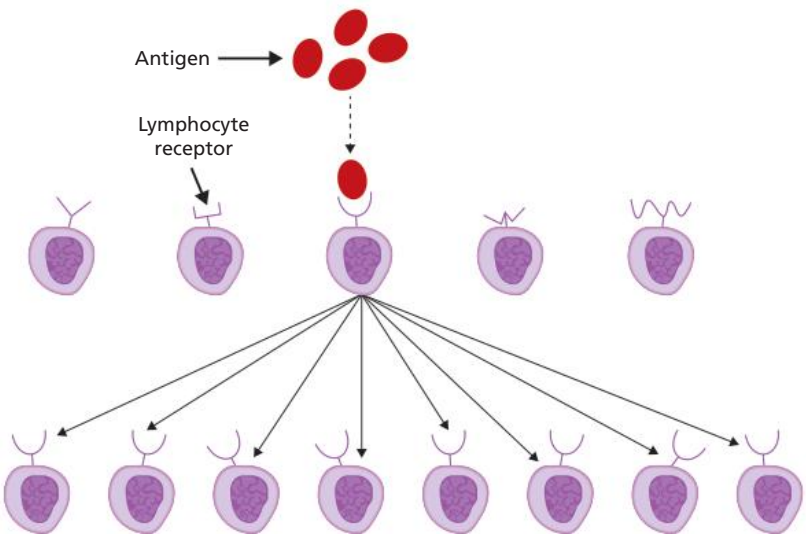
1 Define the term 'self tolerance'.
2 Describe the MHC class I and class II presentation pathways and identify the main differences between them.
3 List two mechanisms that prevent T cells from mounting an immune response against a normal body component in the absence of any true infection or injury.

Clonal selection

B and T lymphocytes originate as stem cells in a process that starts when we are embryos. By the time we are born we have a large number of different types of B and T cells, each with a small number of clones that are able to recognise a specific antigen circulating throughout our blood and lymphatic systems.

A young lymphocyte is released from its 'training ground' into the bloodstream, in which it may encounter an antigen it recognises. Recognition of a specific antigen triggers an impressive response in the cell, causing it to divide rapidly, forming many copies or clones of itself and the specific antigen receptor it carries. These clones can be one of two types of cells: effector cells or memory cells (Figure 6.7).

Random genetic rearrangements allow for a diverse range of lymphocyte receptors to be generated. Clonal selection is responsible for the proliferation of lymphocyte clones that have bound to antigens.



◀ Figure 6.7
The rapid division of a particular lymphocyte clone, once it has bound to an antigen, is termed clonal selection.

Thus, the antigen itself selects which of the millions of different B or T cell clones becomes active. Australian Sir Macfarlane Burnet played a key role in developing this theory, known as **clonal selection**. He was awarded a Nobel Prize in 1960 for his contribution to the field of immunology.

RECALL

- Each B cell and T cell has an antigen receptor that is specific for a unique epitope, so the population of lymphocytes has the capacity to detect a vast range of antigens.
- Lymphocytes with antigen receptors that could recognise self components are deleted or inactivated during their development.
- During infection, only the lymphocytes bearing a receptor that can recognise epitopes on the invading pathogen are activated and proliferate, in the process of clonal selection.

RECAP 6.4

1 Distinguish between the innate and adaptive immune responses.

2 a Draw a diagram to show how B cells bind to antigens and another diagram to show how T cells bind to antigens.

b Describe how antigen–lymphocyte binding is like a lock and key.

3 Briefly explain the principle of clonal selection using non-scientific language that would be accessible to the general public.

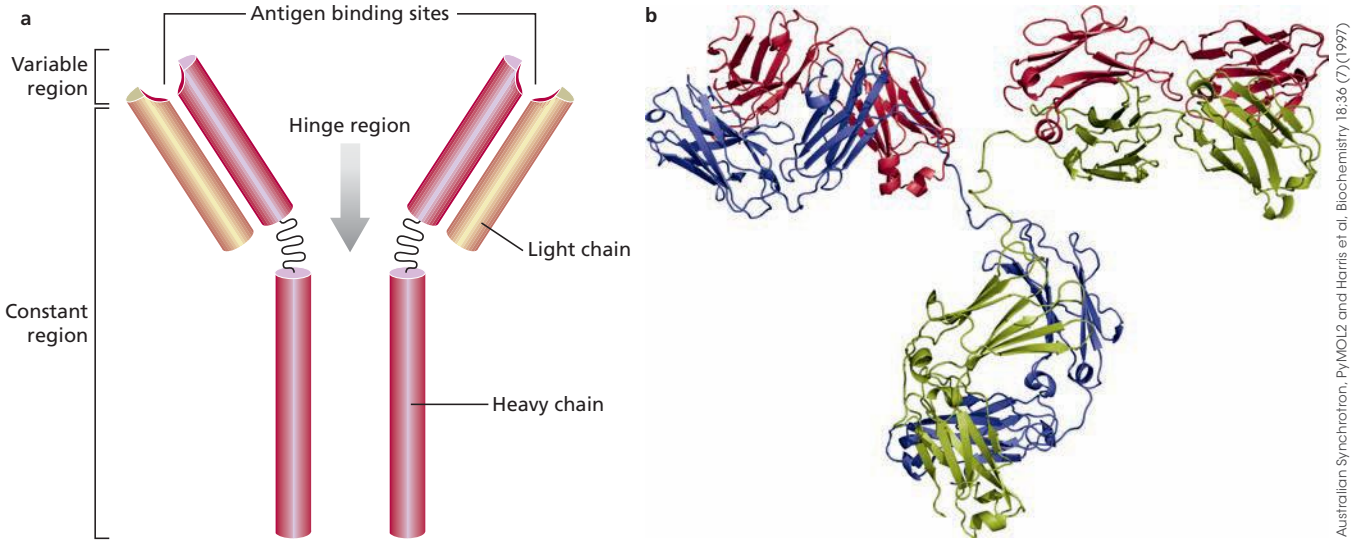
4 Azathioprine is a drug that blocks the production of purine nucleotides (adenine and guanine) in lymphocytes. Predict the effect that azathioprine would have on the process of clonal selection.

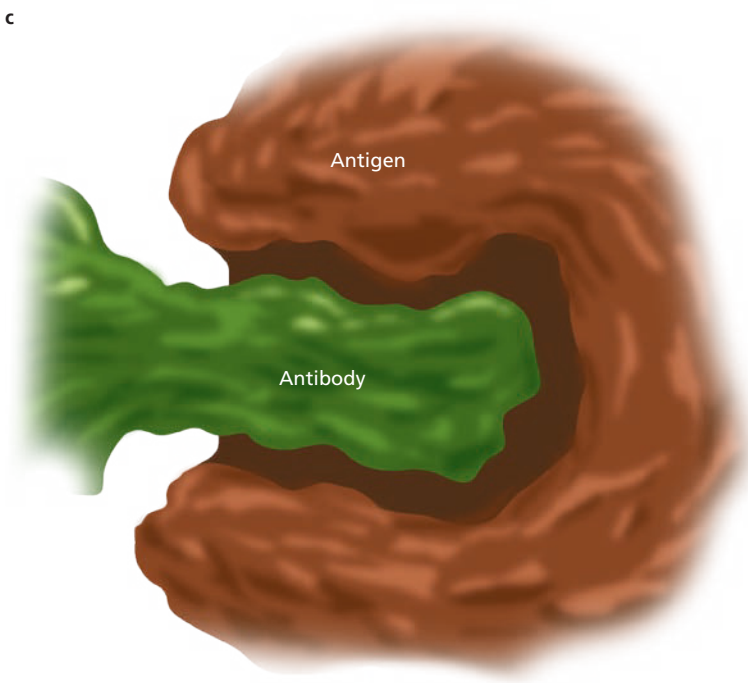
Humoral immunity

The **humoral immune response** is brought about by B cells, which produce an amazing array of different antibodies that attack foreign antigens. The word 'humoral' refers to the fact that the effects of this system are caused by the circulation of antibodies in 'humours', an antiquated concept that roughly means body fluids. You have already learned that antibodies bound to the surface of B cells act as the B cell receptor. Once activated, B cells divide rapidly (that is, they are clonally selected) and produce antibodies that circulate freely in the bloodstream and can lead to the destruction of pathogens.

Antibodies

Antibodies, also known as **immunoglobulins (Ig)**, consist of four polypeptide chains (two heavy chains and two light chains) arranged in the shape of a Y (Figure 6.8). All antibodies have a constant region (most of the Y shape) and variable regions at the two tips of the Y, where there are two identical binding sites that are complementary to a particular antigen. This is the part of the antibody that results from genetic recombination during development. The binding sites work with a lock-and-key system of identification, similar to that of enzymes binding with their substrate (Figure 6.8c).





◀ Figure 6.8
(**a**) The Y-shaped structure of an antibody. The hinge region gives antibodies great flexibility to improve binding to the antigen. (**b**) A ribbon diagram representation of the crystal structure of 1IGT, an antibody of the IgG family produced by plasma B cells (**c**) The active sites on the antibody and antigen molecules are complementary; they fit together like a lock and key.

Antibodies, once bound to an antigen, can lead to the destruction of pathogens in four ways, all of which may occur simultaneously (Figure 6.9). First, antibodies that are bound to antigens are potent activators of the **complement** cascade. The second way is that bound antibodies are able to attract phagocytes, effectively 'tagging' pathogens for phagocytosis and destruction, a process known as **opsonisation**. Some antigens can act as toxins and cause cellular damage. In these cases, antibodies neutralise toxins by preventing them from binding to their target. This is known as **neutralisation** and is the third way that antibodies can act. Finally, the binding of antibodies can also cause **agglutination** of pathogens, meaning that they become stuck together in an antibody–pathogen net. In other words, the pathogens are immobilised and not able to spread. Being clumped together in one spot also makes them more susceptible to destruction by phagocytosis.

See Chapter 5 for details about the complement cascade and how complement, like antibodies, can also result in opsonisation.

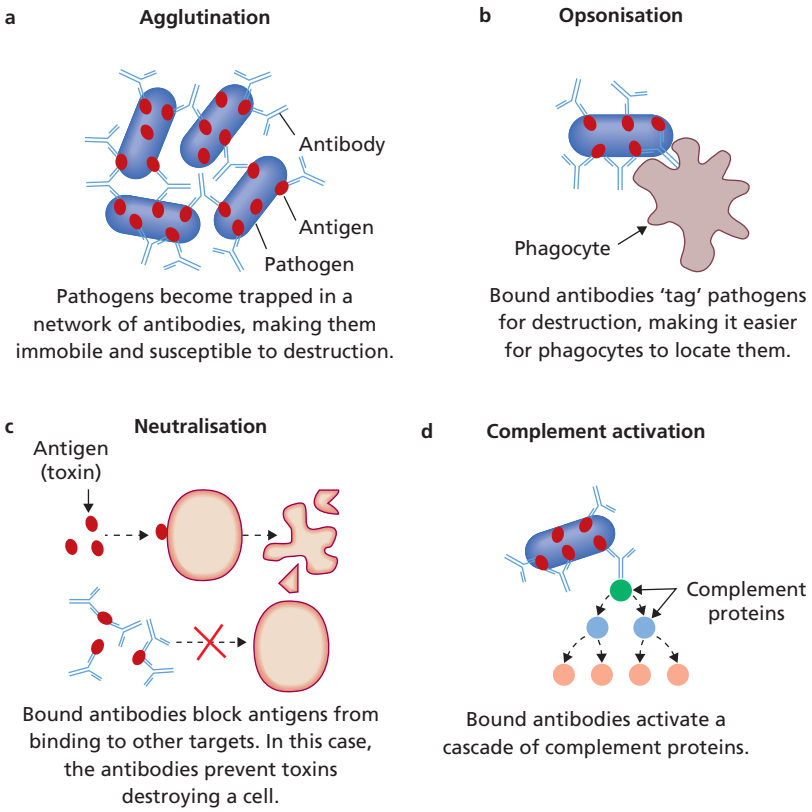


Figure 6.9 ▶ Antibodies can cause the destruction of pathogens in four ways: (**a**) agglutination, (**b**) opsonisation, (**c**) neutralisation and (**d**) complement activation.

Isotypes

Antibodies occur in several different classes, known as **isotypes**. Each isotype has a different heavy chain and constant region, and this allows them to have different functions.

- IgM antibodies are the first to be secreted in an infection. They cause agglutination of cells bearing antigens, causing them to form large clumps that are more easily eliminated by phagocytes. IgM antibodies are also embedded in the phospholipid bilayer, with the two arms sticking out so the antigen-binding sites are exposed. These membrane-mounted antibodies act as the B cell receptors.
- IgG antibodies are produced by effector and memory B cells that have 'matured' after encountering their specific antigen. IgG is responsible for activating complement proteins in the blood and can neutralise toxins directly. Interestingly, this is the type of antibody most commonly passed between mother and baby, either through the placenta before birth or in breast milk later. On a memory B cell, the B cell receptor is usually an IgG antibody instead of an IgM antibody.
- IgA antibodies neutralise pathogens in the respiratory, digestive and reproductive tracts.
- IgE antibodies are important in protection against parasites. It is this type of antibody that also causes allergic reactions to non-pathogenic agents.
- IgD antibodies are found bound to the plasma membrane as the B cell receptor on B cells that also produce IgM. IgD is not secreted into the circulation.

RECALL

- Antibodies are present on the surface of B cells as the B cell receptors and are secreted into the circulation by a type of differentiated B cell called a plasma cell.
- Antibodies function through agglutination, opsonisation, neutralisation and complement activation.
- Antibodies exist in different isotypes that have different specialised functions.

RECAP 6.5

1 Identify what type of compound an antibody is.

2 Draw a diagram of an antibody, labelling where antigens bind.

B lymphocytes and antibody production

To produce antibodies, a B cell needs to be activated by an antigen. When a pathogen enters the body it will encounter a large number of B cells. B cells circulate through the blood and lymphatic system and congregate in lymph nodes where they detect pathogens draining from the tissues or presented on antigen presenting cells. B cells are also found in the spleen, where they can detect and respond to pathogens circulating in the bloodstream. Each B cell has a unique B cell receptor (antibody) on its surface that enables it to bind to a specific epitope. An invading pathogen will only activate B cells bearing a B cell receptor that is complementary to a particular epitope on the pathogen. In this way, the B cell is activated to produce an adaptive immune response. However, B cells are also antigen presenting cells, and carry on their surface pattern recognition receptors (PRRs, discussed in Chapter 5) that recognise foreign pathogens non-specifically. The B cells engulf the pathogens they recognise and present pathogen components on MHC class II molecules for presentation to T_H cells.

If an antigen binds specifically to a B cell receptor, the B cell clone carrying the complementary antibody becomes activated and is clonally selected. It starts rapidly dividing to produce effector and memory B cells (Figure 6.10) in the process of clonal expansion. Following clonal selection and expansion, the B cell clones that have been activated will then be present in much greater numbers than others. This division occurs most effectively with the assistance of T_H cells that have

Further information about the activation and roles of T_H cells is on page 200.

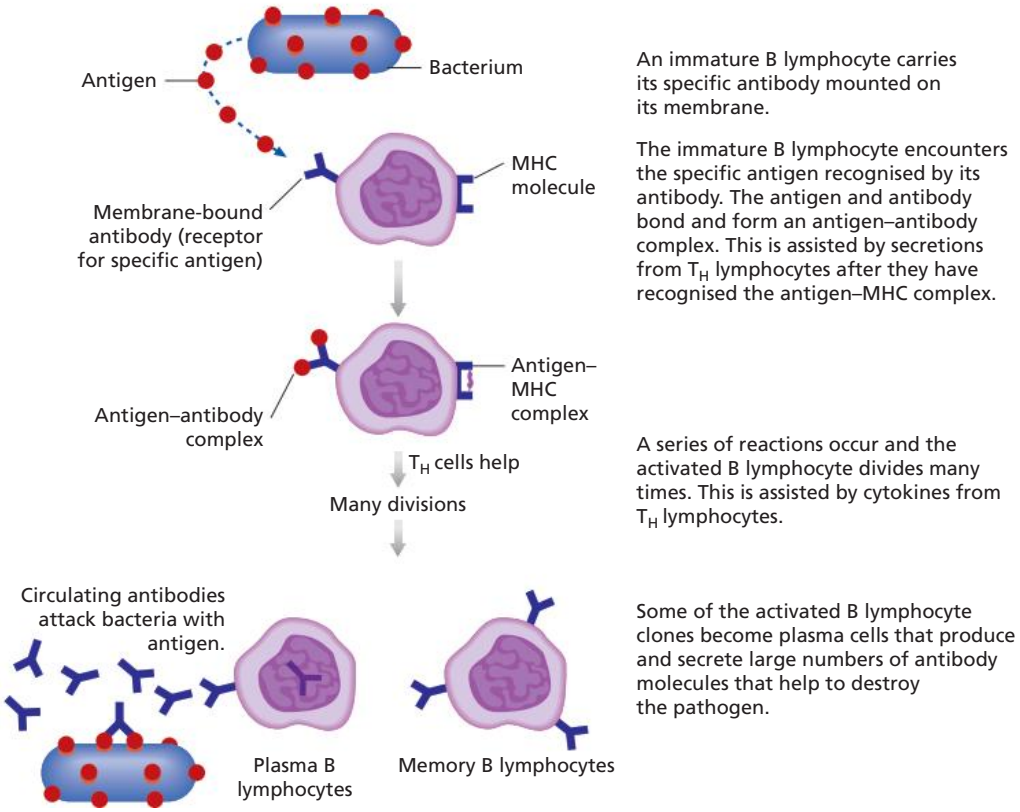
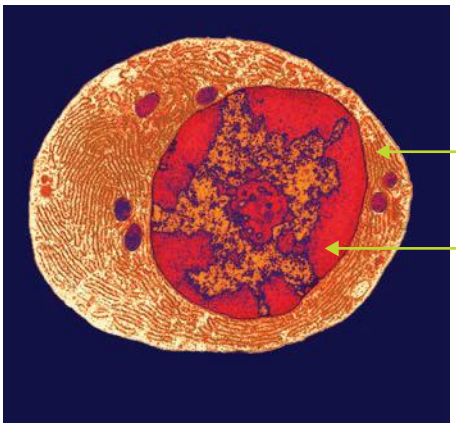


Figure 6.10 Example of an antibody-mediated immune response to a bacterial pathogen, summarising the steps of B lymphocyte activation. This response usually occurs in a lymph node or in the spleen.



amanaimages/Dr Donald Fawcett

Figure 6.11 ▲
Transmission electron micrograph of a plasma cell. There is extensive rough endoplasmic reticulum to allow for the production of antibodies.

been activated by the same antigen. T_H cells assist by producing **cytokines** and contact-dependent signals that promote B cell division and antibody production.

The effector B cells are known as **plasma cells**. Plasma cells have differentiated to become highly specialised for antibody production (Figure 6.11), secreting up to 10 000 molecules of a specific antibody per second into the circulation. These antibodies generally provide protection for up to 28 days but the plasma cells that secrete them can last for years and even decades. Most antibodies will only attack one antigen, but a few will attack a number of different antigens if they are closely related and have similar structures, such as the smallpox and cowpox viruses.

But what of the memory B lymphocytes? These cells persist within the body for months or even years, not secreting antibodies but still carrying them on their plasma membrane. In this way, they are able to recognise the same pathogen quickly should it reinvade the body of the host. Once activated by the revisiting antigen, the B memory cells rapidly divide and form plasma cells that produce large quantities of antibody, often attacking the pathogen before any symptoms of its presence arise.

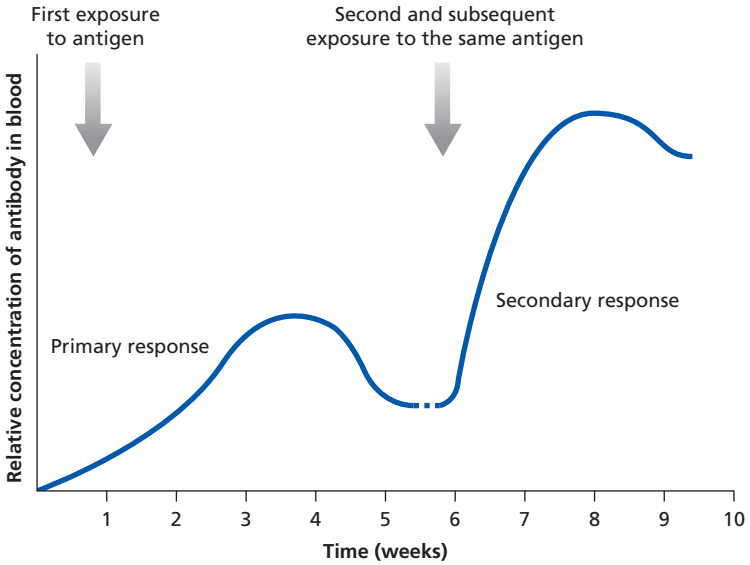


Figure 6.12 ▲
Graph showing antibody levels after an initial (primary) infection by an antigen and after a second exposure to the same antigen

Figure 6.12 shows the speed of antibody production following initial and subsequent exposure to an antigen. Imagine that the antigen in this diagram is from Rubulavirus, which causes mumps. When first exposed to the virus the body produces antibodies, but there is a delay before enough are produced to neutralise the virus. This is why unvaccinated people develop symptoms of mumps when first exposed to the virus, either in wild form or as part of a vaccine formulation. When the same person is later exposed to the virus again, memory cells recognising the virus quickly divide and form plasma cells, which produce antibodies that neutralise the virus while it is still in circulation before it can enter its target cells. You can see in this figure that the **secondary response** is faster (with a steeper response curve) and of a greater magnitude than the **primary response** to that same antigen. This is why people are vaccinated against mumps. After an initial response to the vaccine, they become immune to future infections.

RECALL

- B cells are antigen presenting cells and express pattern recognition receptors. They can take up antigens via PRRs in a non-specific way, or can recognise specific antigens through their BCR.
- For B cell activation to occur, the same antigen must activate T_H cells, allowing them to provide contact-dependent signalling for B cells and secrete cytokines that stimulate B cell survival, activation and proliferation.

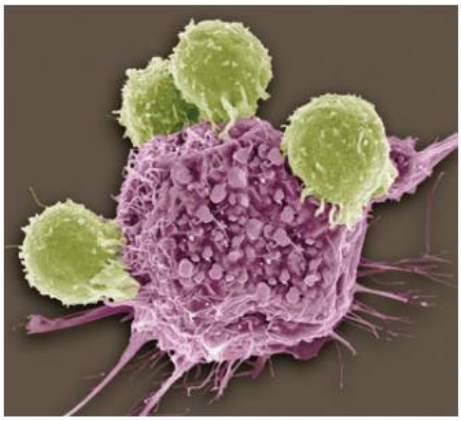
RECAP 6.6

1 What is the difference between clonal selection and clonal expansion?

2 Plasma B lymphocytes possess an extensive rough endoplasmic reticulum, many Golgi apparatuses and many mitochondria. Relate the structure of plasma cells to their function.

3 How do primary and secondary immune responses differ?

Cell-mediated immunity



Science Photo Library/Steve Gschmeissner

▲ **Figure 6.13** Scanning electron micrograph showing T_C lymphocytes attacking a cancer cell

Cell-mediated immunity involves the direct killing of virally infected and cancerous cells by T_C lymphocytes. Like B lymphocytes, they are able to distinguish self from non-self due to membrane-bound receptors, T cell receptors, that interact with antigens. You have already learned that T lymphocytes do not bind with antigens directly but rather bind with the antigens presented on the MHC proteins. MHC class I present antigens from inside the cell and thus flag virally infected or cancer cells.

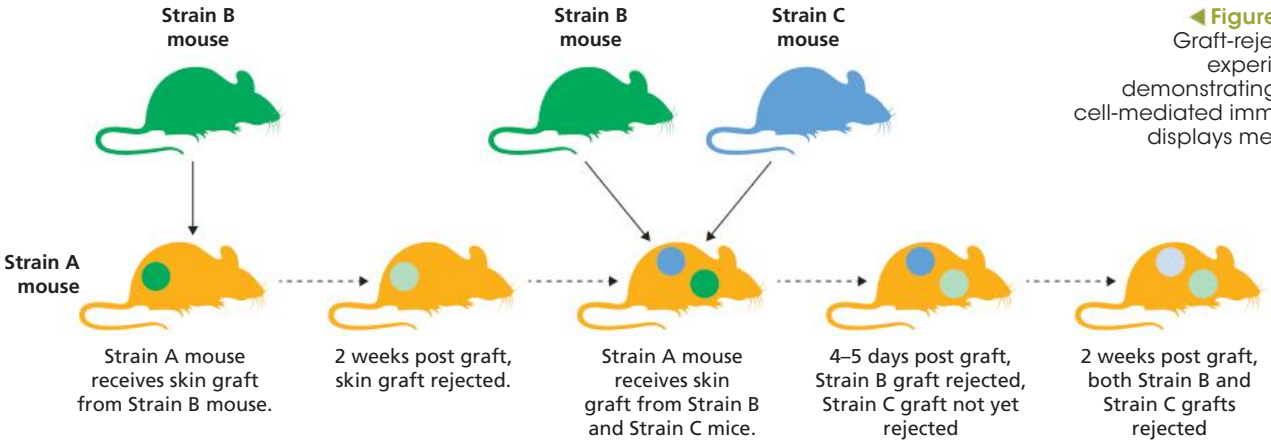
Some viruses have evolved mechanisms to stop or reduce the expression of MHC class I on infected cells. This prevents T_C cells from recognising these cells as virally infected, allowing the virus to evade destruction and continue to divide. In response, natural killer cells have evolved to destroy cells that have low levels of MHC class I on the surface. This is a clear example of how the immune system has influenced the evolution of pathogens and vice versa.

Mechanisms of evolution are discussed in Chapter 8.

Like B cells, activated T_C cells (with the help of contact-dependent signals and cytokines from T_H cells) proliferate by dividing many times to form an army of clones. Some of these clones become effector cells, while others remain as memory cells and migrate in the lymph fluid and through lymph nodes where they can be activated quickly upon a second encounter with the same pathogen.

T_C cells are highly effective killers; they can eliminate infected body cells or tumour cells by releasing powerful cytotoxins when they contact a cell that carries an unrecognised antigen (Figure 6.13). These cytotoxins include proteins called perforin and granzymes that work together to induce apoptosis in the target cell.

The memory of cell-mediated immune responses can be demonstrated in experiments that use skin transplants (known as grafts) in mice. If a mouse is given a skin graft from a non-identical mouse, it will be rejected after around 14 days. If that same mouse later receives a second graft from the same donor mouse, the rejection only takes 4–5 days (Figure 6.14). This is because memory T_C cells formed after the first graft respond more rapidly when they encounter the foreign graft a second time.



◀ **Figure 6.14** Graft-rejection experiment demonstrating that cell-mediated immunity displays memory

As indispensable as these cells are to our immune system, they can also cause problems for patients requiring organ transplants. These cells are the primary cause of transplant tissue rejection as they destroy the transplanted cells directly. Thus, patients receiving transplants must take high levels of immunosuppressant drugs to help counteract this response so that the new organ is not destroyed by the immune system.

Helper T and regulatory T lymphocytes

As the name suggests, helper T cells (T_H cells) assist other cells of the immune system. They do this by secreting chemicals (including cytokines) that induce any activated B or T_C cell to divide and give rise to large numbers of clones that become the effector and memory cells. Cytokines can also stimulate macrophages to engulf invading cells more readily.

Another type of T cell, called regulatory T cells (T_{reg} cells), plays an important role in modulating the action of lymphocytes. T_{reg} cells may enhance or suppress the actions of other lymphocytes. They are also capable of suppressing the action of phagocytes. In this way, they help prevent the immune system overreacting to a stimulus. T_{reg} deficiency causes a very severe autoimmune disease resulting from overactive lymphocytes.

Table 6.2 Three major groups of lymphocytes: B cells, T_H cells and T_C cells

	B lymphocytes	Helper T (T_H) lymphocytes	Cytotoxic T (T_C) lymphocytes
Development of self-tolerance	Occurs in bone marrow	Occurs in thymus	Occurs in thymus
Receptors	BCR (antibody)	TCR	TCR
Antigen recognition	Recognises antigens not presented in MHC	Recognises antigen in MHC class II	Recognises antigen in MHC class I
Undergo clonal selection	Yes	Yes	Yes
Effector functions	Plasma cells produce antibodies	Production of cytokines to aid B cell, T_C cell and macrophage functions	Induction of apoptosis in target cells
Formation of memory cells	Yes	Yes	Yes

Linking the parts of the immune system

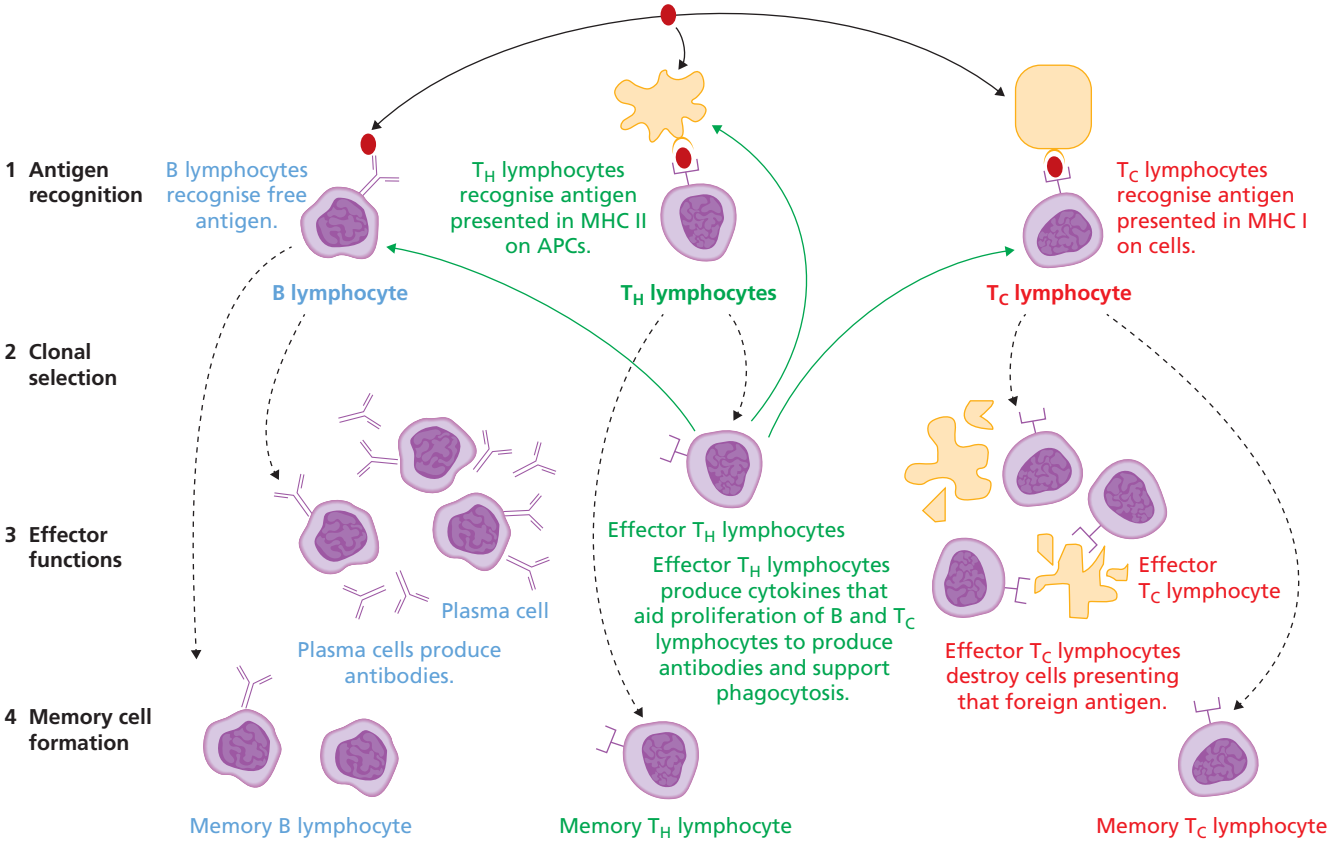
The immune system is a complex network of cells that rely on one another to function properly.

B and T lymphocytes all share a number of features that are summarised in Figure 6.15. They both have a system for generating a diverse range of receptors for different antigens, and they rely on clonal selection to allow for proliferation of relevant clones. Both B and T cells form effector and memory cells. Importantly, specific recognition of antigens and the ability to exhibit memory distinguishes the adaptive immune functions of lymphocytes from the cells of the innate immune system.

The adaptive and innate systems are closely interlinked and do not operate in isolation. Communication between the cells of these systems is critical for

the functioning of both. The following list summarises some of the major connections.

- Antigen presentation by macrophages and dendritic cells allows T cells and B cells to recognise antigens.
- Full activity of T cells and B cells requires cytokine production by antigen presenting cells that have recognised a pathogen-associated molecular pattern or a damage- or danger-associated molecular pattern.
- The binding of antibodies to pathogens can activate complement directly and promote phagocytosis by cells of the innate immune system.
- Phagocytosis is also promoted by cytokines produced by T_H cells.
- Following the destruction of cells by T_C cells, phagocytes play a role in 'cleaning up' the cell fragments produced.
- T_C cells release cytokines that promote destruction of phagocytosed antigens.



▲ **Figure 6.15** Summary of the actions and functions of the cells of the adaptive immune system

RECALL

- T_C cells scan peptides presented on MHC class I molecules. When they detect foreign peptides, with the help of signals from T_H cells, they secrete granzymes and perforin to kill the affected target cell.
- T_H cells scan peptides presented on MHC class II molecules. They express contact-dependent signalling molecules and cytokines to help activate T_C cells and B cells.
- T_{reg} cells are T cells that control the magnitude and duration of immune responses to limit damage to body tissues.
- The innate and adaptive immune system are interlinked with many connections that are essential for proper immune responses.

RECAP 6.7

1 Which of the following statements are true for T_H cells and which for T_C cells?
   a recognise antigens presented by MHC class I molecules
   b undergo clonal selection
   c destroy cells by producing cytotoxic proteins.
2 Describe the role of T_{reg} cells.
3 The adaptive immune system is often described as having memory. Explain what this means, using T_C cells as an example.
4 List three ways that the innate and adaptive immune systems communicate.
5 Explain how T_H cells aid multiple other cells of the immune system in fighting off invaders.

Assisting immunity

The outcome of an infection depends on both host and pathogen factors. Hosts may have an **immunodeficiency**, which occurs to a small extent naturally in the very young or very old, or in people who carry alleles that affect some aspect of their immune system. Certain diseases, such as HIV or some cancers, cause immunodeficiency. Other diseases are treated with drugs that bring about a state of **immunosuppression** in the host. These individuals may not be able to clear an infection that is not usually considered dangerous in healthy adults. However, a healthy individual who becomes infected with a pathogen may be unable to clear the pathogen if the immune evasion mechanisms of the pathogen are too effective. To overcome these factors, we now rely on the use of chemical treatments to support the immune response of the host to fight some pathogens.

Like the specificity of an enzyme for its substrate, and the lock-and-key fit that occurs between them, most drugs are highly specific for their target. They are selected for their specificity, as unwanted side effects can be very harmful to the host. Ideally, antimicrobial compounds should be non-toxic to the patient and have activity against several different species of pathogens.

Preventing infection before it takes hold

Unfortunately, it takes time for the adaptive immune system to locate the invader and mount a defence if that pathogen has never been encountered before. If it is a second infection, memory B and T lymphocytes circulate and lie in wait in the lymph nodes, ready to be activated quickly to destroy the pathogen before symptoms of the disease arise. How, therefore, can we take advantage of this immune memory so that memory cells exist before the first invasion? **Vaccines** have been developed that work by preparing the immune system for the pathogens it may encounter.

A vaccine contains antigens from a pathogen that stimulate the immune system to form memory B or T cells. These memory cells then wait in the lymph nodes ready to detect and respond to the real pathogen bearing these antigens as soon as the pathogen invades. Plasma cells are also formed and these cells then continuously secrete protective antibodies into the circulation.

Substances called **adjuvants** are usually added to vaccines along with the antigen. These adjuvants activate the innate immune system, ensuring that B and T cells receive the assistance that they need from antigen presenting cells to respond to the antigen. In other words, a vaccination gives an organism the experience of a particular pathogen's antigens without the host actually developing symptoms of the disease itself. This means that, if the pathogen is encountered, the immune system is able to mount a secondary response (Figure 6.12).

Active and passive immunity

When the body is infected by a pathogen or stimulated with a vaccination, the memory T and B cells produced will be activated rapidly if that antigen is encountered again. This kind of immunity is known as **active immunity** and generally lasts many years, although the immune system may need booster shots periodically to enhance its army of memory cells.

Active immunity is contrasted with **passive immunity** when antibodies are provided from an external source (Table 6.3). These externally sourced antibodies will provide protection from the pathogen, but only for as long as those antibodies last. As there are no plasma cells or memory B or T cells, the person will not be immune if they encounter the pathogen again.

Table 6.3 Examples of active and passive immunity

	Active immunity	Passive immunity
Naturally occurring	Exposure to a pathogen	Transfer of antibodies from mother to foetus via the placenta Transfer of antibodies from mother to baby via breast milk
Artificial	Vaccination	Anti-venom Antibodies against particular pathogens (e.g. rabies) Mix of antibodies for immunodeficiency

Passive immunity occurs naturally when antibodies pass from a mother to her foetus via the placenta and during breastfeeding. These antibodies are essential for protecting a newborn or very young baby from pathogens. A baby is most vulnerable to infection two to three months after birth, as its own immune system is not yet fully developed and the antibodies it received from its mother via the placenta have disappeared.

In some cases there is insufficient time for antibodies to be produced actively by the patient before death or serious injury occurs. In such instances, a dose of antibodies targeted to a specific antigen is administered directly to the patient. For example, the anti-venom given after a snake or spider bite is a solution of antibodies against the venom. Solutions of antibodies can also be used to prevent the development of disease in somebody who has been exposed to a pathogen. Rabies is a viral disease that is spread in the saliva of infected animals. Untreated, it is a disease that is always fatal once symptoms start because the immune system cannot produce a response quickly enough. However, the development of symptoms can be prevented by quickly administering antibodies against the rabies virus if somebody has been bitten by an infected animal.

Rarely, people are born with or develop a condition in which they cannot produce enough of their own antibodies. As a result these people are very susceptible to infections. A condition such as this where the immune system does not function properly is called an immunodeficiency. A way of treating this type of immunodeficiency is to give the patient a mix of antibodies taken from healthy donors. These will provide protection for only a short period of time, and so these patients will need to have antibody infusions every month or so.

Preparing purified antibodies

Despite having many of the world's most venomous snakes, the number of deaths in Australia from snakebite remains very low. In part this is due to the availability of anti-venom treatment. Anti-venom is a solution of antibodies that are targeted against the venom. In order to use antibodies for anti-venom or to protect against disease, solutions of antibodies need to be produced.

One way to prepare a pure sample is to initially inject the specific antigen into a host such as a rabbit or horse. This induces the animal to produce antibodies, which are secreted into their bloodstream. These are then extracted for use (Figure 6.16). This process is costly and time-consuming, and the purification of the sample is difficult.

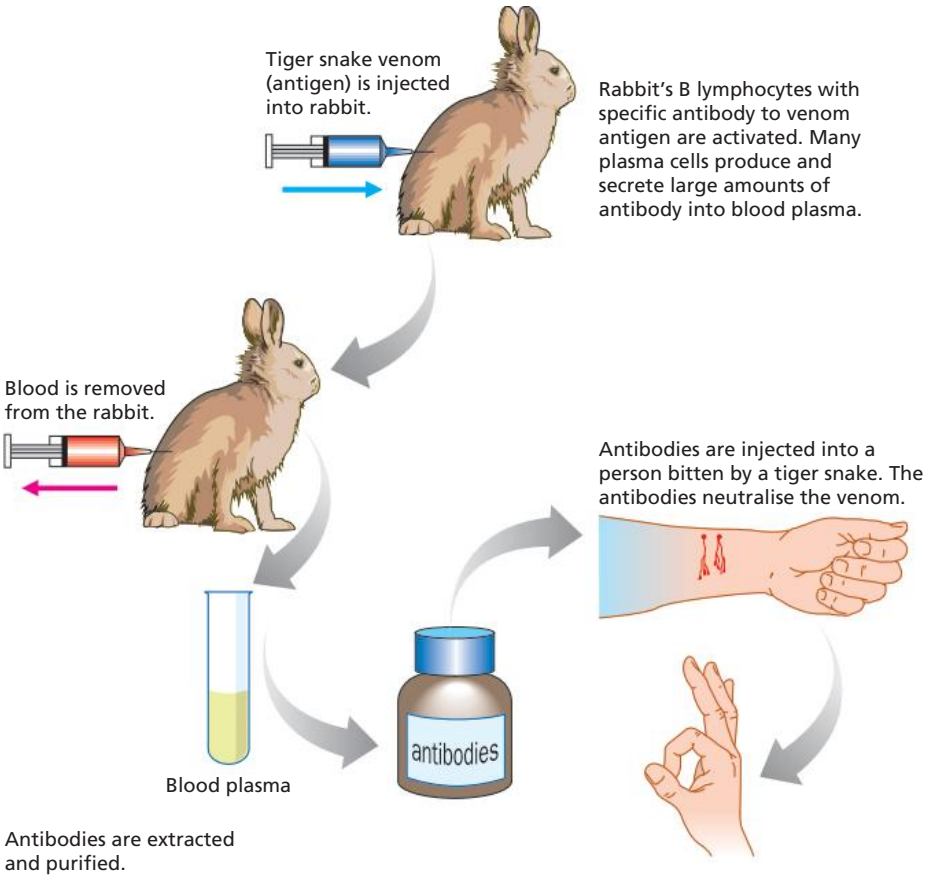


Figure 6.16 ▶
Anti-venom for a tiger snake bite can be produced by collecting antibodies from a rabbit that has been injected with small amounts of venom.

RECALL

- The protection provided by antibodies may be passive or active. Only active immunity provides long-term protection against pathogens.
- Examples of passive immunity include transfer of antibodies from mother to baby and anti-venom to treat snakebite.
- Vaccines stimulate a primary response so that, on encounter with a pathogen, the immune system mounts a more rapid and strong secondary response.
- Vaccines are made from components of the pathogen combined with an adjuvant to stimulate both the innate and adaptive immune responses.

RECAP 6.8

1 Describe how active immunity is acquired from vaccination.
2 Explain why breast milk can confer advantages to the baby's immune system that formula cannot.
3 Discuss why the production of anti-venom is costly.
4 Outline why passive immunity lasts only about 28 days.

Monoclonal antibodies

Another way to produce antibodies is to culture B cells in a laboratory and collect the antibodies they produce. The problem here is that B cells, like most mammalian cells, do not live for very long in culture and so do not mass-produce the specific antibody for any length of time. This can be overcome by fusing the B cell clone that produces the antibody of interest with cells extracted from a plasma cell **tumour**, creating what is called a **hybridoma**. The hybridoma has the ability to produce antibodies coupled with the property of tumour cells to divide repeatedly (the cells have now become 'immortalised'). Each hybrid cell produces many clones of itself, and each clone produces the same antibody. These antibodies are termed **monoclonal antibodies** as they are produced by clones of the same hybrid cell and are thus identical. Hybridomas have revolutionised the production of antibodies.

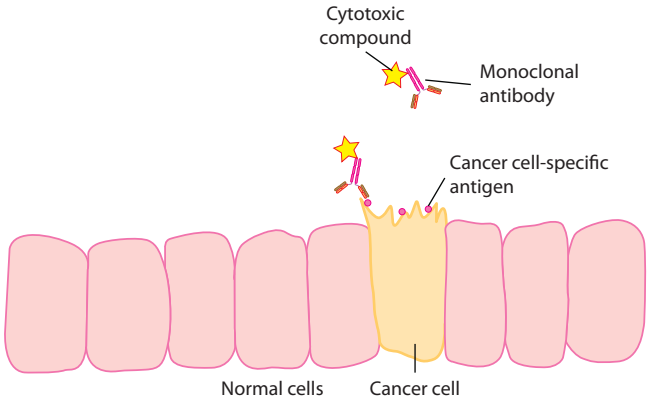
Alamy/Ronald Karpilo

▲ Figure 6.17
A pregnancy test uses antibodies against the hormone human chorionic gonadotrophin (HCG), which is only present in urine if a woman is pregnant. The photo shows an example when HCG is not present.

Antibodies produced this way have a variety of commercial and scientific uses. Their ability to bind to a particular antigen means that monoclonal antibodies can be used to detect whether a substance is present and, if so, to measure it. Urine pregnancy tests are a commercial use of antibody technology (Figure 6.17). These tests work by detecting the hormone human chorionic gonadotrophin (HCG), which is only present in the urine if a woman is pregnant. The tests contain antibodies that bind to HCG. The antibodies have an enzyme attached that causes a colour change if bound. This colour change appears as a strip indicating a positive test.

Monoclonal antibodies for cancer treatment

Antibodies are also being produced to treat some diseases, including cancer. Antibodies that detect antigens unique to cancer cells can be used as anti-cancer drugs. Monoclonal antibodies recognise and attach to a single antigen, such as a receptor for a growth factor that the tumour needs to proliferate. Monoclonal antibodies used as drugs can be designed to trigger an immune response to attack cancer cells, block signals that cause cancer cells to divide, or transport toxic molecules or radioisotopes to cancer cells. An example is the medication trastuzumab, which is a preparation of monoclonal antibodies that can bind to cells of some types of breast cancer, blocking their growth and promoting destruction by the immune system. Ideally, the monoclonal antibody will bind to an antigen that is unique to the cancer and not present on normal body cells so that there are no unwanted side effects of the monoclonal antibody. Unfortunately this situation is rare. Monoclonal antibodies often bind to antigens that are also present on other cells of the body, resulting in various side effects depending on the target cells.



▲ Figure 6.18
Monoclonal antibodies conjugated to cytotoxic compounds can be used to specifically kill cancer cells when the cancer cell expresses a unique antigen.

Table 6.4 Examples of monoclonal antibodies for cancer treatments

Monoclonal antibody	Cancer type	Mechanism of action
Rituximab	Non Hodgkin lymphoma	Binds to the protein CD20 on the surface of cancer cells and activates the immune system to destroy them
Ipilimumab	Advanced melanoma	Binds to and blocks CTLA4, a negative regulator of the immune system, to keep immune cells stimulated
Trastuzumab	Breast cancer	Binds to the growth factor receptor HER2 to block the signal and stop cancer cells growing
Bevacizumab	Bowel and breast and some other cancers	Binds to the growth factor VEGF to inhibit binding to its receptor
Ibritumomab	Non Hodgkin lymphoma	Fused with a radioactive isotope of yttrium, binds to the protein CD20 on the surface of cancer cells, delivering the radioactive compound to the cells.

RECALL

- Hybridomas are immortalised plasma cells that produce large amounts of monoclonal antibodies in tissue culture.
- Monoclonal antibodies have many applications in the laboratory, in diagnostics and in therapeutics.
- Monoclonal antibodies can be used as anti-cancer therapeutics, by triggering an immune response against the cancer, blocking signals to cancer cells, or delivering a cytotoxic molecule to the cells.

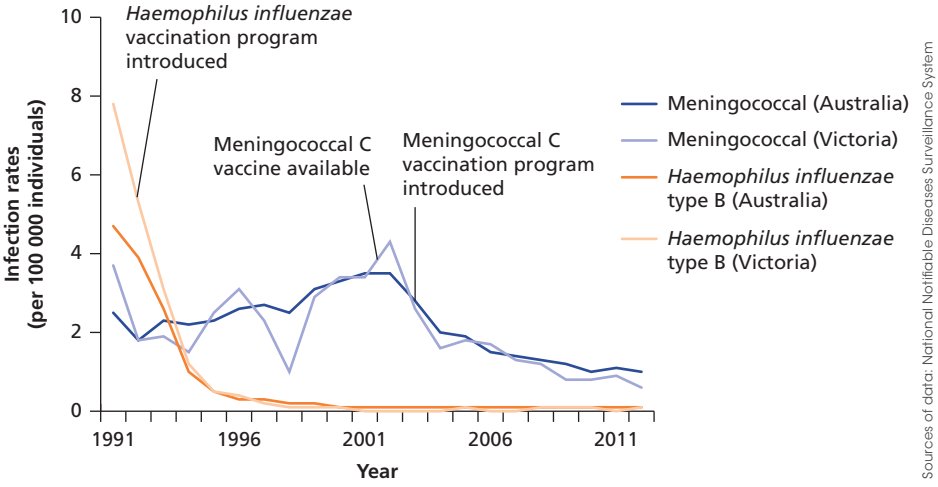
RECAP 6.9

1 List three different commercial uses for antibodies.
2 Describe the advantages of using monoclonal antibodies over antibodies extracted from the serum of immunised rabbits.
3 Under what circumstances might monoclonal antibodies be used as anti-cancer therapy?

Immunisation of populations

Immunisation is a highly effective public health intervention that has substantially reduced worldwide morbidity and mortality from infectious diseases. In Australia, children are routinely vaccinated against a large number of infectious diseases, including hepatitis B, pertussis, measles, tetanus and poliomyelitis. Groups that are at high risk of infection, such as the elderly or chronically ill, may also need additional vaccinations. As new vaccines are developed, immunisation programs against more diseases are being introduced. Figure 6.19 shows the rates of infection of *Haemophilus influenzae* type B and meningococcal C after the introduction of vaccines against these pathogens.

Immunisation programs also have the potential to eradicate diseases by making spread impossible. Smallpox, the first disease for which a vaccine was created, was also the first disease to be eradicated through vaccination. This viral infection, which causes characteristic skin lesions and has a high mortality rate, had been known to infect humans for thousands of years. A coordinated global strategy to eliminate the disease involved mass vaccination as well as targeted vaccination of those who lived near known epidemic areas. In 1980 WHO declared that smallpox

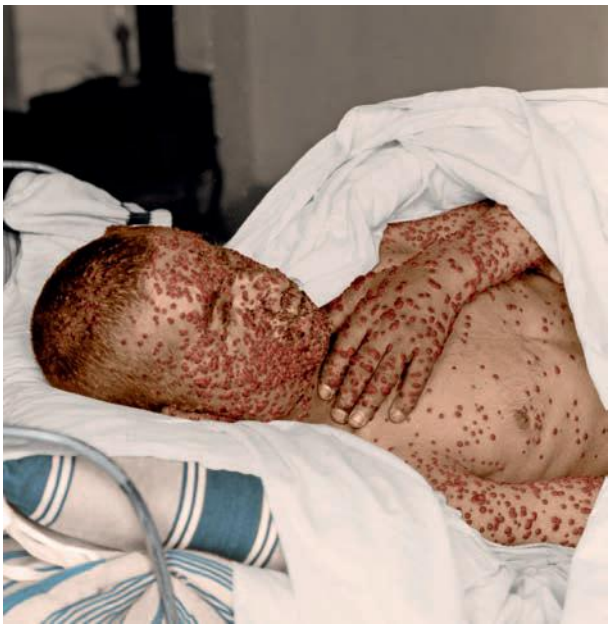


Sources of data: National Notifiable Diseases Surveillance System (NNDSS) and Department of Health, Victoria

◀ Figure 6.19
Rates of *Haemophilus influenzae* type B and meningococcal infection since the introduction of vaccines against these organisms.

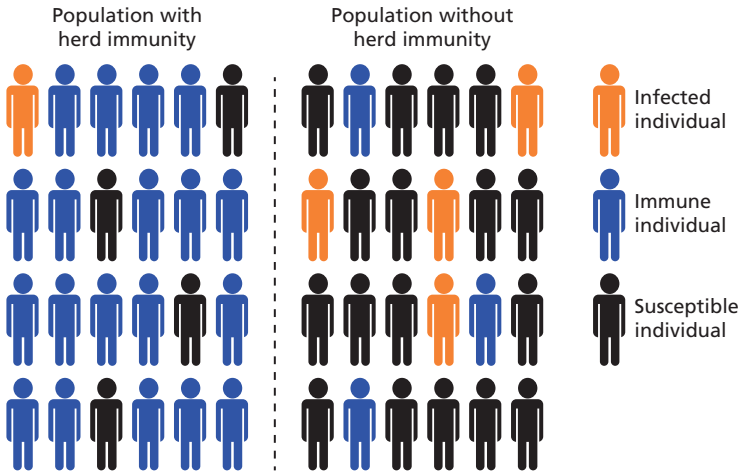
had successfully been eradicated worldwide. Similar attempts to eradicate other diseases such as polio have not yet succeeded.

Not all individuals within a population need to be vaccinated for the spread of a disease to be prevented. If a large enough proportion of the population is immune to a disease, there are too few susceptible individuals to sustain disease spread. This effect is known as **herd immunity**. Figure 6.21 demonstrates why this is the case. Imagine that infected individuals (orange) are only able to spread the disease with those they come into contact with. When there are enough immune individuals (blue), the chance of an infected individual coming into contact with a susceptible individual (black) is so low that the disease cannot spread. For herd immunity to prevent the spread of disease, a high proportion of the population needs to be immune. The exact proportion depends on the virulence and infectivity of a particular disease. Some individuals have health conditions that mean that they cannot be immunised, and so they rely on herd immunity for protection from infection.



Science Source/Robin Treadwell

▲ Figure 6.20
A child infected with smallpox. Vaccination efforts meant that the disease was declared to have been eliminated in 1980.



◀ Figure 6.21
Herd immunity occurs when a large enough proportion of a population is immune to a disease. Disease spread cannot occur as there are too few susceptible individuals.

ACTIVITY 6.1

ADAPTIVE IMMUNE ANALOGIES

Aim

To develop a set of analogies for different parts of the immune system

You will need

- pen
- paper

What to do

1 Working with a partner, brainstorm an analogy for each of the parts of the immune system listed below. Be creative and try to think outside the box! The following is an example of such an analogy for 'vaccine': 'A vaccine is like a trial exam. In a trial exam, exposure to questions trains a student to perform better on the real exam. Similarly, exposure to an antigen in a vaccine trains the body to respond more rapidly and effectively to the real antigen.'

- antibody
- APC
- T_H cell
- T_C cell
- MHC class I molecule
- MHC class II molecule
- phagocyte
- vaccine
- plasma cell
- cytokine
- lymphocyte receptor

2 Combine into groups of three or four and discuss your lists. To what degree does each analogy work? Are there limitations? Decide among the group on the analogy that best fits each term.

3 Present your group's list to the rest of the class. Perhaps you could vote and have a prize for the best analogy.

What did you discover?

Reflect on whether these analogies have helped your understanding of the adaptive immune system. Make a list of things that have become clearer as a result of this exercise.

RECALL

- Immunisation of populations is important to prevent the spread of infections between members of the population.
- Immunisation of populations creates herd immunity, reducing the number of susceptible individuals and preventing disease spread.
- Some individuals with health conditions that prevent them being immunised rely on herd immunity for protection.

RECAP 6.10

1 Some individuals choose not to immunise their children against diseases such as measles and mumps that used to be common in children. How might this affect the prevalence of these diseases in the community?

2 How would you expect the importance of herd immunity to differ between densely and sparsely populated areas?

When the immune system malfunctions

Our immune systems have evolved to provide protection against a wide variety of pathogens. While effective at performing this job, the immune system can also malfunction and cause illness. This may be because the body mistakes a usually harmless substance or its own cells for non-self and launches an attack. The immune system can also fail to defend the body when it needs to, resulting in catastrophic infection. In this section we explore what happens when the immune system misfires.

Allergy

The incidence of asthma and hay fever is around 15% in Australians and is on the increase. These disorders are forms of allergies – exaggerated immune responses to usually harmless antigens.

Most allergic reactions occur when IgE antibodies are produced that can bind specifically to antigens that are normally harmless. These antigens may be pollens, air pollutants, house dust, animal fur, fungal spores, cosmetics or other substances, which are known collectively as **allergens**.

Antibodies that recognise these allergens become attached to the surface of mast cells. When the allergen is present, it binds to the antibodies and causes the mast cell to release its granules full of histamine, cytokines and other inflammatory molecules, in the process of **degranulation**.

These molecules bring immune cells to the affected area, causing localised inflammation. Histamine acts on smooth muscle to cause tightening of the airways, and symptoms also include excessive mucus production in the affected area.

Allergies begin with a **sensitisation** stage, in which the IgE antibodies first begin to be produced. These antibodies arise from an adaptive immune response, involving allergen phagocytosis and presentation to B lymphocytes and T_H cells in a secondary lymphoid organ – in a lymph node or in the MALT, since most allergens are not blood-borne and would therefore not cause a response in the spleen. It is not known why allergens cause an adaptive response in some people and not others, but it seems partly genetically determined and partly environmentally determined. For example, repeated exposure to the allergen can increase the risk of sensitisation.

Allergies can sometimes be treated using a **desensitisation** regime, in which the affected individual is repeatedly exposed (usually by injection) to the allergen in small doses. This brings about a state of **immune tolerance**, stopping or reducing the production of antibodies to the allergen.

In most cases allergies are simply annoying, but they can be life-threatening if they result in **anaphylactic shock**, in which inflammatory responses race through the body leading to constriction of the airways and loss of fluid into body tissues from leaky capillaries. This latter response is due to high levels of histamines and results in a sudden drop in blood pressure, which may lead to a heart attack. Victims of anaphylactic shock need medical treatment urgently to counteract this exaggerated immune response.

RECALL

- Allergies are caused by immune responses against normally harmless antigens, called allergens.
- Antibodies that bind the allergens trigger mast cell degranulation, releasing histamine and other inflammatory molecules that cause the symptoms of allergies.
- Severe allergic reactions can result in anaphylactic shock.

RECAP 6.11

1. List the events that occur during the sensitisation phase of allergies.
2. Discuss why symptoms of allergies are different during the sensitisation phase and later exposures to the allergens.
3. Describe anaphylactic shock and how it differs from less severe allergic reactions.

amanaimages/Corbis

Figure 6.22 ▲
Graves' disease is a form of hyperthyroidism caused by antibodies that bind to the TSH receptor. It can cause bulging of the eyes.

Autoimmunity

Our immune system is carefully tuned to remove or suppress any B or T cells that may respond to antigens in our own body, but sometimes this system of self-tolerance fails and the immune system starts to react against our own tissues. In other words, it responds to self as non-self. This gives rise to what is called an **autoimmune disease**. There are many different types of autoimmune diseases and almost any part of the body can be affected, but symptoms commonly involve the skin, kidneys and joints. The effects the autoimmune disease has on the body depend on which self antigen (known as an auto-antigen) the body is reacting to.

A striking example is Graves' disease, a type of hyperthyroidism. It is caused by antibodies that bind to the thyroid-stimulating hormone (TSH) receptor on thyroid cells. TSH is responsible for stimulating thyroid hormone production within these cells. The binding of these antibodies has the same effect as the TSH and causes extremely high levels of thyroid hormones. Thyroid hormone levels are usually maintained in a narrow range, but because this is an abnormal stimulus the normal feedback mechanism cannot compensate. The high thyroid hormone levels can cause weight loss, diarrhoea, a tremor and anxiety. Graves' disease can also cause inflammation around the eyes, causing them to bulge out of their sockets (Figure 6.22).

While some autoimmune diseases are caused by an error in only one component of the immune system, such as the auto-antibodies that develop in Graves' disease, others can involve many parts of the immune system, particularly where inflammation contributes to the development of the disease. In multiple sclerosis (MS), the oligodendrocytes forming the myelin sheath that surrounds nerve axons come under attack from the immune system and are destroyed. The myelin is therefore a source of **auto-antigens** in MS ('auto' = self, 'antigen' = antibody generator, but also meaning immune response generator in this context). The destruction of myelin, or **demyelination**, impairs the transmission of action potentials along the axon of the neuron, causing neurological problems that can eventually affect almost all aspects of the autonomic and somatic nervous systems.

The causes of MS are not known, but are believed to involve interactions between genetic and environmental factors. Expressing particular MHC alleles may increase an individual's risk of developing MS, as they may for other autoimmune diseases including lupus and type 1 diabetes. This is because normal self-peptides may be more likely to appear as foreign to T_H cells when presented on these MHC alleles.

In MS, T_H cells mistakenly recognise components of oligodendrocytes as foreign and become activated. The blood–brain barrier becomes damaged, perhaps as a result of an

infection, which allows uncontrolled entry of the T_H cells and other lymphocytes into the central nervous system. Once in the brain, the T cells stimulate the activation of macrophages and dendritic cells, which secrete cytokines that attract more leukocytes and other proteins that have direct, damaging effects on the myelin.

The destruction of oligodendrocytes can release more auto-antigens into the circulation and this, in the presence of the inflammatory cytokines, can stimulate the formation of auto-antibodies and more activated T cells. Once this cycle begins, it is very difficult to stop. Some of the drugs used to treat MS aim to block the actions of the cytokines involved or to prevent lymphocyte entry into the CNS, either by keeping cells in lymph nodes or by preventing their migration from circulation through the blood–brain barrier and into the brain.

Immune modifying drugs

Many autoimmune diseases are treated with glucocorticoids, a class of steroid hormone that binds to an intracellular glucocorticoid receptor. The hormone acts as a transcription factor with a range of anti-inflammatory effects, suppressing the immune system and the processes involved in autoimmunity. However, it also has several unwanted side effects, which include weight gain, muscle wasting, osteoporosis, diabetes, cataracts and glaucoma, among others. Alternative drugs can suppress T cells, deplete B cells and inhibit components of the inflammatory response. The immunosuppression caused by these therapies can make patients more susceptible to infections and can impair the ability of their T_C cells to detect and destroy cancer cells.

As scientific knowledge of the causes of disease grows, new drugs are being created that specifically target aspects of autoimmune disease that are essential for its continuation. Cell communication – both within cells and between cells and tissues – is a key part of the development and persistence of autoimmune diseases. Some new drugs that target cell communication include signal transduction inhibitors, monoclonal antibodies that bind to and neutralise cytokines, and drugs that act as a decoy receptor for cytokines, preventing them from binding to receptors on their target cells. **Recombinant cytokines** are also used to modify immune responses in autoimmune diseases. They are produced in genetically modified organisms such as bacteria, yeast and insect cells so that their production can be carefully controlled and scaled up for commercial purposes. Recombinant interferon beta (IFN-β) is used to treat people with early signs of MS to prevent demyelination and inflammation in the central nervous system.

Several other therapies are in use for MS. Some small molecule compounds help to treat MS but their modes of action are unknown. Other drugs have been designed and developed based on a great deal of research into the mechanisms of disease. For example, monoclonal antibodies are now used to bind and block the cell adhesion molecules necessary for T_H cell entry into the central nervous system.

RECALL

- Autoimmune diseases occur when the immune system recognises self-antigens as non-self. These self-antigens are also called auto-antigens.
- Multiple sclerosis is an example of an autoimmune disease. The auto-antigens are found within myelin, and the myelin is destroyed by innate and adaptive immune processes.
- Steroid hormones have been used to treat autoimmune diseases by bringing about a state of immunosuppression. Monoclonal antibodies and recombinant cytokines are among newer classes of drugs that can more specifically target autoimmune disease processes.

RECAP 6.12

1 Name four types of autoimmune diseases.
2 List the processes that occur to cause MS.
3 Describe three different therapies for autoimmune diseases.

Immunodeficiency

In some people, the immune response is not sufficient to fight off pathogens that are usually easily defeated. Conditions where a defective immune system renders someone vulnerable to infection are known as immunodeficiency. Immunodeficiency may be caused by genetic defects or acquired later in life.

Primary immunodeficiency

Immunodeficiencies that are caused by genetic factors, and therefore inherited, are **primary immunodeficiencies**. For example, severe combined immunodeficiency (SCID) is an inherited condition that results in a severely reduced or totally absent army of B and T lymphocytes. People with SCID may develop infections that generally have little effect on most of the human population. The main way of treating SCID is with a bone marrow transplant, which provides the patient with a new immune system.

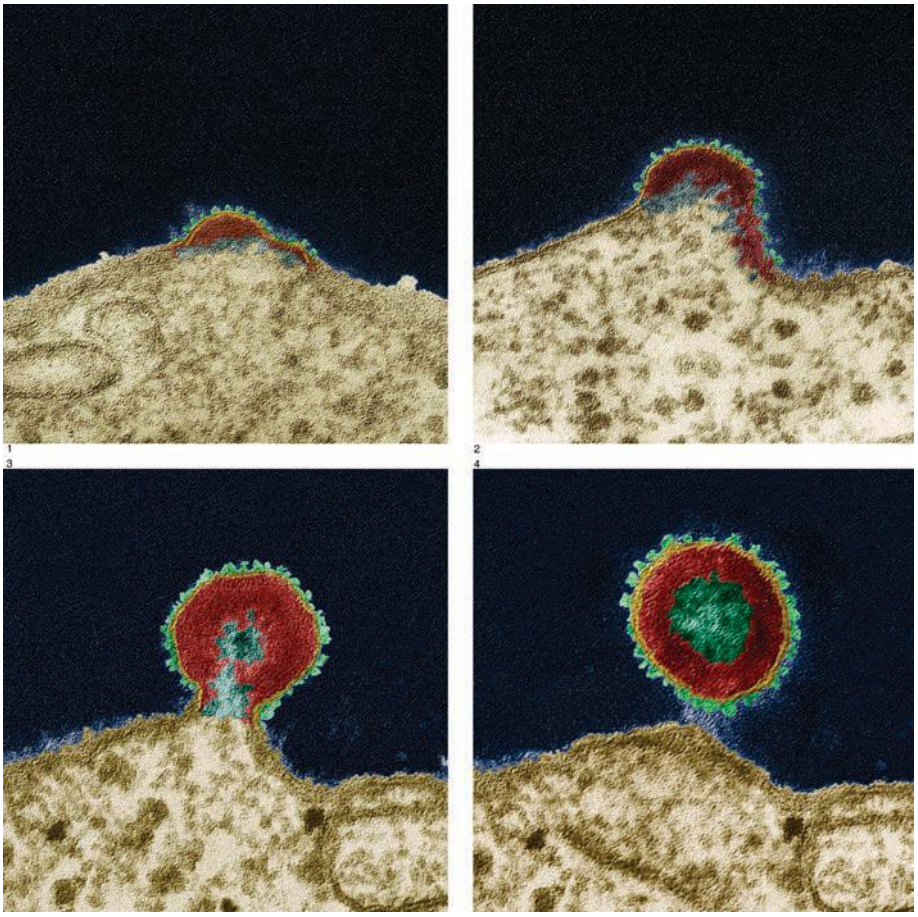
For a bone marrow transplant, the patient is given high levels of chemotherapy designed to destroy their bone marrow. The next step is harvesting bone marrow or stem cells from the blood of a donor. The donor may or may not be related to the patient, but needs to have matching MHC proteins; otherwise the MHC proteins presenting self peptides may appear to present foreign peptides to T_H cells, thereby acting as auto-antigens. The donor cells with matching MHC alleles are infused into the patient; the cells settle in the bone marrow and begin to divide.

By replacing the bone marrow stem cells, the procedure replaces all of the cells of the immune system. As such, the patient will now be able to produce functional lymphocytes and fight infections. Sometimes this new immune system recognises the body as non-self and mounts an immune attack of its own, called graft-versus-host disease (GVHD). This is the opposite of the immune system rejecting a transplanted organ – the transplanted immune system is rejecting the body. Because of GVHD and other risks associated with bone marrow transplants, scientists and doctors are also trialling forms of gene therapy to try to replace the faulty gene.

Acquired immunodeficiency

Immunodeficiency may also be acquired, such as by contracting a disease or by taking immunosuppressive drugs following organ transplantation. This type of immunodeficiency can be classified as a **secondary immunodeficiency** and is not primarily genetically determined. For example, the human immunodeficiency virus (HIV) is a viral infection that results in a severe form of immunodeficiency known as acquired immunodeficiency syndrome (AIDS). More than 35 million people are infected worldwide and this number is steadily increasing. HIV is a major cause of mortality in parts of Sub-Saharan Africa where infection rates are extremely high.

HIV generally targets T_H cells by binding to specific receptors on these cells and injecting its RNA. The T_H cell is then stimulated to produce more viral particles, which bud from the host cell's own plasma membrane, ready to infect other T_H cells (Figure 6.23). Eventually, this kills the T_H cell. The virus spreads through the immune system, slowly depleting the army of T_H cells, eventually causing AIDS.



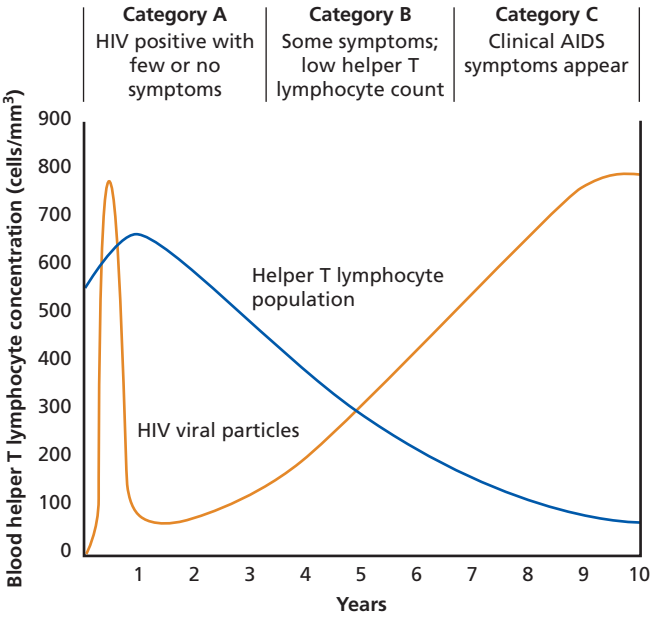
Science Photo Library/Eye of Science

◀ **Figure 6.23**
Transmission electron micrograph of HIV budding out of a T_H lymphocyte

Early in an HIV infection the immune system is capable of producing a response against the virus. However, as more and more T_H cells become disarmed, the effectiveness of humoral and cell-mediated responses decreases and the number of viral particles present increases (Figure 6.24).

After several years, an infected individual becomes prone to multiple infections from pathogens that would be relatively harmless under normal conditions. Many sufferers die from simple yeast or bacterial infections, pneumonia, or unusual tumours and cancers.

As yet there is no cure for HIV infection. Drugs have been developed to reduce the spread of the virus in the host, although they do not destroy it. These drugs can prevent viral replication and the development of AIDS, and their discovery has significantly improved the lifespan of people living with HIV. Despite this, financial and logistic barriers mean that these drugs remain inaccessible to many people infected worldwide.



▲ **Figure 6.24**
Stages of infection by HIV

Advances in microbiology, genetics and molecular biology may produce more effective treatments and possibly a cure. The best defence at the moment is prevention of transmission. HIV is found in human body fluids and spreads directly, primarily through sexual contact and through entry into blood by sharing of syringes and transfusion of contaminated blood. It can also be transmitted to infants during pregnancy and during birth. Knowing the method of transmission assists control of the spread of disease.

RECALL

- Primary immunodeficiencies are genetically determined, whereas secondary deficiencies are acquired.
- Primary immunodeficiencies can be treated by bone marrow transplant, for severe cases, or by gene replacement therapies.
- HIV causes immunodeficiency as it specifically infects and destroys T_H cells.

RECAP 6.13

1 Explain why children born with SCID had to be contained in an isolated environment.
2 Describe why HIV causes immunosuppression of many aspects of the immune system even though it targets only a single cell type.
3 List three ways of preventing HIV transmission.

Biological knowledge and society: Tracking the flu to prevent a pandemic

Influenza (the flu) is a contagious disease of the respiratory tract caused by infection with influenza virus. Globally, approximately 500 million people suffer with the flu each year. In Australia, 1500 deaths are associated with flu infections annually. The very young, the sick and the elderly are most susceptible. Influenza symptoms of fever and muscle ache require bed rest, which impacts the Australian economy through lost days at work.

How influenza enters and leaves host cells

Human influenza virus infects cells of the respiratory system. Once inside the host, a protein on the virus surface called haemagglutinin (H) attaches to sialic acid residues located on the tip of glycoproteins that protrude from the host cell membrane (Figure 6.25). H is the key required to gain entry into the host cell. Once the virus has attached, receptor-mediated endocytosis is triggered so the human cell engulfs the virus. Inside the human cell, the virus tricks the cell into replicating viral components. Virus capsids assemble, enclosing a set of virus genes. Hundreds of viruses start to bud from the host cell. But as they try to leave, their H proteins get stuck to the host cell membrane. A second protein on the surface of the virus, an enzyme called neuraminidase (N), acts like scissors to cut the virus free from the cell. The virus then infects another cell in the respiratory system, or leaves the host in mucus drops as an infected person coughs, sneezes or talks. If a susceptible host is infected, it will become sick.

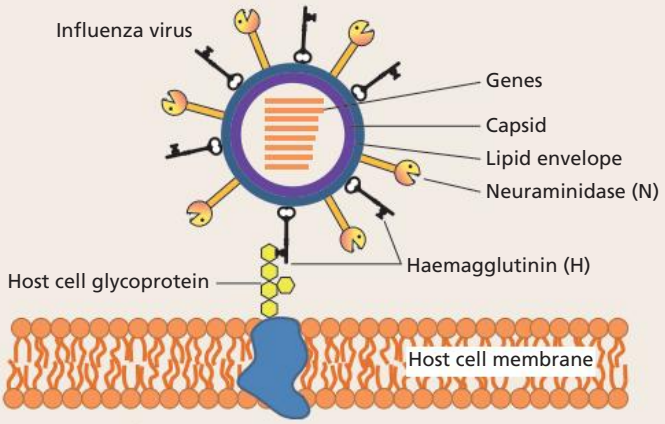


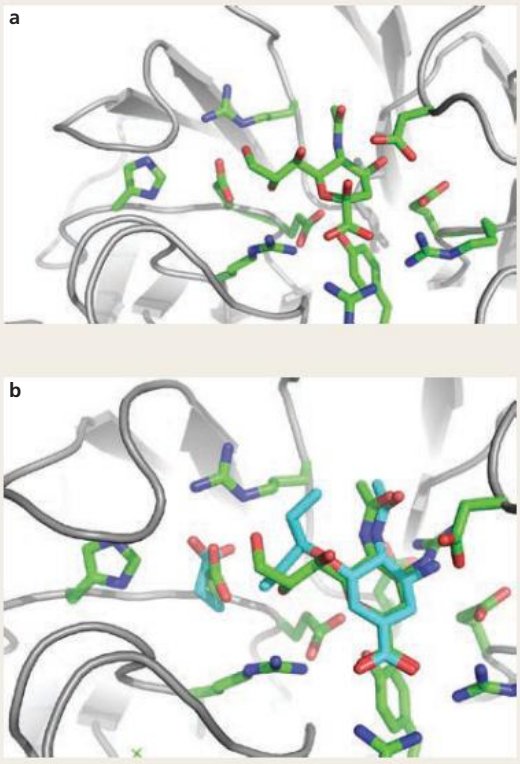
Figure 6.25 ▲
Influenza virus uses haemagglutinin (H) protein to enter a host cell. A second protein, an enzyme called neuraminidase (N), is required to release budding viruses from the host cell membrane.

Exploring rational drug design for making a flu antiviral treatment

Antibiotics work only against bacterial infections. There are relatively few antiviral

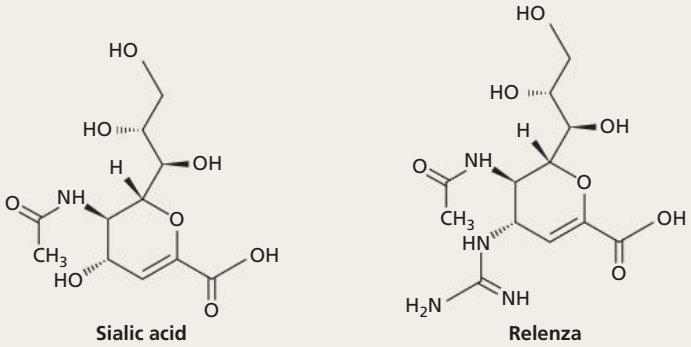
drugs available, especially for treating influenza. A team of scientists led by Professor Peter Colman became interested in the N protein in a search for a drug against influenza. They wondered if they could discover a way to stop N from working. If they could, then this might stop the virus from spreading to new cells, thus stopping the infection. The team cloned and sequenced the N gene of two flu viruses, the first from a strain that had caused the Asian influenza pandemic in 1957 and the second from a strain that had caused an epidemic in 1967. The team also used X-ray crystallography to help determine the three-dimensional structure of the N protein. On comparing the two structures they discovered a part of the protein that was highly conserved. This means there were not changes in this part of the protein between the 1957 and the 1967 strain. They had discovered the active site. The active site of N has since been found to be highly conserved in all strains of influenza infecting humans and other animals.

The normal substrate of N is sialic acid. It is found at the terminus of the cell membrane glycoproteins that H binds to. When sialic acid enters the active site of N, bonds in the glycoprotein become stressed and break, cutting the virus free of the host cell so it can go off to infect more host cells. The team investigated how the substrate, sialic acid, interacts with the conserved amino acids in the active site of N (Figure 6.26). They then looked for an inhibitor molecule with a similar structure to sialic acid. However, they wanted this inhibitor to bind irreversibly to the active site so that N would no longer be able to cut the virus free of the host cell. This process is known as rational drug design: using knowledge of the structure and properties of a protein active site to find an effective inhibitor.



Peter M. Colman, The Walter and Eliza Hall Institute of Medical Research

▲ **Figure 6.26**
(**a**) The product of the neuraminidase-catalysed reaction, sialic acid, bound to the enzyme. (**b**) Overlay of zanamivir (green) and oseltamivir carboxylate (cyan) bound to neuraminidase.



▲ **Figure 6.27**
Sialic acid is the natural substrate for N. Relenza is a drug designed to inhibit the action of N.

Over to you

1 The team designed an inhibitor and called this drug Relenza. Sialic acid and Relenza are shown in Figure 6.27. Describe any differences you can see on the Relenza drug molecule compared to sialic acid, the natural substrate of N.

2 These differences make it bind to the active site of N more strongly, making Relenza an inhibitor of N. Referring to Figure 6.25, draw a diagram to represent how Relenza works as an influenza preventative by stopping virus particles from leaving a host cell.

Using Relenza to control a pandemic

Relenza is not available to treat influenza in Australia unless a person is at risk of severe flu, such as if they are pregnant or have asthma. One reason for this is to decrease the likelihood that the influenza virus will evolve resistance to this drug. A second reason is to ensure adequate supplies in the case of a pandemic. The drug can be taken as a preventative in the case of an outbreak. The government will give the drug to personnel involved in jobs required to ensure society does not break down.

Over to you

3 Make a list of ten services essential to society that you would supply with Relenza in the case of a flu pandemic. Defend your choices.

Controlling influenza epidemics

There are three types of influenza that can infect humans. Type A and type B can cause epidemics, while type C only causes mild infections. Type A viruses can infect a number of different species and are further divided into subtypes based on their H and N surface proteins. There are 18 different H and 11 different N. These proteins vary slightly in structure as a result of mutations that have arisen in the H and N genes. H and N proteins are the antigens that trigger an immune response to the virus. If these surface proteins change, the new flu virus may not be recognised by our immune system and can cause serious illness before an immune response develops. Melbourne is generally the first city in the southern hemisphere to succumb to new strains of influenza. Figure 6.28 reveals the peak outbreaks of influenza infection during the 2009 H1N1 pandemic.

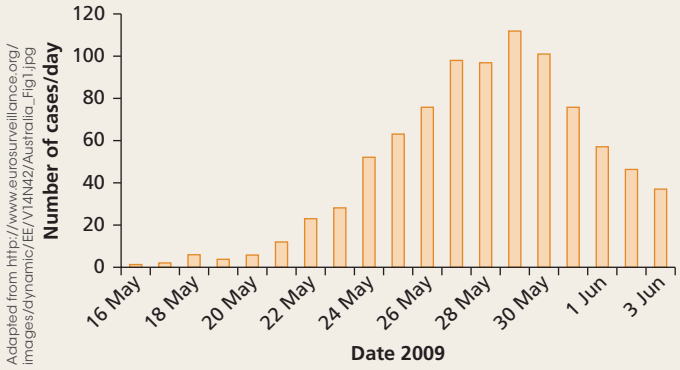
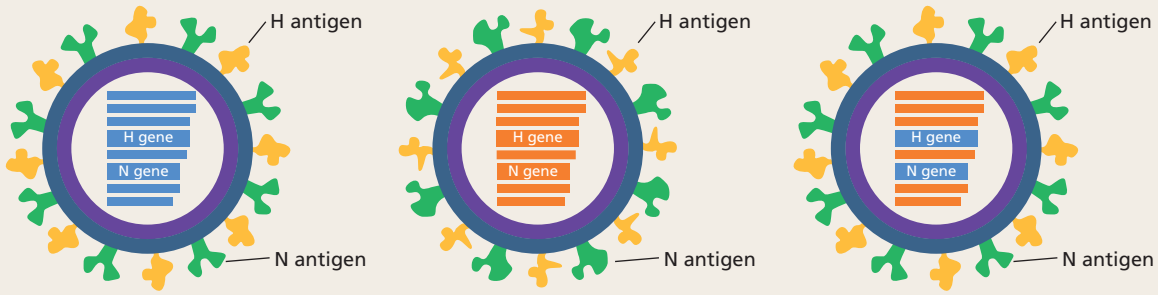


Figure 6.28 ▲
Confirmed cases of influenza A/H1N1 in Victoria each day during the 2009 pandemic

The World Health Organization (WHO) tracks influenza outbreaks globally. They find out when and where outbreaks are occurring, determine the type of influenza circulating, and measure the impact it has on hospitalisations and death. The most severe strains can be controlled by producing a vaccine. Information about influenza in the northern hemisphere can be used to prepare vaccines against flu strains that will arrive in the southern hemisphere. Figure 6.29 reveals how flu vaccines are prepared.



1 Virus we want protection against

2 Harmless flu virus that grows well in chicken eggs but cannot infect human cells

3 This virus cannot infect humans but the H and N antigens are the same as those in the virus we want protection against. It is used in vaccines.

Figure 6.29 ▲
To make a vaccine against a new strain of influenza virus, take the genes for H and N from that virus, virus 1, and put them into a virus that cannot infect humans, virus 2. You now have a new virus, virus 3, that is harmless to humans but produces the H and N antigens from the virus we want protection against. Infect chicken eggs with virus 3 so it replicates. Use this virus to make the vaccine.

When a vaccine is injected into your arm, your body mounts an immune response against the H and N antigens of the viruses in that vaccine. As a result you produce memory cells that will provide a faster and larger immune response if you encounter that same antigen again. You will rapidly produce antibodies that control the infection. You probably will not experience any flu symptoms.

Over to you

4 Construct a flow chart to show the sequence of events that occur when you are infected with influenza. On your flow chart show both vaccinated and unvaccinated responses.

Over to you: Investigating an ethical question

There is increasing pressure to make annual vaccinations against influenza mandatory for healthcare workers. The Peter MacCallum Cancer Centre has had a mandatory influenza vaccination program since 2009. However, there are no punitive consequences for staff who refuse to participate.

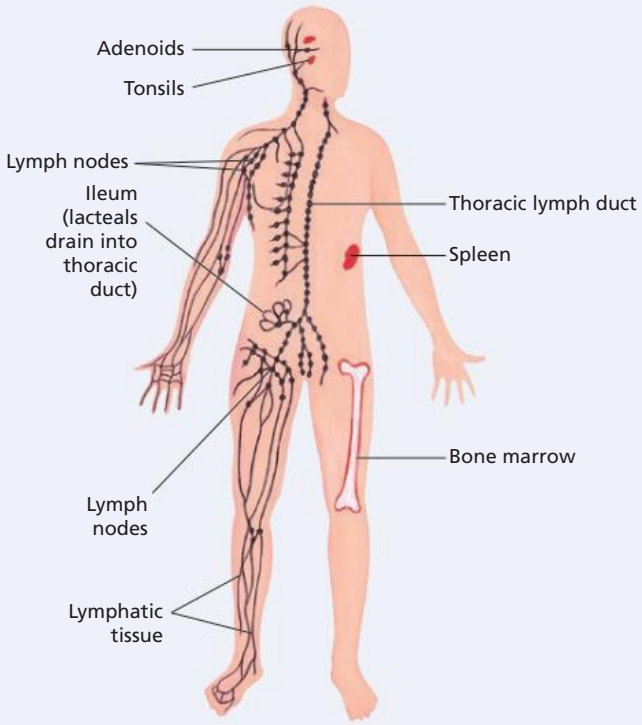
5 Do you think that annual influenza vaccinations for everyone should be mandatory? What social and ethical issues do you see? Use the guiding principles in Table 6.5 to assist you to formulate a decision on this issue.

Table 6.5 Guiding principles used as a framework for analysing issues in biology

Guiding principle	Description	Considerations
Individual rights	Every individual has the right to autonomy – to be their own person and choose their own course of action.	Are the individual rights of all individuals considered and respected? Is informed consent provided? Is there respect for privacy and confidentiality?
Beneficence	A duty to do more good than harm. To consider the welfare of the research participant.	Who will benefit from this technology? How will they benefit? (can include physical, psychological, economic or social benefits) How many will benefit?
Non-maleficence	The duty not to cause harm	Who might be harmed by this technology? How are they harmed? (can include physical, psychological, economic or social harms) How many are harmed? Is it possible to minimise the harm?
Justice and equity	Fair and equitable treatment for all	Can everyone have equal access? How can discrimination be avoided?

CONCEPT SUMMARY

Lymphatic system



Key components

Interstitial fluid	Lymph
Primary lymphoid organs	Secondary lymphoid organs
Bone marrow	Thymus
MALT	Spleen
Lymph vessels	Lymph nodes

One of the ways an adaptive immune response is initiated is by migration of pathogen-activated dendritic cells into lymph nodes, where they present the pathogen antigens to B and T lymphocytes.

T cells

T lymphocytes have a T cell receptor (TCR). This recognises peptide antigens carried on MHC molecules. T helper cells recognise peptide antigens presented on MHC class II molecules (only on professional antigen presenting cells: dendritic cells, macrophages, B cells). Cytotoxic T cells recognise peptide antigens presented on MHC class I molecules (on all nucleated cells). T cells only recognise antigens presented on the MHC molecules expressed by that individual – a phenomenon known as MHC restriction. Self-peptides should not initiate a T cell response. Non-self peptides are recognised as foreign and initiate a T cell response.

When T helper cells recognise their specific epitope, they become activated and can:

- become clonally selected and undergo clonal expansion
- secrete cytokines and express membrane proteins that stimulate cytotoxic T cells and B cells (via paracrine and contact-dependent signalling)
- secrete cytokines that assist macrophages in their phagocytic function
- limit the magnitude of inflammation and the adaptive response (T regulatory cells).

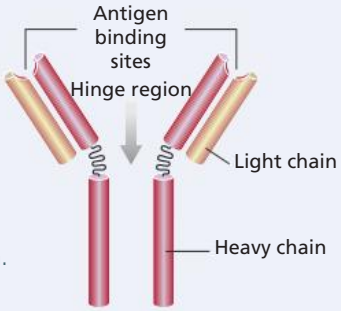
When cytotoxic T cells recognise their specific epitope, they:

- require T helper cells to become properly activated
- become clonally selected and undergo clonal expansion
- release perforin, a protein that forms pores in the target cell membranes that allows granzymes, proteins that induce apoptosis, to diffuse from the T cell into the target cell
- express death receptors that initiate the extrinsic (external) pathway of apoptosis.

This is the basis of cellular immunity.

B cells

B lymphocytes express antibodies as secreted proteins or as membrane-bound proteins (B cell receptor). Antibodies have different isotypes: IgM, IgD, IgG, IgE, IgA. These differ in their constant regions and have different functions including opsonisation, complement activation, neutralisation and agglutination. The antibody's variable regions determine the specificity of the epitope they can recognise.



When B cells recognise their specific antigen, they:

- can undergo clonal selection and expansion (this is improved by T helper cell assistance in the form of cytokines and contact-dependent signals)
- can produce plasma cells, which produce great amounts of secreted antibodies
- can produce memory B cells, which enter the circulation and are able to differentiate into plasma cells rapidly upon subsequent exposure to the same antigen.

This is the basis of humoral immunity.

The adaptive and innate immune responses are closely linked

- Antigen presenting cells
  - Present antigens for activation of B and T cells
  - Produce cytokines for activation of B and T cells
- Antibodies binding to pathogens can stimulate their recognition and phagocytosis
- T cells (T_H and T_C) can produce cytokines that stimulate phagocytosis and destruction of phagocytosed pathogens
- Phagocytes clear apoptotic debris resulting from T_C action.

Assisted immunity

Assisted immunity is particularly necessary when hosts are immunodeficient or immunosuppressed.

Immunisation

This process induces active immunity in an individual using a vaccine to stimulate a primary response. Vaccines include antigens from the pathogen (either killed or inactivated) together with an adjuvant to provide PAMPs. Herd immunity can protect individuals that are not immune, but this relies on a large enough proportion of protected individuals in the population.

Passive immunity

Examples include:

- transfer of antibodies from mother to foetus via the placenta
- transfer of antibodies from mother to baby via breast milk
- anti-venom
- purified solutions of antibodies against particular pathogens, such as rabies, ready for injection
- mix of antibodies for immunodeficiency.

Monoclonal antibodies

Monoclonal antibodies are produced by a hybridoma. They are widely used in the laboratory, in diagnostics and in therapeutics. In cancer, monoclonal antibodies are used to bind to cancerous cells to stimulate their destruction by TC cells or phagocytosis, bind and block signalling between growth factors and their receptors on cancer cells, bind an inhibitory receptor to block negative regulation of T cells, and deliver radioactive substances to the cancer cells.

Immune system malfunctions

Allergy

Allergy occurs when IgE antibodies are produced that can bind specifically to antigens that are normally harmless (allergens), such as pollen. It involves degranulation of granulocytes (mast cells and basophils), releasing histamine and initiating inflammation. There is a sensitisation stage, and in some cases desensitisation can treat the allergy.

Autoimmunity

Autoimmunity occurs when B or T cells respond to antigens in our own body, failing to recognise 'self'. There are many different autoimmune diseases, depending on the autoantigens involved.

Multiple sclerosis:

- The autoantigen is found within myelin.
- An inflammatory immune response causes demyelination and neurodegeneration.
- Treatments for MS include recombinant cytokines that modify the immune response, and monoclonal antibodies that block chemotaxis of T cells or their passage through the blood–brain barrier.

Immunodeficiency

- Results in a defective immune system that leaves the individual susceptible to infection.
- Primary immunodeficiencies are genetically determined and include severe combined immunodeficiency.
- Secondary immunodeficiencies are acquired as a result of disease or immunosuppressive drugs. An example is acquired immunodeficiency syndrome (AIDS), which results from infection with the human immunodeficiency virus (HIV), which infects and destroys T_H cells.

CHAPTER GLOSSARY

active immunity the immunity formed by stimulation of the immune system with an antigen and the generation of effector and memory cells; it is contrasted with passive immunity

adaptive immune response an immune response that is directed against a specific antigen and retains memory of that antigen, responding with a secondary response on subsequent exposure to the same antigen

adjuvant a substance added to a vaccine along with an antigen that contains pathogen-associated molecular patterns to improve the immune response to that antigen

agglutination when antigens or pathogens become stuck together because of antibody binding

allergen an antigen that is normally innocuous but in some circumstances causes allergy

anaphylactic shock a severe form of allergic reaction that causes widespread swelling, including of the face and neck, which can make breathing difficult

antibody a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called immunoglobulin

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antigen presenting cell (APC) a cell that displays peptides derived from processed antigens on MHC class II molecules for presentation to T_H cells; includes B cells, macrophages and dendritic cells

auto-antigen (auto means 'self') a normal body component that activates an immune response in autoimmune disease

autoimmune disease when the body's own immune system attacks a normal body component

B cell receptor (BCR) a surface-bound antibody that serves as a receptor so that B cells are able to detect antigens

B lymphocyte/cell a class of lymphocytes; once activated, they are characterised by the production of antibodies

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

cytokines signalling molecules that coordinate inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

cytotoxic T (T_C or killer T) lymphocyte/cell a class of lymphocytes that destroys virally infected or cancerous cells by secreting proteins that cause apoptosis

degranulation a cellular process where the granules of the neutrophils, mast cells, basophils or eosinophils are emptied into the extracellular surroundings

demyelination destruction or removal of the myelin sheath that surrounds the axons of neurons

dendritic cells antigen-presenting cells that phagocytose and present antigens to cells of the adaptive immune system

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

epitope a small part of an antigen, that is specifically bound by antigen receptors such as B cell receptors and T cell receptors

helper T (T_H) lymphocyte/cell a class of lymphocytes that aids T_C cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling

herd immunity the concept that unvaccinated people are protected from diseases if almost everyone else (around 95% of people) is vaccinated

humoral immune response an immune response mediated by antibodies

hybridoma a hybrid cell created by fusing a B cell clone with cells from a plasma cell tumour; produces monoclonal antibodies and divides repeatedly

immune system a complex network of cells, tissues and organs in the body that detects differences between self molecules and foreign organisms and mounts an immune response that results in formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

immunisation the introduction of a vaccine to generate antibodies and memory lymphocytes that respond rapidly on encounter with the pathogen

immunodeficiency a state in which the immune system does not function properly, leaving a person susceptible to infections a healthy immune system could normally fight off

immunoglobulin (Ig) see **antibody**

immunosuppression reduction of the activation or efficacy of the immune system, for example by drugs that prevent transplant rejection or treat autoimmune diseases

innate immune response a response to a pathogen that is not specific and does not generate antibodies or memory lymphocytes

interstitial fluid a fluid that lies in the spaces between cells and drains into the lymphatic system; also known as tissue fluid or extracellular fluid

isotype a subtype of immunoglobulin; each isotype (IgG, IgM, IgA, IgE and IgD) performs a different function

lymph a colourless fluid that originates from the extracellular (tissue) fluid

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and in removing foreign matter

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes for presentation to lymphocytes and initiation of an adaptive response

major histocompatibility complex (MHC) protein markers found on the cell surface that are important in distinguishing self from non-self. There are two classes: MHC class I is found on all cells and MHC class II is found only on antigen presenting cells

MALT mucosal-associated lymphoid tissue; secondary lymphoid tissue in which adaptive immune responses occur

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

monoclonal antibody antibodies produced by a hybridoma with specificity against a single antigen; their specificity is identical to the antibodies produced by the original cell

neutralisation the process by which antibodies prevent toxins from acting; that is, by binding to them and blocking them from binding to their targets

opsonisation a process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

passive immunity immunity characterised by the transfer of antibodies from one individual to another; this type of immunity does not generate immunological memory

plasma cell an effector B cell that has differentiated to become highly specialised for producing antibodies

primary immunodeficiency an inherited immunodeficiency resulting from mutations in the genome

primary lymphoid organs the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

recombinant cytokines cytokines manufactured in bacteria using genetic engineering techniques, see Chapter 11

regulatory T (T_{reg}) lymphocyte/cell a class of lymphocytes that helps to negatively regulate the immune response

secondary immunodeficiency immunodeficiency acquired as the result of an environmental factor such as HIV infection

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and MALT

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self antigens to prevent an immune response to these antigens

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens presented on MHC proteins

tumour an abnormal growth of tissue

vaccine an injected solution of dead or weakened antigens or pathogens, together with an adjuvant, that is used for the process of immunisation

CHAPTER REVIEW QUESTIONS

Remembering

1 Identify two functions of the MHC proteins.

2 Recall where the following cell types undergo their development:
- a T cells
- b B cells

3 The ability to distinguish between self and non-self antigens is crucial to the functioning of the immune system.
- a Define what is meant by the term 'self antigen'.
- b Outline how the lymphocytes learn to distinguish between self and non-self antigens.
- c Discuss the problem that can occur if the immune system responds to self antigens.

4 Draw a diagram comparing the amount and speed of antibody production in response to an antigen after the first and second exposures.

5 List the different ways that antibody binding can inhibit pathogens.

6 a Describe the role of T_H cells.
- b Identify how they are able to perform this function.

7 List three ways that dysfunction of the immune system can cause disease, giving specific examples.

8 Identify whether the following statements are true or false.
- a Immunodeficiency can be inherited or acquired.
- b An autoimmune disease is one where the immune system attacks the body's own cells.
- c People born without B cells can mount a fully functional adaptive immune response against a virus.
- d An allergy is caused by the body's immune response to a self antigen.

Understanding

9 Describe what is meant by the term 'clonal selection', using B cells as an example.

10 Millions of different antibodies are able to be made by our B cells, even though our genome has only around 30 000 genes. Explain how this is able to occur.

11 Passive immunity does not display memory. Present an argument as to why this is the case.

12 Draw a diagram to illustrate one way that antibodies can be produced for commercial uses.

13 Explain how vaccinations work to prevent infection, giving a specific example.

Applying

14 Liver, heart and kidney transplants are now fairly common procedures in many hospitals. However, recipients of these transplants face the problem of rejection of these organs.
- a Explain why the immune system rejects these organs.

Transplant patients are usually prescribed immunosuppressant drugs to prevent transplant rejection. Many immunosuppressant drugs work by interfering with DNA synthesis.
- b Suggest a negative effect that these drugs may have on the health of the patient.
- c Explain how a drug that interferes with DNA synthesis can prevent transplant rejection.
- d A patient with kidney failure was successfully 'cured' with a kidney transplant from his identical twin brother. He was concerned that the doctor did not prescribe immunosuppressant drugs. Are the patient's fears warranted? Justify your response.

15 Australia has many venomous snakes. One species, commonly called the death adder (*Acanthophis antarcticus*), has one of the most dangerous bites in the world. The active component of the venom is an alpha-neurotoxin that binds to the receptor sites for acetylcholine. Paralysis of muscles results and death can occur when the muscles of the diaphragm become paralysed and breathing is prevented.
- a What type of substance is acetylcholine?
- b Describe the function of acetylcholine.
- c Explain how the alpha-neurotoxin prevents acetylcholine from working.

Fortunately, there is an anti-venom available to people who have been bitten by a death adder. If the anti-venom is injected quickly enough, it prevents the paralysis. Anti-venom is prepared by injecting tiny amounts of snake venom into a horse over a long period of time. The amounts of venom injected are so small that the horse is unaffected; however, there is a response by the horse's immune system.

d Name the substances the horse would produce in order to counteract the snake venom in its body.

e Name the cells in the horse that would be responsible for the formation of this substance.

f Explain why small amounts of venom are injected into the horse over a long period of time.

g Outline the steps involved in the formation of these substances.

h After 10–12 months blood is extracted from the horse and the plasma can be injected into snakebite victims. Identify what term is given to the use of horse plasma as a treatment for snakebite.

i Explain how this is effective in treating the snakebite victim.

16 Monoclonal antibodies are commonly manufactured using a hybridoma. A hybridoma is made from a B cell fused to a cell from a plasma cell tumour.

a Describe two situations in which monoclonal antibodies may help save a person's life.

b Patients with plasma cell tumours have an abnormally high level of a protein (called a paraprotein) in their blood, which can 'clog' the fine capillaries of the kidneys where blood is filtered to produce urine, and cause kidney failure. Predict what type of protein this paraprotein consists of.

c Explain what property of tumour cells makes them useful for fusing to B cells for monoclonal antibody production.

17 Immune thrombocytopenic purpura (ITP) is an autoimmune disease where the platelet counts of those affected drops extremely low. These patients may develop bruising, rashes and, in extreme cases, severe internal bleeding. Antibodies against platelet surface markers can often be found in the bloodstream of patients with ITP.

a Recall the role of platelets.

b Explain how the formation of anti-platelet antibodies may lead to the symptoms described.

c Platelets from blood donations can be given to patients as a transfusion. Predict whether or not these would be effective at preventing symptoms in patients with ITP. Justify your response.

18 Explain how the body's ability to distinguish between self and non-self is important in the development of autoimmune diseases.

19 Explain how HIV infection impacts on the immune system.

20 Figure 6.30 shows the response to two different doses of a vaccine against tetanus.

a Explain the body's primary response.

b Explain the trend shown following the second dose of antigen.

c Copy the graph and add a second line that shows the expected response if the same person was exposed to a first dose of a vaccine against diphtheria at 60 days.

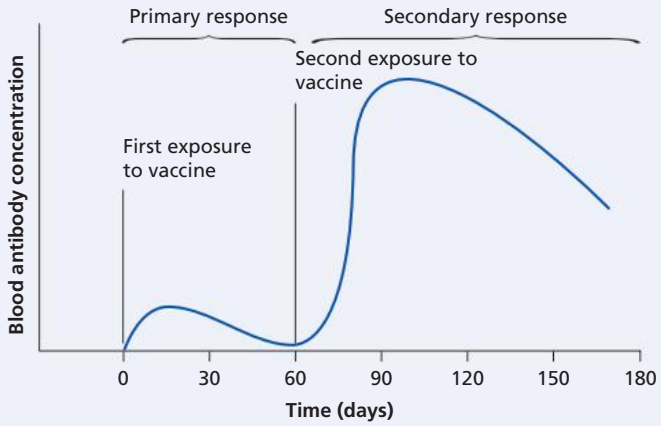


Figure 6.30 ▲
Response to vaccination against tetanus

Analysing

21 Compare the roles of the humoral and cell-mediated immune responses with regard to the type of pathogen targeted and how pathogen destruction is brought about.

22 Compare and contrast the MHC class I and MHC class II molecules.

23 Figure 6.31 is another graft-rejection experiment that builds upon the one you have seen in Figure 6.14 on page 199. The aim of this experiment is to determine if the memory that the immune system exhibits with regard to graft rejection can be transferred between individuals.

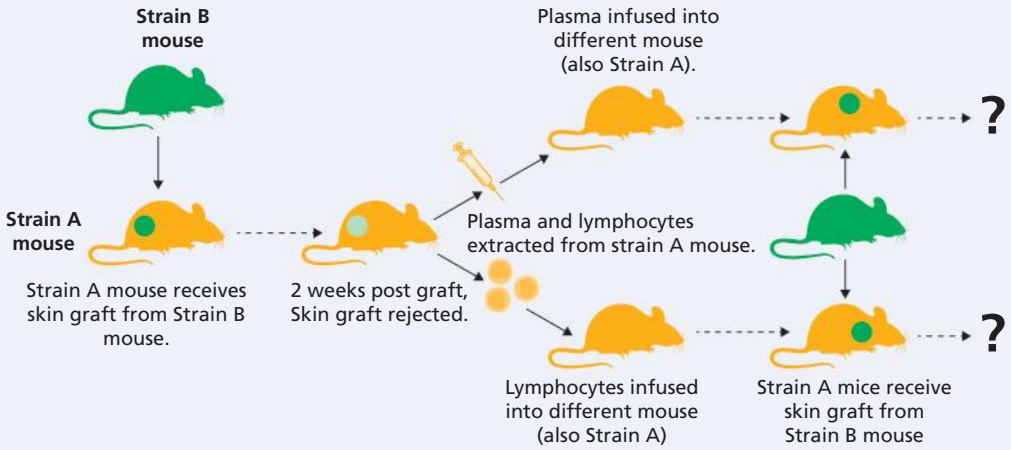


Figure 6.31 ▲
Experimental set-up to see if immune memory can be transferred between individuals

- **a** Name the components of the immune system that are responsible for graft rejection.
- **b** Identify which part(s) of the blood (plasma, lymphocytes or both) would be expected to contain these components.
- **c** Predict how long it will take each mouse (the one that has received the plasma and the one that has received the lymphocyte infusions) to reject the skin graft. Explain your reasoning.
- **d** In this experiment the infused lymphocytes are not rejected by the recipient's immune system. Explain why this is the case.

24 A mutation in a single gene found on the X chromosome can prevent B cells from maturing. This causes the condition known as X-linked agammaglobulinaemia (XLA) in which patients produce extremely low levels of antibodies.

- **a** Predict whether XLA is an immunodeficiency or autoimmune condition.
- **b** Draw a line graph to show the normal response to first and second exposure to a vaccine. Add a line to show the response to the same vaccine you would expect in somebody with XLA.
- **c** Miriam is planning to start a family but her father had XLA and she is concerned she could pass on the condition to her children. Use your knowledge of genetics to calculate the risk of Miriam's children developing XLA. Does it make a difference whether Miriam has a boy or girl?

Evaluating

25 The adaptive immune system is sometimes described as more 'sophisticated' or 'important' than the innate immune system. Evaluate whether either or both of these adjectives fits.

26 Provide an argument for or against the following statement: 'The importance of antibodies as technological tools has almost come to surpass that of their original function.'

Creating

27 Draw a diagram that shows all the different defences encountered by an antigen when it enters the body. Be sure to indicate how these different defences communicate.

28 Consider the workings of the immune system. Synthesise your knowledge of biology and immunology to create a presentation discussing some of the different challenges medical researchers face when tackling diseases such as cancer, influenza and multiple sclerosis.

29 Now that you have completed your studies of the innate and adaptive immune systems, prepare a brief summary that distinguishes between the two. You may like to use a table or Venn diagram.

30 'Humans have evolved to not generate lymphocyte receptors that can bind to self-antigens.' Do you agree with this statement? Justify your response.

Reflecting

31 Describe how learning about some of the commercial applications of antibody technology has impacted upon your understanding of how antibody structure relates to its function.

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES OVER TIME?

Area of study 1:
How are species related?

Area of study 2:
How do humans impact on biological processes?

Area of study 3:
Practical investigation

Getty Images/globestock

CHAPTER 7

CHANGES IN BIODIVERSITY OVER TIME

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

KEY KNOWLEDGE

Changes in biodiversity over time

- significant changes in life forms in Earth's geological history including the rise of multicellular organisms, animals on land, the first flowering plants and mammals
- evidence of biological change over time including from palaeontology (the fossil record, the relative and absolute dating of fossils, types of fossils and the steps in fossilisation), biogeography, developmental biology and structural morphology
- patterns of biological change over geological time including divergent evolution, convergent evolution and mass extinctions.

KEY SCIENCE SKILLS

Communicate and explain scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them
- use clear, coherent and concise expression

Figure 7.1 ▶
An artist's rendering of *Anomalocaris*, an extinct sea-dwelling organism fossilised in the Burgess Shale

One day in 1886, a construction worker in the Canadian Rockies stumbled upon what is now regarded as one of the world's most significant fossil sites – the Burgess Shale. Previously unknown fossils of seemingly headless shrimp with odd appendages and other wondrous creatures baffled observers. Charles Walcott from the Smithsonian Institute visited the site and collected more than 60 000 important fossils, giving us a window to life in the past.

We can find out about the past by looking at the traces left in the earth. This has helped us understand the processes that establish change in a group of organisms. We now know that, with enough time and enough variation, a group of organisms can change beyond recognition – or die out.

The diversity of life on Earth today is astonishing. It is the result of **evolution**, the scientific explanation for the mechanisms that drive species to change over time. How evolution works can be complex. The contemporary view of evolution, the 'modern evolutionary synthesis', has developed through more than 150 years of research and observations.

Research in the fields of genetics and earth sciences, as well as countless newly discovered species of living and fossil organisms, all provide evidence that builds on early ideas. Emerging technologies can help with identifying new discoveries and can also be used to identify mechanisms that drive evolution and patterns within the process itself. But the scale of time involved can be difficult to comprehend, so to understand the history of life on Earth we first need to understand the history of Earth itself.

The ages of the Earth

Human lives are determined by hours, days, years and decades. This framework works for our own lives, but when we try to understand how old Earth is, or how long it takes for species to diversify, our terms of reference quickly become meaningless. We need something bigger than our own life span to measure the time scales of these changes. Instead of understanding the planet's history in years, we have devised other ways of measuring 'deep time' in segments covering millions, sometimes billions, of years, such as **periods**, **eras**, **epochs** and **eons**. These measurements are known as geological time and are expressed in millions of years ago (**mya**).

Earth has changed significantly over billions of years; far from being stable, it is changing right now as you read, and it will continue to change. Our dynamic planet can harbour only equally dynamic inhabitants; any organism's failure to respond and adapt to Earth's changes results in its extinction. Life has flourished and dwindled throughout various stages of Earth's history, responding to the changes by either adapting or dying out.

It is possible to track a number of physical and climatic changes that have occurred during the history of Earth. Some of these changes in the past were rapid and dramatic, causing major changes to sea levels and vegetation patterns; others occurred more slowly over time. In turn, these affected animal populations. Key events that have occurred so far in Earth's timeline are summarised in Table 7.1.

Table 7.1 Geological timeline and key events

Eras and eons	Periods and epochs	mya	Continental associations	Organisms
Precambrian eon		4560–570		First Archaea First bacteria First eukaryotes First multicellular organisms
Palaeozoic	Cambrian	570–510	Landmasses aggregate at equatorial zone Australia part of Gondwana North America and Greenland part of Laurentia Europe part of Baltica	First invertebrates Arthropods, including trilobites, brachiopods dominant
	Ordovician	510–439	Northern landmasses form supercontinent Laurasia	Diverse marine communities, reef-forming organisms Brachiopods and cephalopods Jawless fish
	Silurian	439–408		First land plants and arthropods Jawed fish
	Devonian	408–362	Gondwana moves south	First trees Land plants and fish spread First land vertebrates (tetrapods) descend from lobe-finned fish
	Carboniferous	362–290		Ferns dominant Swamp forests Insects dominate as the first winged animals First reptiles and amphibious tetrapods abundant
	Permian	290–245	Laurasia and Gondwana unite to form Pangaea	Reptiles dominant, rise of reptilian ancestors of mammals
Mesozoic	Triassic	245–208		Catastrophic mass extinction eliminates most life. Surviving organisms start to diversify, including dinosaurs and marine reptiles First mammals
	Jurassic	208–146	180 mya: Pangaea begins to break up 160 mya: Africa breaks from Gondwana	Dinosaurs dominant Cycads and conifers Flying reptiles (pterosaurs) *Archeopteryx* (first dinosaur–bird fossil to be found) dies and fossilises in Bavaria, southern Germany

Table 7.1 *(Continued)*

Eras and eons	Periods and epochs	mya	Continental associations	Organisms
	Cretaceous	146–65	120 mya: India breaks from Gondwana, moves north	First flowering plants Arrival of marsupials in Australia via Antarctica
			Gondwana breaks from Laurasia and drifts south	Dinosaurs populate huge rift valley between southern Australia and Antarctica
			Gondwana breaks up Late Cretaceous: Australia and Antarctica still attached	Cool temperate forest of podocarps, celery pines, proteas Southern beech (*Nothofagus*) established
Cenozoic	Paleogene			
	• Palaeocene	65–54		Dinosaurs now extinct Flowering plants, birds and mammals radiate into newly vacant niches left by dinosaurs
	• Eocene	54–40	50 mya: Australia begins to break from Antarctica and drifts north Inland seas form as eastern highlands lift Antarctic ice cap begins to form	
	• Oligocene	40–23	30 mya: separation of Australia and Antarctica complete	First *Eucalyptus* species
	Neogene			
	• Miocene	23–5	Slow drying of southern parts of the Australian continent	Rainforests contract to the equator First *Acacia* species Large marsupials are well established
	• Pliocene	5–2.6		Australia close enough to Asia to allow exchange of plants and animals (e.g. bats, rodents)
	Quaternary			
	• Pleistocene	2.6		Major ice ages First humans arrive and increase in range
	• Recent (Holocene)	(10000 years)		8000 years before present: formation of Great Barrier Reef begins

RECALL

- The variety of life on Earth has changed significantly over the course of geological time.
- Geological time is expressed in periods, eras, epochs and eons, which are expressed in millions of years ago (mya).
- The rise of multicellular organisms, the appearance of animals on land, the first flowering plants and mammals all occurred at specific geological times.

RECAP 7.1

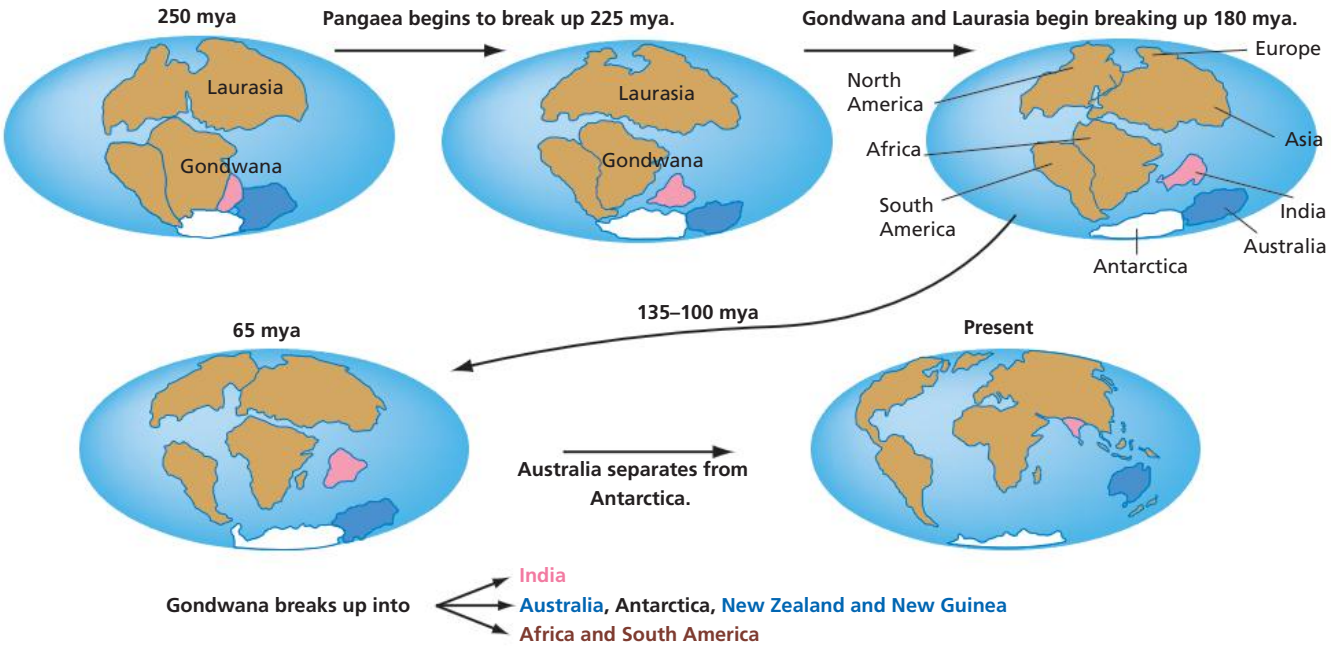
1 List in order from most distant to most recent the geological eras and the periods within them. Next to each, draw three images that represent the dominant or important organisms that existed during those periods.

2 Name the eon and period in which the first plants appeared on land, and state what type of plants dominated for the following 100 million years.

3 Name the period in which mammals appeared and list three other features of the geographical landscape during that period.

The changing face of the planet

The major plates of Earth's crust float on the fluid mantle that lies over the core of Earth, resulting in a process called **continental drift**. These plates are in constant movement, tearing apart or colliding and causing uplift of Earth's crust. (For example, the plate on which Australia sits is moving north at about 5–7 cm each year.) This movement is known as plate tectonics and may result in earthquakes, volcanic activity, or a combination of the two. If an earthquake happens under the sea, a tsunami can occur. Colliding plates can also create major mountain ranges where sections of continents crush together, forcing soft rocks upwards. The Himalayas are an example of this, where the upwardly thrust rocks were once sea-floor sediments.



▲ Figure 7.2 The breaking up of the supercontinent Pangaea to form the present-day continents

Geological (Figure 7.2) and fossil evidence (Figure 7.3) show that 200 mya a single supercontinent known as Pangaea existed. Over a period of about 20 million years, Pangaea broke up into two landmasses, the northern continent Laurasia (which would later give rise to North America, Asia and Europe) and the southern continent Gondwana (which would eventually become Antarctica, Australia, New Zealand, India, Africa and South America). Gondwana then broke up over the following 100 million years. Africa and India drifted north, and South America, Antarctica and Australia initially stayed together. By the Eocene epoch (45 mya), Australia and Antarctica were all that remained of the once great southern landmass of Gondwana. Finally, Australia broke free and began its trip northward, a journey we are still on.

Figure 7.3 ▶
Distribution of fossil evidence for former Gondwana land mass

Geological and biological evidence has helped construct maps of continental movement. Another line of evidence comes from observing changes in Earth's magnetic field; over millions of years, our magnetic field has changed frequently, alternating between 'normal polarity' such as we see today and 'reversed polarity', where the magnetic poles were reversed. Also, some iron-rich minerals found in rocks originating from lava flows are magnetised, serving as 'frozen compasses', effectively recording their orientation relative to the position of the magnetic poles when the rocks first formed or solidified.

Temperature, climate and sea levels

Over the course of its history, Earth's climate has oscillated between hot, humid periods and cold, dry periods. Evidence for this is found in ice cores drilled in Greenland and Antarctica. Some ice cores are several kilometres long and contain a record of climate change dating back 100 000 years.

For much of its history, Earth was much warmer than it is today and the temperature gradation from the equator to the poles was not as wide. Evidence suggests that past fluctuations have been dramatic.

Towards the end of the Precambrian era (570 mya), during the Carboniferous and Permian periods (245–362 mya) and during the Oligocene epoch (23 mya), snow, glaciers and sheets of ice covered much of Earth. Between these cold, dry periods there were long periods, millions of years, of warmer temperatures when the ice melted, the sea level was higher (pink areas, Figure 7.4), humidity was generally higher and

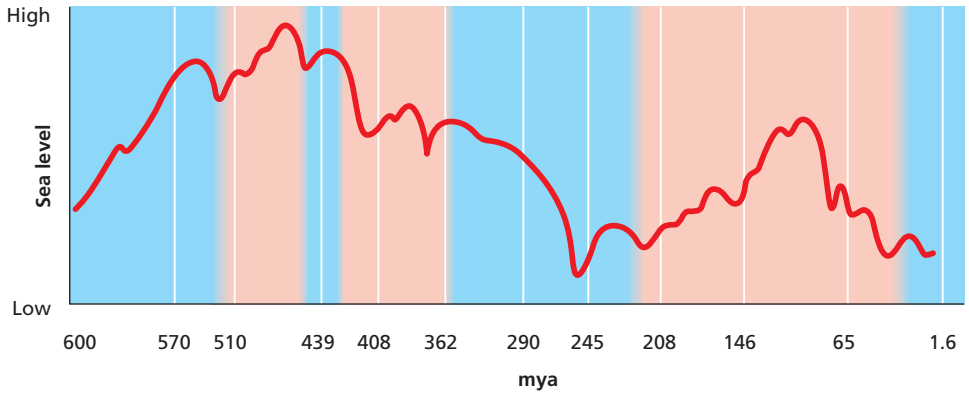


Figure 7.4 ▶
Sea levels have changed repeatedly with the changing temperature of Earth. Fossils record some mass extinctions, where dramatic variations in climate and sea levels appear to have influenced the severity of the extinction events.

vegetation was generally more tropical. Such climate variations inevitably affect life in some way or another, and it is possible to track some of these changes through the fossil record, especially fossil plants.

Oxygen levels

A critical environmental factor for life is the composition of the atmosphere: it affects all living things worldwide, including anaerobes. The first atmosphere of Earth most likely had very little oxygen. Evidence from sediment cores shows that the oxygen concentration began to increase about 3 billion years ago. By 1.5 billion years ago the level was about 1% of the present level, making up about 0.2% of the atmosphere. By 600 mya it had risen to 5% of the present level, making up about 1% of the atmosphere. This increase in atmospheric oxygen came from large numbers of cyanobacteria or 'blue-green algae' in the oceans. These algae are able to use water as a resource in photosynthesis, converting carbon dioxide to organic compounds and producing oxygen as a waste product. A few descendants of Earth's original 'atmosphere engineers' are very much alive today and continue to form stromatolites in places such as Hamelin Pool in Western Australia, just as they did hundreds of millions of years ago.

RECALL

- Continental drift occurs as the plates of Earth's crust move. This is called plate tectonics and causes earthquakes, volcanoes and the formation of mountain ranges.
- 200 mya a supercontinent, Pangaea, existed and subsequently broke up to form Laurasia and Gondwana. What is now Australia originated in Gondwana, as did Africa, India, South America and Antarctica.
- Temperature, sea levels and weather have had major impacts on the life forms present on Earth. Photosynthesising cyanobacteria produced large amounts of oxygen, which formed the atmosphere that allowed animals to prosper on Earth.

RECAP 7.2

1. Briefly describe Pangaea, Laurasia and Gondwana. Identify how each relates to the others.
2. Identify the type of evidence that would support the idea that Antarctica and Australia were once connected.
3. Suggest what process might explain how fossils that died on the sea floor could be found high in the Himalayan mountains.
4. Recount how the level of oxygen in the atmosphere has changed since Earth was formed. How has this change affected the type of organisms – in terms of cell type and cell size – that were able to survive as time progressed?

Evidence for evolution: the fossil record

What are fossils? Put simply, **fossils** are the preserved remains and traces of organisms. They provide evidence of past life. These remains can be hard parts, such as teeth, bones and shells, or impressions in the rock where the organism's tissue has decayed. Most fossilised 'hard parts' of animals or plants are found in rocks that have been derived from sediment,

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Figure 7.5 ▲
An immaculately preserved fossil of the extinct fish *Ceratoichthys*: a rare example of a complete fossilised skeleton

that is, sand, silt or clay. Along with animal bones, such as the skeleton of *Ceratoichthys* (Figure 7.5), fossils can also include footprints, burrows and even preserved waste products such as coprolites – fossilised faeces.

So what can the fossil record tell us? Much of our knowledge of the changes that have occurred in living things over time is derived from fossils. Only a very small percentage of organisms leave fossilised remains. Many fossils are destroyed by natural processes such as weathering and erosion.

Fossilisation

The process of fossilisation requires very specific and rare conditions. The remains of the vast majority of long-extinct animals may never be found, and consequently the fossil record is incomplete and biased toward organisms that lend themselves more easily to the fossilisation process. To become a fossil, organic matter needs to be deposited and covered in sediments in an environment that lacks oxygen. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay, depriving the remains of oxygen, as can happen in the beds of lakes and rivers or in calcium-rich sea beds. In many cases, minerals from the sediments have replaced the natural bone or shell material, making the remains harder and more likely to fossilise. This type of fossilisation is called mineralisation.

Fossils can form when organisms are covered with sedimentary material, such as mud, silt or sand, that is generally carried by rivers and streams and deposited. These materials are consolidated to form sedimentary rock. The overlying sedimentary material protects organic matter from scavengers and also slows its decay long enough for it to fossilise. The resulting fossils are generally only the 'hard parts' of organisms (such as bones and teeth that are slow to decay) and rarely more delicate tissue such as feathers. Fossils of this type are not found in volcanic rocks because molten lava solidifies at about 1000°C, which is hot enough to burn any organic material, but they can be found in sedimentary layers of eroded volcanic ash. Metamorphic rock does not usually bear fossils, as the pressure and heat of metamorphism generally (although not always) destroys any trace of a fossil.

Thin tissue, such as leaves and muscle, is sometimes preserved as films or impressions left in the sedimentary rock. Fossils are also formed when soft material, such as volcanic ash, fills an impression, or when minerals later form in a pocket in sedimentary rock left by a decomposed organism, which can result in fossils composed of opal. A 3-million-year-old set of footprints from a family of early humans, including children, is preserved in this way in the Afar triangle region of Africa. Dinosaur footprints can also be found in sandstone and mudstone.

There are several other ways fossils can form. They can be formed as a result of freezing and subsequent dehydration. Plants are also commonly fossilised. The original plant material may be partly dissolved and some tissue replaced with dissolved salts, which petrify the material; that is, they turn it to rock. Entire tree trunks have been preserved by petrification in fossilised forests in Arizona and Antarctica. As a consequence, fossilisation can tell us a great deal about past life and how it differs from what we see in the world today.

Transitional forms and the pace of evolution

Close examination of the fossil record reveals interesting evidence of intermediate states between an organism's ancestral form and that of its descendants, such as the famous bird/dinosaur *Archaeopteryx* (Figure 7.6). These intermediate states are called transitional forms. Transitional forms give us copious evidence for evolution, documenting change over time on a broad scale. Transitions between species are harder to identify due to the bias of the fossil record.

There is always bias in the fossil record. Given the specific requirements for fossilisation and thus the nature of the remains that can be fossilised, there will always be chapters missing from the story. Even with this bias, it is possible to observe a gradual change over time of organisms as their shape or size transitions, in some cases to different forms. In other cases no such gradual transition is evident and the changes seem sudden and inexplicable. The change appears as a burst of evolutionary speed. The burst of evolution suggested by a gap in the fossil record may be explained by aspects of two theories of evolutionary patterns: **gradualism** and **punctuated equilibrium**.

▲ **Figure 7.6**
A reconstructed model of the bird-like dinosaur *Archaeopteryx*, an example of a transitional form between feathered dinosaurs and modern birds

Gradualism

The concept of gradualism assumes that evolution occurs as a steady, slow divergence of lineages (ancestral tree branches). Gradualism states that apparently sudden bursts of evolution implied by the fossil record are not a real indication of an evolutionary history, but an illusion of the fossil record. Evolution only appears as a burst because of the absence of sediments containing fossils that document this transition, or perhaps a change in conditions that made fossilisation impossible. Even if a small section of potentially fossil-bearing sediments were absent, this may account for fossils missing from millions of years in the fossil record. Were this section of strata still present, the fossils within it would show a divergence pattern that is slow, even and steady, in other words, gradual.

Punctuated equilibrium

In contrast to gradualism, the theory of punctuated equilibrium states that the apparent burst of evolution is not an illusion, but real. It states that species remain fairly stable for long periods of time but may swiftly change to a new species; for example in response to rapid changes in the organism's environment. Like gradualism, punctuated equilibrium accepts the existence of transitional forms between species, but for such brief periods that they were not preserved as fossils. Punctuated equilibrium is thought to be a successive process of stasis followed by a period of rapid change of a subset of the population. This theory does not imply that the natural selection theory is incorrect. Examples of both gradual and punctuated bursts of change are seen in the fossil record.

RECALL

- Fossils are preserved remains of organisms or traces of their existence. The conditions for fossilisation occur rarely and this can cause a bias in the fossil record.
- Transitional forms in the fossil record provide evidence for evolutionary relationships between organisms and document change in organisms over time.
- Gradualism is a theory of evolution that assumes that slow and steady changes occur in organisms over time. Punctuated equilibrium assumes that species remain fairly stable for periods of time but undergo changes in rapid bursts.

RECAP 7.3

1. Recount the steps involved in the process of fossilisation.
2. Identify two ideal types of environment or scenarios in which fossilisation could probably occur.
3. Most of our knowledge of the evolution of sharks is based on the remains of fossilised shark teeth. Suggest why other fossilised body parts of sharks have not been found in abundance.
4. Palaeontologists have found tracing the evolution of sea jellies ('jellyfish') to be very challenging. With your knowledge of fossils and the process of fossilisation, suggest why this may be the case.
5. What is the difference between gradualism and punctuated equilibrium? Are these patterns of evolution mutually exclusive?

Fossil dating methods

To make sense of the fossil record and examine it for evidence of evolution, we first need to understand some basic information about the fossils and their geological settings. How old are the fossils, which organisms arose first, and which organisms lived together? These questions can only be answered if we are able to determine the age of the evidence accurately. A combination of comparative and absolute dating techniques is used to estimate the ages of sedimentary rocks and the fossils within them.

Dating techniques that work comparatively

Comparative dating (also called relative dating) is used to determine the age of a rock, or a fossil contained in the rock, relative to other rocks or fossils found nearby. This approach to dating relies on our understanding of how sedimentary rock is formed.

Sedimentary rock, as you would expect, is composed of sediment: weathered material from Earth's surface, such as gravels, silts, sands and muds that have been transported by water and deposited in river beds, flood plains and sea floors. Sediment transport and deposition is an ongoing process; it has been occurring continuously on Earth for billions of years and can still be observed today. Over time, these deposited sediments form defined layers that consolidate into sequences of sedimentary rock. These sequenced layers are called strata, and a section showing successive layers of sedimentary deposition is called stratification. Strata are deposited in a time sequence, with the oldest on the bottom and the youngest on the top. Assuming natural processes like tectonic movement have not twisted or inverted the layers, palaeontologists can assign relative ages to fossils based on the strata in which they are found. While this technique cannot give an age in years, it enables the ages of the sequence of the strata to be estimated relative to each other.

Dating techniques that work 'absolutely'

Absolute dating (or chronometric dating) is a technique that assigns a numerical age in years to a fossil or rock. There are three main types of absolute dating: radiodating, electron spin resonance and luminescence.

Unlike comparative dating, absolute dating is based on the physical or chemical properties of materials in the rock, rather than the assumption-based sequences that relative dating provides. The most common method of absolute dating is radiometric

dating, which is based on the predictable rates of decay of naturally occurring radioactive isotopes present in a rock or fossil. By testing for the presence of different radioactive isotopes, an age in years can be estimated for the sample.

Radiometric dating

Some elements occur as **isotopes**: they have the same atomic number (the same number of protons) but a different atomic mass (different numbers of neutrons). For example, carbon has three isotopes: carbon-12, carbon-13 and carbon-14. Carbon-12 (^{12}C) has six protons and six neutrons in each nucleus, and carbon-14 (^{14}C) has six protons and eight neutrons. Some isotopes have an unstable nucleus that splits up and emits energy in the form of radioactivity (alpha, beta or gamma rays) at a measurable rate. This process is referred to as radioactive decay. The half-life of an isotope is the time taken for half of the radioactive atoms in an initial sample to decay.

Carbon-14 is a radioactive isotope that forms when cosmic rays strike nitrogen atoms in the upper atmosphere, and it decays at a known rate to produce nitrogen-14 (^{14}N) (Figure 7.7). Its measurable rate of decay is the basis of carbon dating.

When an organism dies, the carbon-14 it contains decays at a steady rate. Using the half-life of carbon-14 and the ratio of carbon-14 to carbon-12 in the sample we can determine the age of the sample – in other words, the time taken for the original ratio to decay to the present ratio.

In its simplest form, carbon dating assumes that the proportion of carbon-14 in the atmosphere is constant, but data from tree rings shows that it can change with time. For this reason, the calculated age has to be corrected into calendar years. Ages are expressed with their degree of accuracy (usually as plus or minus *x* years).

The older the object, the greater the margin of error. Carbon dating – corrected for atmospheric variation – is thought to be accurate for samples up to about 12 000 years old. After this time it is difficult to measure the level of carbon-14 accurately and so other radioisotopes, such as potassium-40 (which decays into argon), are used instead (Table 7.2).

Carbon-14 dating is generally not applied to fossils for two main reasons: in most cases fossils have been mineralised, meaning that the organic (carbon-containing) tissue has been chemically altered or replaced, and the process of fossilisation generally takes longer to occur than the maximum age of accuracy for carbon-14.

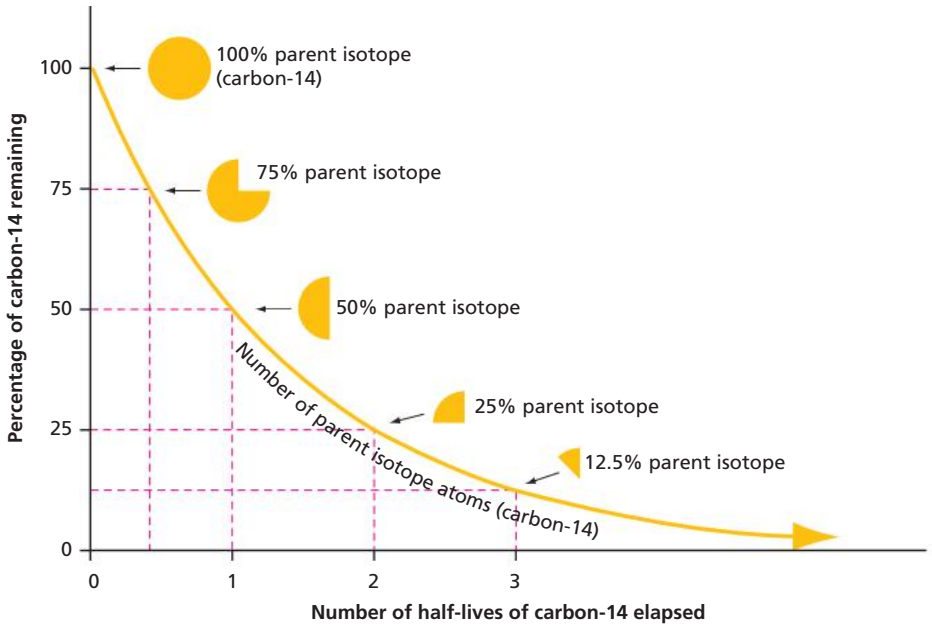


Figure 7.7
Graph of the half-life of carbon-14

Table 7.2 Half-life and product of decay of some elements used in radiometric dating

Element	Product	Half-life (years)
carbon-14 (^{14}C)	nitrogen-14 (^{14}N)	5730
uranium-235 (^{235}U)	protactinium-231 (^{231}Pa)	34300
uranium-234 (^{234}U)	thorium-230 (^{230}Th)	80000
potassium-40 (^{40}K)	argon-40 (^{40}Ar)	1.25 billion
thorium-232 (^{232}Th)	lead-208 (^{208}Pb)	14 billion
rubidium-87 (^{87}Ru)	strontium-87 (^{87}Sr)	48 billion

Electron spin resonance

Electron spin resonance is a relatively new absolute dating technique that measures the properties of electrons in the crystals of minerals. Some common minerals 'collect' electrons in their crystal lattice at a predictable rate, either from radioactive sources in the surrounding rocks or by absorbing cosmic rays. The electrons become fixed within these crystalline lattices and the trapped electrons are mildly magnetic. This magnetism of the trapped electrons can be measured to give an electron spin resonance reading, which in turn gives a measure of the amount of radiation the sample has experienced since its formation. As the amount of radiation received increases with time, the accumulated amount of radiation can be divided by the background dose rate to determine the age of the sample.

The sensitivity of the technique depends strongly on the nature of the sample and the environment it has experienced, but it can be used to measure ages from a few thousand years to 1–2 mya for samples such as teeth and shells, and for quartz-based minerals that have had their 'clocks' reset by exposure to intense heat.

Luminescence techniques

Thermoluminescence and optically stimulated luminescence are two other forms of absolute dating techniques that are used in Australia. Both measure characteristics of minerals within sedimentary rock. When radiation strikes a mineral crystal, energy from the radiation can be trapped within the crystal and is released only when the mineral is exposed to heat or light. The basis of thermoluminescence is the measurable light that is emitted from a mineral when it is heated; optically stimulated luminescence is the light that is emitted from a mineral when it is exposed to visible light. Both techniques are useful for dating the last exposure of the minerals to heat or light and the occurrence of mineralisation, but they cannot generally provide an estimate for the absolute age of a sedimentary rock.

RECALL

- Comparative dating methods can determine the age of a fossil or fossil-bearing rock in relation to the surrounding rock, and are assisted by our knowledge of how rock strata are formed.
- Absolute dating methods include radiometric dating, electron spin resonance and luminescence techniques.
- Different techniques of dating fossils may be used, depending on the type of fossil, its age and where it was found. A combination of methods may be used if possible.

RECAP 7.4

1 List the methods used to determine the age of fossils and give the pros and cons of each.

2 Which isotopes would be measured to radiodate a fossil that comparative dating suggests is approximately 50000 years old?

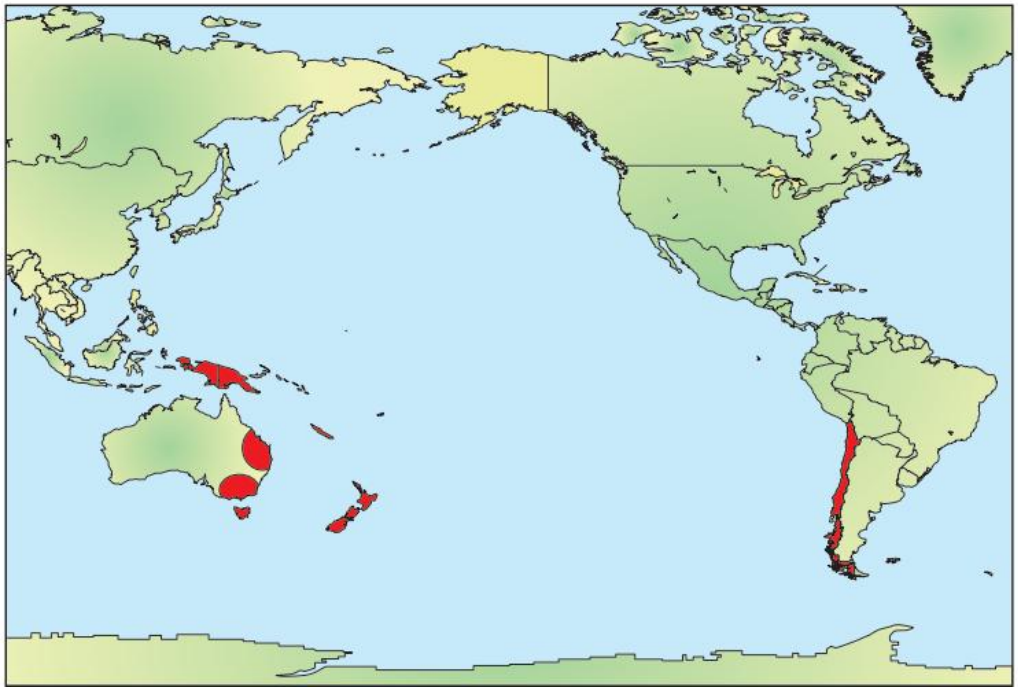
Many lines of evidence for biological change over time

So far we have looked at the evidence for evolution and given it context in terms of timing, geology and climate. However, our understanding of the mechanisms that explain what we see around us today has itself undergone many historic changes to reach this point: the concept of evolution has itself evolved. Our understanding of evolution now shows that additional evidence – patterns observed in the world today – gives even greater explanatory power to the process of evolution.

Biogeography

Biogeography is the study of the distribution of organisms and ecosystems across the world and through geological time. The fauna and flora of Australia owe their uniqueness to the relatively recent isolation of the landmass. However, Australia and other landmasses in the southern hemisphere share many plant and animal groups.

Many genera of Indian plants are similar to those of the monsoonal environments of northern Australia. Some Malaysian rainforest genera occur in the rainforests of tropical eastern Australia. Southern beech trees, *Nothofagus*, are found as both living and fossil specimens in mainland Australia, Tasmania, Papua New Guinea, New Caledonia, New Zealand and South America (Figure 7.8), and as fossils in Antarctica. The mountains and dry valleys of Antarctica have fossils of *Glossopteris* seed ferns (embedded in rocks and coal seams)



◀ Figure 7.8
The red areas show where southern beech trees, *Nothofagus*, are found as both living and fossil specimens in Australia, Papua New Guinea, New Caledonia, New Zealand and South America.

that are the same as those found in coal deposits of India, South America, South Africa and Australia. These were all laid down in ancient forests prior to and including the Permian period of the Palaeozoic era, that is, up to 245 mya. The far-flung distribution of these groups provides evidence that Gondwana once existed.

Wallace drew the line

Alfred Russel Wallace (1823–1913) is known as the 'father of biogeography'. He was a self-taught naturalist, a professional collector of flora and fauna, and an important intellectual of the 19th century. Wallace's travels took him to the Amazon for four years and, later, Malaysia and Indonesia for eight years. During his time in Southeast Asia, he collected more than 126 000 specimens. In Indonesia, he was struck by the stark difference between the bird families he encountered in the islands of Bali to the west and those in Lombok to the east, a distance of 25 km. On Bali, the birds were more closely related to those of the larger islands of Java, Sumatra and mainland Malaysia, the 'Asian' fauna. Those on Lombok were related to the 'Australian' fauna of Papua New Guinea and Australia.

Birds were only one example of the faunal differences Wallace found. Further observations of some species of fish and large mammals indicated a distinct break between two regions. The line between them became known as Wallace's line (Figure 7.9), but the reason behind this dividing line remained mysterious for many years. The mystery of 'the line' was later solved with the theory of plate tectonics, as the line approximates the collision zone between the Australian and Asian plates.

Figure 7.9 ▲
Wallace's line shows the demarcation of Asian fauna from Australian fauna.

Comparative anatomy

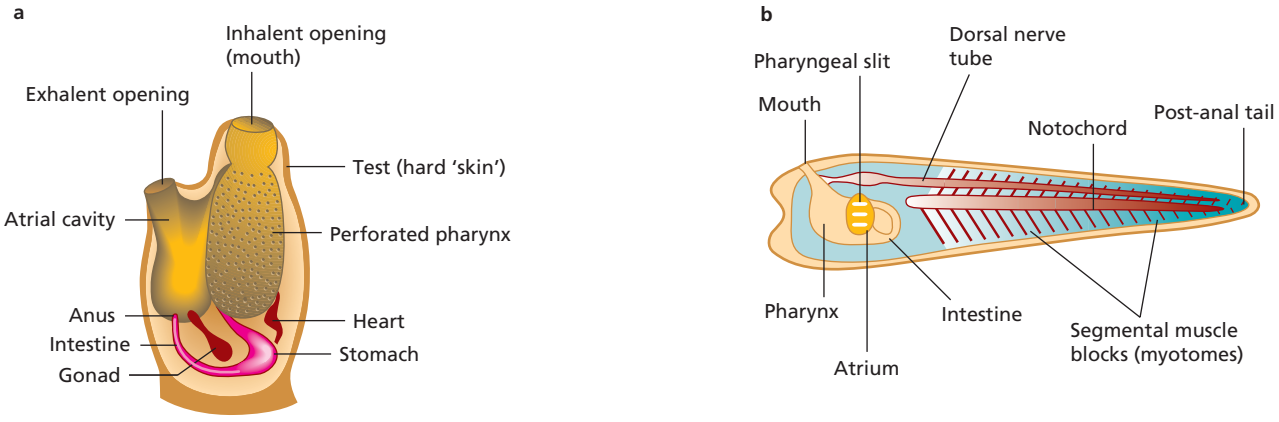
While we now know that all life shares a common genetic code and relies on similar physiological and biochemical processes, early evolutionary theorists could only use evidence that they could observe at the time. Even to the casual observer, it is evident that different species can appear vastly different, seeming to bear very few similarities to each other, while others appear so similar that their shared common ancestor must have

existed relatively recently. Closer examination of the physical characteristics of species, at both the embryonic and adult stages, can reveal further evidence for evolution.

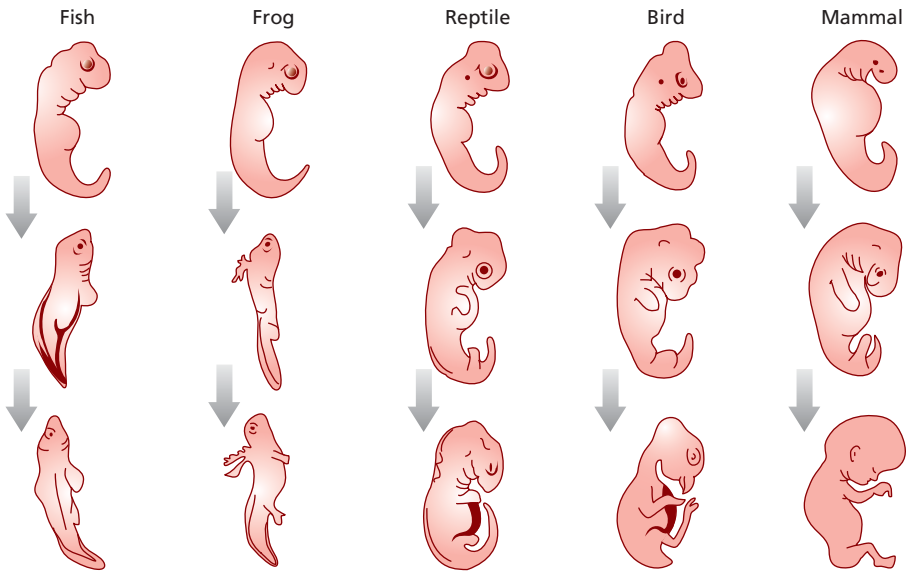
Embryology

Comparative anatomy is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of embryos. For example, all members of the phylum Chordata have, at some stage of their development, a dorsal notochord (a solid tissue running along the back), pharyngeal slits (which turn into gill slits in fish), a dorsal nerve chord and a tail that extends past the anus. Sea squirts are the most unlikely members of this phylum; the adults look more like marine invertebrates than the more closely related vertebrates (Figure 7.10a). Sea squirt larvae, however, have the requisite characteristics, including a notochord (Figure 7.10b). Vertebrates have lost the notochord and it is replaced with vertebrae.

The similarities observed between embryos of fish, humans and many other organisms are suggestive of a shared ancestor from which all these species have evolved (Figure 7.11). No other theory can adequately explain why the same structures occur in all chordate embryos, whose adult forms are so diverse.



▲ **Figure 7.10**
(**a**) Adult sea squirts show few characteristics of chordates. (**b**) The free-swimming larva of the sea squirt shows the characteristic features of chordates, revealing its evolutionary affinity with chordates.



◀ **Figure 7.11**
Similarities between chordate embryos suggest a common ancestor.

Homologous structures

Homologous structures are common physiological structures shared by different organisms that stem from their descent from a common evolutionary ancestor. When an adaptive radiation occurs, organisms retain the same basic structures because they have the same genetic history. For example, all lizards have scaly skin; this is a defining characteristic of their classification. However, the scales can differ in colour, hardness and shape in relation to the habitat that they occupy, and may function in defence, temperature maintenance or camouflage. The different types of scales are examples of homologous structures.

The example of homologous structures in lizards is one that has a relatively recent evolutionary history, but some homologous structures have evolved from a much more distant common ancestor and may have very different functions. The wing of a bird, the wing of a bat, the leg of a crocodile, the flipper of a whale and the arm of a human all have the same basic structure: the pentadactyl limb, a hand or foot with five fingers or toes. However, in each species the limb has been modified to suit a variety of ways of life, demonstrated by the different bone lengths and coverings of the limbs (Figure 7.12).

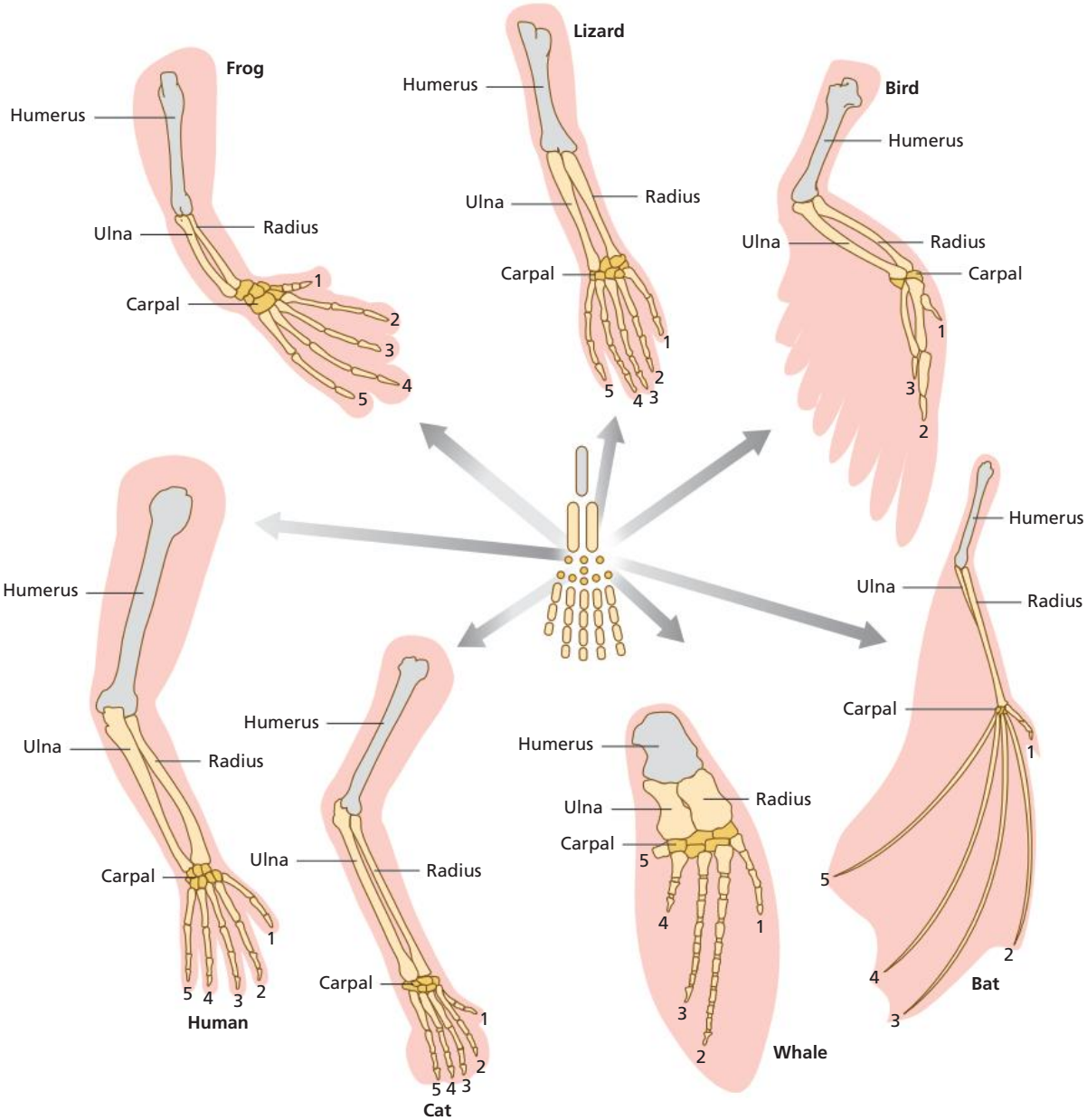


Figure 7.12 ▼
The principle of homologous structures can be illustrated by the adaptive radiation of the forelimb of a selection of vertebrates, which all show the basic pentadactyl pattern modified for different uses.

The leaves of land and aquatic plants all have the same basic components but the structure shows enormous variety in size, shape, colour and function. Some leaves function as coloured petals, some as support structures in buds and others act as defensive spines or fleshy water stores (Figure 7.13).

Homologous structures can be used to infer phylogenetic (i.e. evolutionary) relationships because only organisms with a common ancestor can have the structures with the same basic arrangement.

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◀ Figure 7.13 Homologous structures derived from leaves. (**a**) The spines of a cactus and (**b**) the bracts of *Heliconia* are derived from the same basic structure but now have different forms. In this case, the spines serve different functions; they are homologous structures. In other examples, homologous structures can share functions, but different environments can influence how these functions are necessarily performed.

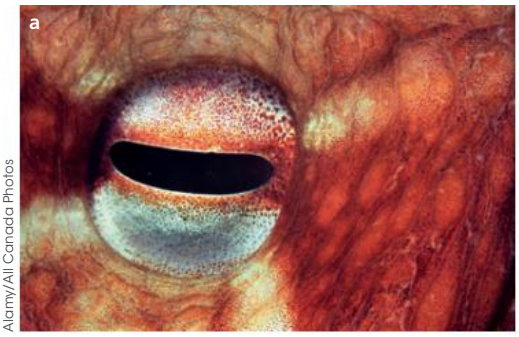
Vestigial homologous structures

In some cases, homologous structures stemming from a common descent can eventually cease to provide a functional use for an organism; the structure may not necessarily impede a particular **adaptation** of an organism, but at the same time the structure no longer serves a 'useful' purpose. These structures are called **vestigial structures**. Vestigial structures can take a variety of forms, including skeletal structures on vertebrates, soft tissue such as organs, or even at the cellular and molecular levels.

Wherever vestigial structures may be found, they are usually either rudimentary or atrophied. Vestigial structures are quite common and are yet another line of evidence that points to shared ancestry. Among humans, some features that are thought to be vestigial include the coccyx (tailbone), the muscles that allow some people to wiggle their ears, and the palmar grasp reflex that causes babies to grip tightly onto something placed in the palm of their hand.

Analogous structures

Analogous structures are features of organisms that have the same function but not the same basic structure. The eyes of octopuses and vertebrates are remarkably similar, even down to fine points of detail, and an observer could conclude that they are homologous structures (Figure 7.14). However, there is one telling difference. In the vertebrate eye, the nerve fibres lie in front of the sensory cells of the retina, whereas in the octopus eye they lie behind them. Because of this, the vertebrate eye has a blind spot where the optic nerve emerges from it, whereas the octopus eye lacks one. The reason for this difference lies in the ways the two eyes developed, which indicates that they are the products of two distinct lines of evolution.



Alamy/All Canada Photos

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Figure 7.14 ▶
(**a**) Octopus eyes and (**b**) human eyes are the solution to the same problem with similar adaptations.

RECALL

- Biogeography is the study of the geographical distribution of organisms with a consideration of the location of the continents over geological time. It can provide evidence that the ancestors of similar organisms once lived on the same historical land mass.
- Comparing the development and anatomy of organisms can provide evidence that organisms developed from a common ancestor.
- Homologous structures evolved from the same ancestral form but have developed different forms or functions. Analogous structures evolved from different ancestral forms to have a common function and therefore show some fundamental differences.

RECAP 7.5

1 Explain the significance of *Glossopteris* plant fossils in understanding the biogeography of Gondwana.
2 Why does embryology provide evidence for a shared common ancestor of all chordate organisms?
3 List two examples of homologous structures and two examples of analogous structures.

EXPERIMENT 7.1

HOMOLOGOUS STRUCTURES

Charles Darwin noted that many animals shared similarities in body structure. He argued that this seemed to suggest that the structures had developed from a common ancestral form. Are the similarities in structures as obvious as he suggested?

Aim

To investigate homologous structures in the pentadactyl limb of various vertebrates

Materials

- four examples of vertebrate pentadactyl limbs. These could be actual skeletons, models, photographs or illustrations of the limbs (e.g. frog, bird, dolphin, dog, cat)

Procedure

For each of the samples that you have been given, complete as many observations as possible and note them in your results.

1. Examine the forelimbs and hind limbs of each specimen carefully and make a quick sketch in your results. Create a table to record the number of bones that make up each individual digit on the forelimbs and another table for the hind limb. Include the hand/foot area, wrist/ankle area, forearm/shin area and the upper arm/thigh area.
2. Describe any other differences that you may have observed in each specimen when it is compared to the generalised diagram of a pentadactyl limb (Figure 7.15).

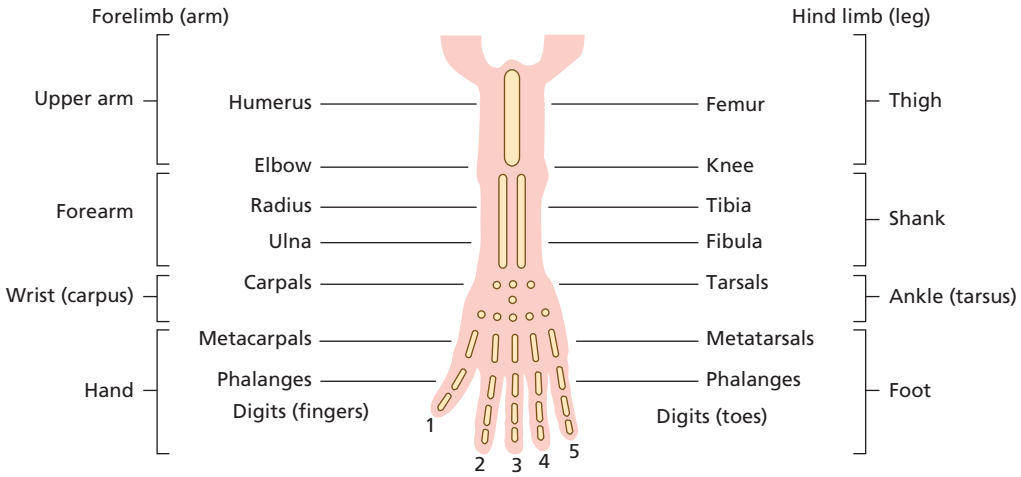

Figure 7.15▲
Generalised pentadactyl limb

Results

Your results should include:

- name of organism
- sketch of forelimb
- sketch of hind limb
- summary table of counts
- descriptions of differences.

Discussion

1. Analyse how the number of bones in each area of your specimens compares to the generalised pentadactyl limb.
2. Other than bone numbers, identify and explain what other differences you find in the limb structures.
3. Suggest and explain reasons for the differences noted for each particular animal.
4. Suggest what advantage these differences might offer to the species concerned.
5. Identify the basic similarities in the different limbs and explain how these can be found in so many different species that may occupy a variety of different habitats.

Taking it further

Use the Internet to examine the limb structure of other animals to see how they compare to the ones you have examined in this experiment. Do the similarities or differences reflect how closely animals are related to each other?

Conclusion

Write a conclusion that summarises your findings.

Making sense of the evidence

In the late 1600s, Western civilisations believed in the idea of 'natural theology' – that every 'kind' of organism has essential, unalterable characteristics. As biological studies blossomed, naturalists began noticing variability in species, and the discovery of the remains of animals unlike anything seen before introduced the idea of extinction, which challenged natural theology: where did these giant animals come from, and where did they go?

Questions like this prompted naturalist Jean-Baptiste Lamarck to devise the 'transmutation of species by spontaneous generation'. Lamarck suggested that organisms pass on to their offspring characteristics that they acquire during their lifetimes; that is, individual efforts during the lifetimes of organisms were the mechanism that drives adaptation. Although now discredited, this was the first, albeit flawed, theory that embraced evolution.

In the 1850s, two naturalists, named Alfred Russel Wallace and Charles Darwin, were studying and collecting forms of life in different parts of the world. By coincidence, they both arrived at the same idea about how species 'came to be'. To refute Lamarck's theory, Darwin proposed a new theory of evolution and 'called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection'. Originally published in 1859 as *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*, Darwin's work is now commonly known as *The Origin of Species*. The basis of Darwin's theory of evolution was that individuals within a population showed a range of variation in their characteristics. Those with characteristics, or traits, most suited to their environment would have an advantage over other individuals, making them more likely to pass these alleles (which encode these favourable traits) to the next generation. In each generation, favourable alleles become more common and the population gradually changes to become better suited to its environment. Darwin provided evidence for descent with modification (branching evolution) based on patterns in variation of domesticated and wild species, and patterns of species distributions in time and space. This was a new approach to understanding evolution. Much of the previous work had viewed relationships between organisms to be 'ladder-like' – that life can be organised in a hierarchy of lower to higher organisms. Darwin proposed a more 'tree-like' scenario, where life's lineages can be mapped on a branching diagram. In this analogy, the forks in the branch mark points at which new species arise – evolutionary events that occurred when populations became so different from other populations of the same species that they could no longer interbreed. This important concept is the basis of **phylogeny**. Phylogeny seeks to reconstruct the evolutionary history of any given group of organisms by studying the patterns of relationships among them.

Phylogeny will be discussed more in Chapter 9.

Divergent evolution

Divergence is a pattern of evolution where differences between groups of organisms accumulate to a critical point that leads to **speciation**, the development of a new species. This pattern is usually the result of the dispersal of a single species to different environments; that is, groups from the same species become isolated from each other. The isolation stops the gene flow between these separated populations. A group of organisms that has a recent common ancestor may evolve different adaptations in response to a range of environmental pressures.

As members of the population develop adaptations over successive generations, they may diverge enough to become new species. The process is referred to as **adaptive radiation**.

For example, koalas (tree-dwelling herbivores), Tasmanian devils (ground-dwelling carnivores) and marsupial moles (dune-burrowing insectivores) are related because they have a common marsupial ancestor (Figure 7.16). However, they show quite different feeding structures that adapt them to different diets. These animals are an example of adaptive radiation. Also, because they have evolved into separate species, they are an example of **divergent evolution**.

You can learn more about the mechanisms of evolution and speciation in Chapter 8.

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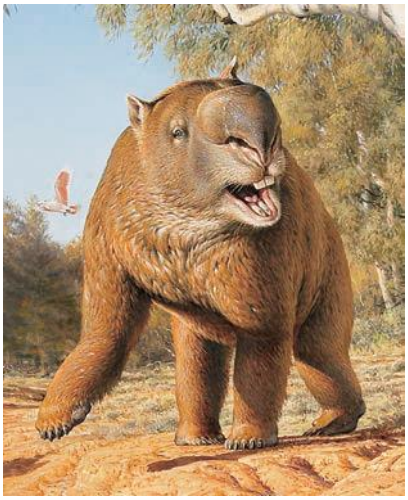
◀ **Figure 7.16**
(**a**) Koalas, (**b**) Tasmanian devils and (**c**) marsupial moles evolved from a common ancestor that probably lived during the Eocene epoch. They are examples of the divergent evolution of marsupials.

Adaptive radiation

Adaptive radiation is a pattern of divergent evolution where organisms rapidly diversify into numerous new forms, particularly when environmental changes trigger the availability of new resources and environmental **niches**. A clear example of this can be found in Australia's fossil record, which indicates that during the Middle Miocene epoch (approximately 15 mya) dense tropical forests covered central Australia where the Simpson Desert is now.

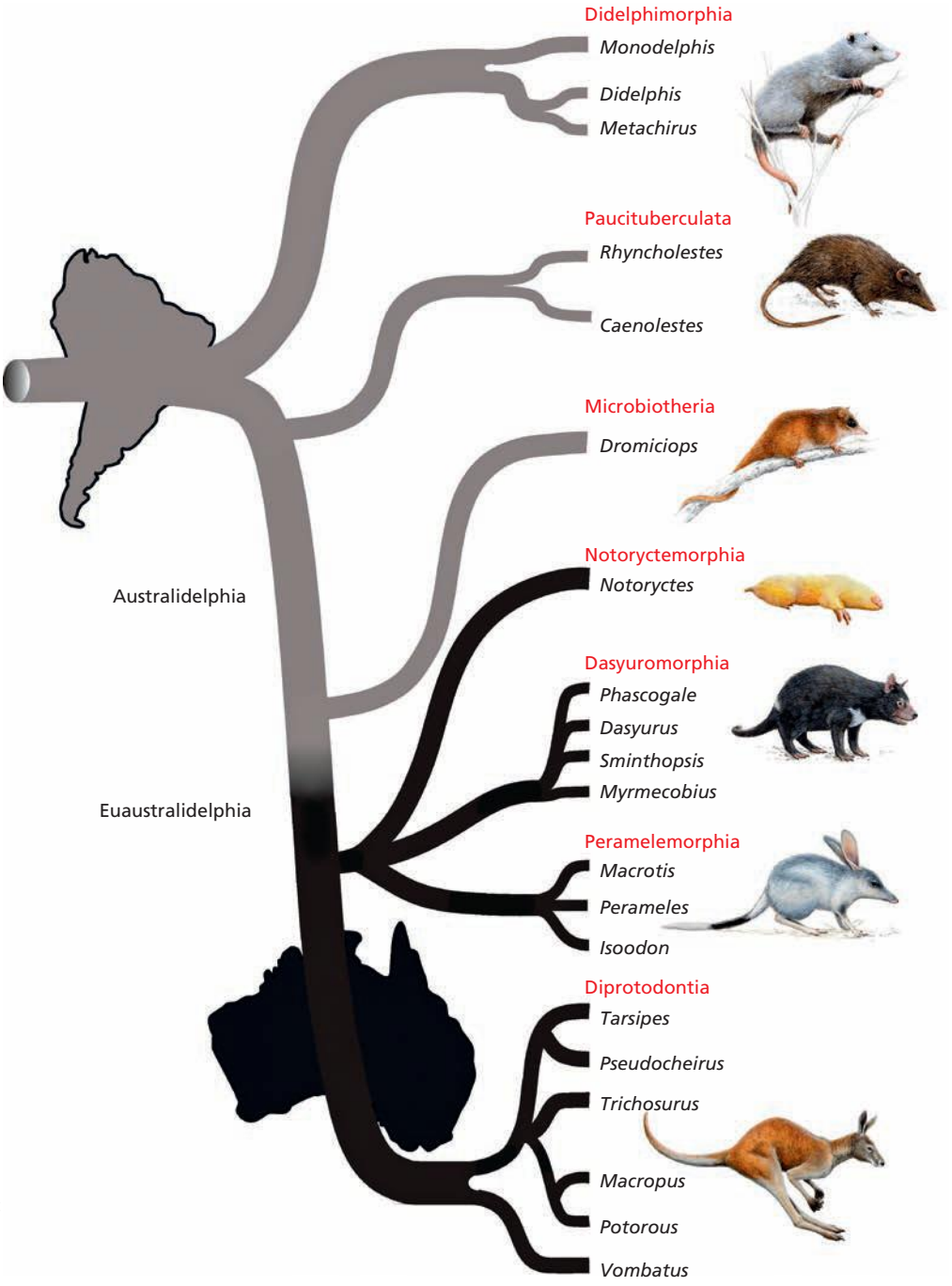
Forests, lakes and permanent rivers provided a lush habitat for marsupials such as giant koala-like possums, shrewish insectivores and sheep-sized browsers. Flamingos, crocodiles, turtles and dolphins flourished in the waterways. The range of habitats allowed the extensive radiation of animal species that adapted to the available resources, and is therefore an example of adaptive radiation.

Slowly, the tropical centre of Australia began to dry out during the Pliocene epoch (approximately 5 mya). This brought an end to the tropical habitat, which gave way to broad grasslands. Large browsing mammals called diprotodontids (Figure 7.17) and a variety of possums could not survive with the reduction of trees and the subsequent limited food available.



▲ **Figure 7.17**
The giant *Diprotodon optatum* was a type of megafauna that browsed on leaves.

As the tropical forests retreated from central Australia, the animals they once supported were forced to compete for diminishing resources and became vulnerable to extinction. Remnants of these forests and their inhabitants are now confined to Papua New Guinea and pockets of northern Queensland. The grasslands that replaced the forests provided new habitats that allowed for adaptive radiation of other Australian mammals: the kangaroos and wallabies.



Nilsson MA, Churakov G, Sommer M, Tran NV, Zemann A, Brosius J, et al. (2010) Tracking Marsupial Evolution Using Archaic Genomic Retroposon Insertions. PLoS Biol 8(7): e1000436. doi:10.1371/journal.pbio.1000436. © 2010 Nilsson et al. open-access article Creative Commons Attribution License

Figure 7.18 ▶
Adaptive radiation of marsupials began in South America, which was then joined to modern Australia in the supercontinent Gondwana. Most surviving marsupials are now restricted to the Australian continent.

RECALL

- Evolution is gradual: small genetic changes driven by natural selection accumulate over long periods that are consistent with known genetic mechanisms.
- The many lines of evidence of evolution also give important insights into patterns of evolution.
- Divergent evolution describes a pattern of evolution in which species accumulate many changes, becoming new species with characteristics that differ from those of the parent species. When this occurs on a large scale it is called adaptive radiation.

RECAP 7.6

1 Define 'divergent evolution' and give an example of how it has affected the evolution of species.
2 In a group of species that arose from a common ancestor through divergent evolution, would you expect to see homologous structures or analogous structures?
3 Give an example of a situation that leads to adaptive radiation of species.

Convergent evolution

Convergent evolution is a pattern that occurs when unrelated organisms evolve similar adaptations in response to their environment. An example of convergent evolution is provided by anteaters. Many animals eat ants and termites and have developed similar structures, even though they are not closely related.

Modern anteaters include echidnas, which are monotremes; numbats, which are marsupials; and aardvarks and pangolins, which are placentals (Figure 7.19). All of these species have an elongated snout that functions as a smelling and digging device, a long, extendible tongue that can extract ants from crevices, and powerful claws that are used for digging up ant and termite nests.

The different species of ant-eating mammals have a common ancestor, but not a recent one; they belong to different orders. They have developed ant-eating habits independently and coincidentally, rather than as a legacy from their common ancestor. The first mammal-like animal probably emerged in the Triassic period, around 208 mya.

The results of convergent evolution often show up as analogous structures: adaptations of very different types of structures that solve a problem in a similar way.

Extinction of species

The fossil record shows that nearly all species that ever lived are now extinct. In most cases they represented the end of an evolutionary lineage and left no descendants. Although extinction occurs regularly there have been periods when the rate of extinction has been very high. These are referred to as mass extinctions.

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Figure 7.19 ▶
Ant-eating mammals including (**a**) echidnas (monotremes), (**b**) numbats (marsupials) and (**c**) pangolins (placentals) show convergent evolution with ant-eating structures.

Five mass extinctions are documented in the fossil record within the last 500 million years and there may have been many more before that. The Cretaceous mass extinction, 65 mya, is the best known and has received a lot of attention. It saw the demise of the dinosaurs, which had dominated the land for the previous 180 million years.

The most dramatic mass extinction event, often called the 'Great Dying', appears to have occurred at the end of the Permian Period 250 mya. This appears to coincide with one of the most extensive periods of volcanic activity Earth has ever seen. In fact some scientists believe that this event went close to wiping life out completely. However, one of the few survivors of this catastrophe was the ancestor of the dinosaurs, one of the most successful vertebrate groups ever to have evolved. They were able to colonise the newly vacant environmental niches that were left by the extinct species, and rapidly formed many new species with features that allowed them to exploit the new environmental conditions.

Adaptive radiation following mass extinctions is covered more in Chapter 8.

The sixth mass extinction

Most biologists agree that Earth is facing a loss of species at a rate that rivals many of the previous mass extinctions. They refer to this as the sixth mass extinction event. The cause of this extinction event appears to be our own species, *Homo sapiens*, and the event continues today with modern agriculture and development practices. The first phase of this extinction event began around 50 000 years ago, when modern humans first spread out of Africa across Asia, Europe and Australia. These modern humans brought significant technology and skills with them; they were very effective hunters and many animal species faced predatory pressures that they had not faced before. But modern

humans also brought another powerful technology with them wherever they went: fire. The extinction of several species of megafauna coincided with the first arrival of humans and a different fire regime in Australia, but the exact reasons for their disappearance are an area of ongoing research.

Getty Images/SPL Creative

Figure 7.20
Dromornis (*Dromornis stirtoni*), believed to be the heaviest bird to occupy Earth, lived in Australia from the late Miocene (6 mya) to the early Pliocene (1.8 mya).

RECALL

- Convergent evolution occurs when unrelated organisms (or organisms with a very distant common ancestor) evolve structures or adaptations to perform a similar function in response to a particular environmental cue.
- Such structures are analogous structures.
- Extinctions and mass extinctions are patterns of evolution and have been important in determining the species that are present today. Mass extinctions open up many niches, and this allows adaptive radiations to occur.

RECAP 7.7

1 Define 'convergent evolution' and give an example of how it has affected the evolution of species.
2 Would homologous structures be present in organisms that have evolved analogous structures?
3 Name an example of a mass extinction event and how it changed the subsequent profile of organisms on Earth.

CONCEPT SUMMARY

Evolution

Evolution is the scientific explanation for the mechanisms that drive species to change over time, resulting in the diversity of life on Earth today.

Geological time

Precambrian eon

- 4560–570 mya: First archaea, bacteria, eukaryotes and multicellular organisms

Palaeozoic era

- Cambrian period, 570–510 mya: First invertebrates
- Ordovician period, 510–439 mya: Diverse marine communities: brachiopods, cephalopods and jawless fish
- Silurian period, 439–408 mya: First land plants and arthropods, and jawed fish
- Devonian period, 408–362 mya: First trees, spread of land plants and fish, as well as the first land vertebrates
- Carboniferous period, 362–290 mya: Ferns dominant, swamp forests, insects as first winged animals, first reptiles and amphibians
- Permian period, 290–245 mya: Reptiles dominant, reptilian ancestors of mammals

Mesozoic era

- Triassic period, 245–208 mya: Mass extinction event. Diversification of survivors (dinosaurs, marine reptiles), and first mammals
- Jurassic period, 208–146 mya: Dinosaurs dominant, flying reptiles and Archeopteryx. Cycads and conifers
- Cretaceous period, 146–65 mya: First flowering plants, cool temperate forests of podocarps, celery pines and proteas. Southern beech established. In Australia, mammals arrive from Antarctica and dinosaurs populate huge rift valley between Australia and Antarctica.

Cenozoic era

- Paleogene period, 65–23 mya: Dinosaurs extinct. Flowering plants, birds and mammals radiate into niches left by dinosaurs. First Eucalyptus species (Oligocene epoch).
- Neogene period, 23–2.6 mya: Large marsupials well established. Australia and Asia close enough to exchange plants and animals.
- Quaternary period, 2.6 mya–Present: Major ice ages. First humans arrive and increase in range (Holocene epoch). Great Barrier Reef forms (8000 years ago).

Continental drift

- Movement of the tectonic plates of Earth's crust.
- Geological and fossil evidence show the disintegration of Pangaea into Laurasia (later giving rise to North America, Asia and Europe) and Gondwana (eventually becoming Antarctica, Australia, New Zealand, India, Africa and South America).
- Details of the past are found by measurement of magnetic pole orientation in volcanic rock, and in ice cores showing a record of temperature, climate, sea levels and atmospheric oxygen.

The fossil record

- Preserved remains, including teeth, bones and shells, or impressions in the rock where the organism's tissue has decayed, and traces of organisms, such as footprints, burrows and coprolites
- Usually found in sedimentary rock
- Fossilisation requires specific, rare conditions: organic matter needs to be deposited and covered in sediments in an environment that lacks oxygen.

Transitional forms

Intermediate states between an organism's ancestral form and that of its descendants, such as the famous bird/dinosaur transitional form *Archaeopteryx*

Gradualism

Concept that evolution occurs as a steady, slow divergence of lineages

Punctuated equilibrium

Concept that evolution occurs in bursts; a successive process of stasis followed by a period of rapid change of a subset of the population

Fossil dating

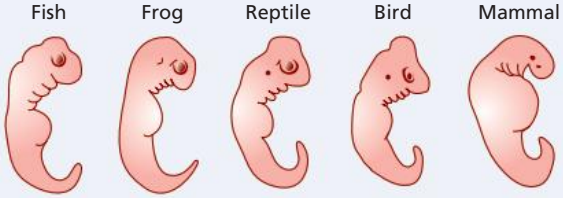
Comparative dating is the relative dating of fossils found in different strata, which are sedimentary layers deposited in a time sequence.

Absolute dating can assign a numerical age to a fossil. It is divided into three types:

- Radiometric dating uses measurements of the relative proportions of isotopes (such as ^{14}C:^{12}C) and the known half-life of the radioactive isotope to determine the age of a sample, expressed with a degree of accuracy. Carbon dating is not usually applied to fossils since the half-life of ^{14}C is too short.
- Electron spin resonance measures the magnetism of electrons trapped in crystalline lattices of some minerals. measurement of the magnetism of electrons trapped in crystalline lattices of some minerals.
- Thermoluminescence and optically stimulated luminescence measure characteristics of minerals within sedimentary rock. Thermoluminescence measures the light emitted from a mineral within sedimentary rock when heated. Optically stimulated luminescence measures light emitted when the mineral is exposed to visible light. Both techniques can provide an estimate of the date of mineralisation but not the absolute age of the sedimentary rock.

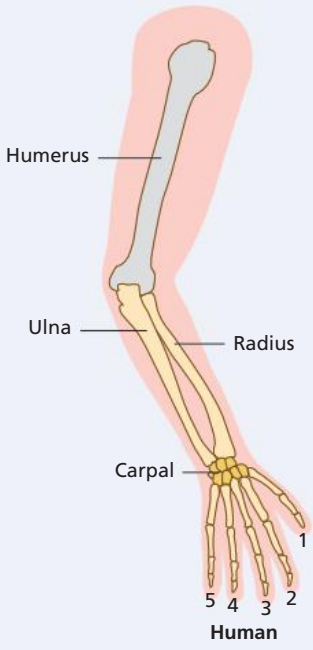
Evidence for evolution

- Biogeography is the study of the distribution of organisms and ecosystems across the world and through geological time. For example, Australia and other landmasses in the southern hemisphere share many plant and animal groups and identical fossils, which were deposited in the Permian period. The distribution of these groups provides evidence that that Gondwana once existed.
- Wallace's line is located between Bali (similar fauna to Java, Sumatra and Malaysia) and Lombok (similar fauna to Papua New Guinea and Australia). This is now known to be the collision zone between the Australian and Asian plates.
- Comparative anatomy examines the physical characteristics of species, at both the embryonic and adult stages.
- Embryology indicates that the similarities observed between embryos of fish, humans and many other organisms are suggestive of a shared ancestor: all members of the phylum Chordata have a dorsal notochord (a solid tissue running along the back), pharyngeal slits (which turn into gill slits in fish), a dorsal nerve chord and a tail that extends past the anus.
- Vestigial structures and homologous structures



Patterns of evolution

- Divergent evolution: When differences between groups of organisms in response to environmental pressures accumulate to a critical point that leads to speciation.
- Adaptive radiation: Rapid diversification into numerous forms
- Convergent evolution: When unrelated organisms evolve similar adaptations in response to their environment
- Analogous structures: Adaptations of very different types of structures that solve a problem in a similar way



Extinction of species

Nearly all species that ever lived are now extinct. Extinction occurs quite regularly. Mass extinctions are periods when the rate of extinction has been very high. The sixth mass extinction began around 50 000 years ago and coincides with the spread of modern humans.

CHAPTER GLOSSARY

absolute dating the process of determining the age in years of rocks and their contained fossils on the basis of the physical or chemical properties of materials in the rock

adaptation a developed characteristic that enhances an organism's survival in its natural environment

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

analogous structures features of organisms that have the same function but not the same structure

biogeography the study of the distribution of living things over a geographical area through geological time

comparative dating the process of determining the age of rocks and their contained fossils relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

continental drift the relative movement of Earth's continental landmasses, which appear to drift or 'float' over Earth's mantle

convergent evolution a process whereby unrelated organisms evolve similar adaptations in response to their environments

divergent evolution evolution in which a single ancestral species diverges, or splits, to eventually become two or more descendant species

eon a division of geological time that can be divided into periods, epochs and ages

epoch a division of geological time that is shorter than a period and is marked by one or more significant events

era a division of geological time comprising periods and epochs

evolution the process of gradual change in the characteristics of a population of organisms that results in new species

fossil the preserved remains or traces of an organism

gradualism a theoretical model of the pace of evolution occurring as a steady, slow divergence of lineages at an even speed, irrespective of gaps in the fossil record

homologous structures features of organisms that have the same general structure but different functions

isotope atoms of an element that have the same number of protons but different numbers of neutrons and therefore different relative atomic masses

mya millions of years ago, sometimes expressed as millions of years before present (myBP), or simply millions of years (my); for example, a fossil dated as being 5 million years old lived 5 mya

niche an organism's habitat; or way of life or function of an organism in its environment

period a division of geological time; periods and epochs together make up eras

phylogeny evolutionary relationships that exist between species, often expressed as a tree-like diagram

punctuated equilibrium a theoretical pattern of evolution in which changes in organisms occur in bursts separated by periods of stasis; the rapid changes occurring during the bursts may not be preserved in the fossil record

speciation the evolution of one or more new species from an ancestral species

vestigial structures structures found in organisms that have lost most, if not all, of their original function in the course of evolution; in ancestral organisms the structures served a purpose, but in their descendants the structures become atrophied or rudimentary

CHAPTER REVIEW QUESTIONS

Remembering

1. List the categories of evidence for evolution.
2. Recall Lamarck's theory of evolution.
3. Define the following terms:
   - a gradualism
   - b punctuated equilibrium
   - c biogeography
   - d vestigial structures
   - e homologous structures
   - f comparative genomics.

4 The fossil record is a vital stream of evidence in the modern evolutionary synthesis, but it is patchy and incomplete. Recall the reasons for this patchy record.

Understanding

5 The thylacine (marsupial) and the American grey wolf (placental) evolved independently of each other in remote biogeographic locations, but both animals had a similar appearance and occupied similar ecological niches. The similarities between the two organisms are most likely a result of which evolutionary pattern?

6 Describe the fundamental difference between Darwinian evolution and its precursor theories in terms of how descent should be viewed.

7 Both birds and bats have wings, while mice and crocodiles do not. Explain if this means that birds and bats are more closely related to one another than to mice and crocodiles.

8 Consider the effect on large land herbivore populations of warmer, wetter global conditions in contrast with cooler, drier conditions.

9 Forty per cent of the world's species of fruit fly are found on the islands of the Hawaiian archipelago.
- **a** Propose why the Hawaiian archipelago might provide a suitable habitat for so many different species of fruit flies.
- **b** Explain how adaptive radiation may have been involved in the evolution of Hawaiian fruit flies.
- **c** Describe three ways that ancestral fruit fly genes may have been transported from one island to another.

10 The sugar glider and the flying squirrel have a similar appearance. Both have a flap of skin between the forelimbs and hind limbs that enables them to glide from branch to branch. The flying squirrel is a placental mammal found in the northern hemisphere and the sugar glider is a marsupial found in Australia.
- **a** Name the process that has resulted in these species having similar features.
- **b** Name and describe the evolutionary pattern that accounts for the similarity of these two species.
- **c** Suggest how these two animals – one a placental and one a marsupial – are different in other ways.

11 Draw your own timeline illustrating the geological eras and periods shown in Table 7.1, then complete the following tasks.
- **a** Identify the era in which life first appeared.
- **b** List all periods in which dinosaurs existed.
- **c** Determine whether *Eucalyptus* species would be expected in Africa. Explain your reasoning.

Applying

12 Embryological studies show bird embryos develop a fourth finger and a fifth toe that vanish as the foetus develops. This vestigial developmental structure is evidence for common descent.
- **a** Explain what this evidence explicitly says about the characteristics of the ancestors of birds.
- **b** Explain whether you would expect a complete fossil skeleton of a common ancestor showing this characteristic to have been found.

13 Stone tools have been found with campfire charcoal. Explain how the technique of carbon dating could be used to determine the time at which the tools were made.

14 Explain the basis of the technique of electron spin resonance.

15 A fossilised fish skeleton is found in sandstone, 1 m below the surface, at location X. A very similar skeleton is found at location Y, 2 m below the surface and 1 km away from location X. Another similar skeleton is found at location Z, 3 m below the surface and 3 km away from location X. Describe what can be inferred about:
- **a** the way in which the rocks were formed
- **b** the age of the fossil at location Y.

Analysing

16 New Zealand has no large native land mammals, but has been home to some highly specialised bird species. Many of these birds have lost the ability to fly, and in the case of the five species of kiwi, have developed some distinctive features. Mammal-like characteristics such as a keen sense of smell, bone marrow (which makes bones heavy and unsuitable for flight) and a pair of functional ovaries in females (most birds have only one functional ovary) are highly unusual for birds. Research the five species of kiwi and explain how they show examples of:
- **a** divergent evolution
- **b** convergent evolution
- **c** adaptive radiation
- **d** analogous structures.

17 Identify a limitation of luminescence in dating sedimentary rock.

18 There is a variety of types of tortoise on the Galápagos Islands. One species has a domed shell and a short neck and is found on islands with high moisture content. Another has a shell that flares up at the front so that the tortoise can lift its long neck up. The long-necked tortoise is found on the more arid islands.

The main food of the tortoises is the prickly pear cactus. On the islands with no tortoises, the prickly pear has a low spreading form with soft spines. On the islands with the long-necked tortoise, the prickly pear has a tall form with hard spines.

- **a** Explain how the tortoises could have first reached the Galápagos Islands.
- **b** Assess and explain if it is likely that the ancestor tortoises would be identical to the modern tortoises.
- **c** Explain why prickly pear would grow in different plant forms on different islands.
- **d** Define and explain what type of evolution is illustrated by the association of the long-necked tortoise and the tall prickly pear.

Evaluating

19 The hoatzin (*Opisthocomus hoazin*) is a remarkable bird from South America. It has only one known fossil ancestor, a 10-million-year-old skull fragment found in Colombia. The age of the fossil demonstrates that hoatzins were endemic to South America; the fossil pre-dates the land bridge between North and South America by 8 million years.

Genetic analysis of the living hoatzin has shown it is unique, perhaps because of its extensive history of geographic isolation, and it has its own suborder. Chicks of the hoatzin show a characteristic seen in no other living bird: a pair of claws on their wings, a characteristic similar to those seen on the bird-like dinosaur *Archaeopteryx*, which had three wing claws.

From the above description, identify lines of evidence for evolution from the disciplines of:

- **a** palaeontology, via the fossil record
- **b** biogeography
- **c** developmental biology
- **d** morphology
- **e** genetics.

Creating

20 The 1861 discovery of the Jurassic-age fossil skeleton of the feathered dinosaurian bird ancestor *Archaeopteryx* from Germany was a key moment in the development of Darwinian theory. The discovery of the pigeon-sized animal was brought to the attention of Charles Darwin, who commented that 'hardly any recent discovery shows more forcibly than this how little we as yet know of the former inhabitants of the world'.

The skeleton of *Archaeopteryx* clearly shows that it had claws on its forelimbs, well-developed feathers on its wings (allowing for weak gliding flight), teeth and a long bony tail.

- **a** Define which of these characteristics point to a relationship to birds on the basis of:
  - **i** embryology
  - **ii** homologous structures
  - **iii** analogous structures.
- **b** Explain how the relationship of *Archaeopteryx* to dinosaurs and birds has limitations based on molecular homology and comparative genomics.
- **c** Predict how the potential diet (as influenced by the climate, continental associations and other animals and plants) would have affected the *Archaeopteryx* in terms of its:
  - **i** teeth
  - **ii** size
  - **iii** locomotory adaptations.

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CHAPTER 8
MECHANISMS OF EVOLUTION

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

KEY KNOWLEDGE

Changes in the genetic makeup of a population

- the qualitative treatment of the causes of changing allele frequencies in a population's gene pool including types of mutations (point, frameshift, block) as a source of new alleles, chromosomal abnormalities (aneuploidy and polyploidy), environmental selection pressures on phenotypes as the mechanism for natural selection, gene flow, and genetic drift (bottleneck and founder effects) and the biological consequences of such changes in terms of increased or reduced genetic diversity
- processes of evolution including through the action of mutations and different selection pressures on a fragmented population and subsequent isolating mechanisms (allopatric speciation) that prevent gene flow
- the manipulation of gene pools through selective breeding programs.

KEY SCIENCE SKILLS

Communicate and explain scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them
- use clear, coherent and concise expression

Science Source/John Devries

Alamy/Frank Hecker

Figure 8.1 ▲
The peppered moth, *Biston betularia*, has (**a**) a white speckled *typica* form and (**b**) a dark *carbonaria* form.

The peppered moth, *Biston betularia*, is widespread in Britain. Historically, the standard moth form, *typica*, was white, liberally speckled with black. During the 1800s British cities and the countryside were transformed by the Industrial Revolution. Hundreds of coal-powered factories produced large quantities of airborne soot and other pollutants. By 1895, 95% of moths in industrial regions of Britain, such as Manchester, were black (form *carbonaria*). A well-known lepidopterist, J. W. Tutt, proposed an evolutionary link between the Industrial Revolution and the moth population. Dark pigmentation was part of the natural, inheritable variation of the *B. betularia* population, but very rare. Blackening of tree trunks by soot presented a new environmental pressure for the moth population. The dark-coloured moths were better able to evade bird predation than the common white speckled form. Over time, black moths came to dominate the population.

Since 1950, when clean air legislation was passed, the situation has reversed; once again dark-coloured moths are suffering greater predation on the naturally white tree trunks and their presence in the population is less common. Both dark and white forms continue to exist in the population.

Like the peppered moth example, individuals in any population express a range of different **phenotypes**. This is because members of a population have variation in **genotypes** that causes variation in their phenotypes. This genetic variation is **inheritable**; it can be passed to the next generation and under certain circumstances may give an individual an advantage in survival and reproduction compared to the rest of the population. In the case of the peppered moth, a **mutation** in genotype produced a dark-coloured form in this population. This dark phenotype conferred a survival advantage in the changed environment. On the other hand, the genotypic variation may also give a disadvantage or have no effect at all. Either way, genetic mutation introduces new **alleles** and, therefore, new variation into populations.

Mutations are the source of new variation

The basis of evolutionary theory is that favourable traits become more common in each successive generation. Those members of a population that survive and reproduce in their habitat carry the traits most suitable for their circumstances and so, over time, the population becomes more suited, or better adapted, to its habitat. But what

happens when the habitat changes? In most cases where there is genetic variation in the population, some members will survive the changes and pass on their genes. For example, some members of a locust population may be resistant to local pesticides. Those members would survive seasonal crop spraying and pass on their genes. If no members in the locust population possessed a genotype that resisted pesticides, the local locust population would not survive.

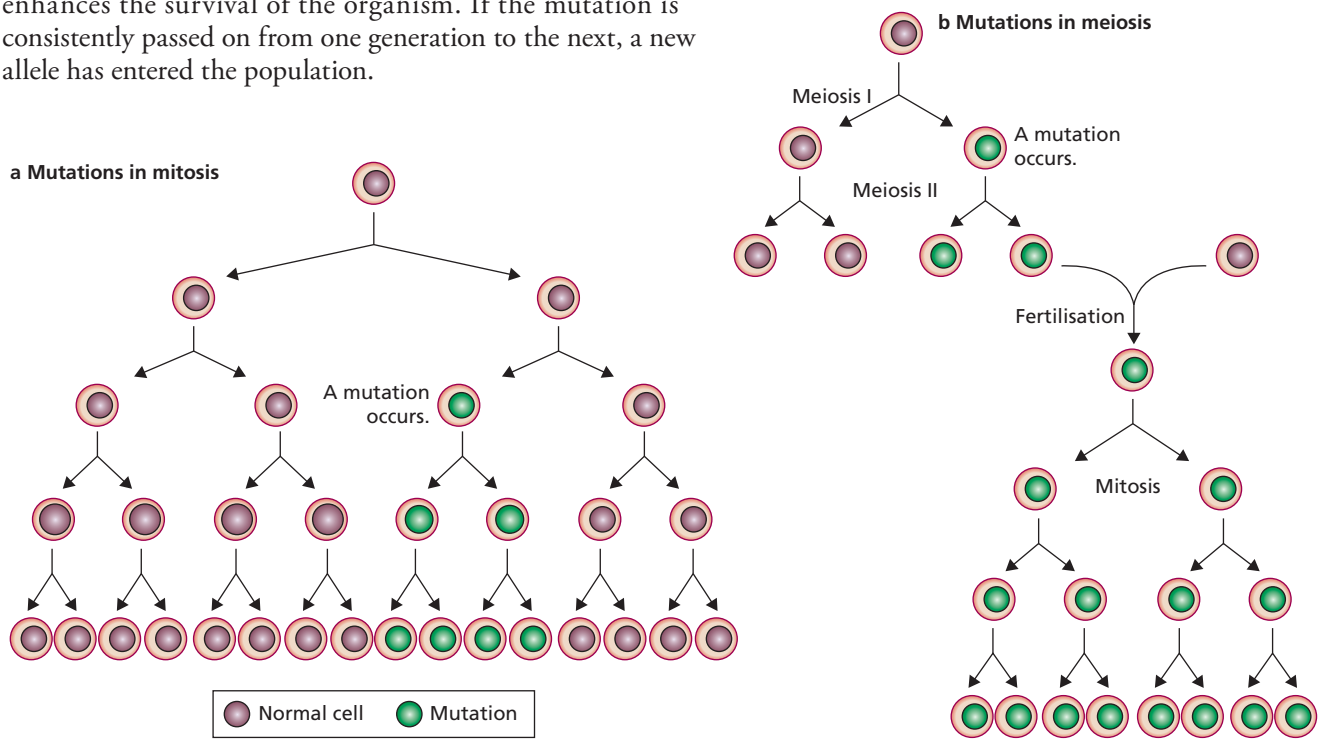
Such variation in the gene pool is essential for the survival of populations. So where do new alleles come from? The answer is that they generally come from old alleles through mutation. Mutations are rare and mostly produce harmful effects. In a large population they are barely noticeable. But despite this they are essential to evolution because they are the ultimate source of variation within populations.

Mutations are changes to DNA. A **spontaneous mutation** may arise during cell division, or mutation may be induced by physical or chemical **mutagens**, or through the action of biological agents. Mutations that occur in genes often affect the translated proteins they code for. These effects are sometimes subtle. More often they are severe, with potentially catastrophic effects for the survival of the organism that bears them. Rarely, they can enhance the function of the protein or make it better suited to the environment the organism inhabits. The effect of a mutation depends upon whether it has occurred in non-reproductive (body, or **somatic**) cells or in the reproductive (**germ-line**) cells (Figure 8.2).

A mutation in a somatic cell occurs only in the affected body cell and the daughter cells produced from it by mitosis. All other cells of that organism lack the mutation. Cancer is a salient consequence of mutations in somatic cells. The mutations accumulate in particular genes or regions of the DNA that accelerate the rate of cell division, abolish the cell's ability to undergo apoptosis or increase the rate of mutations within the cell.

Mutations that occur in germ-line cells affect gametes and have the potential to be inherited, or passed on to the next generation, so that they are incorporated into every cell of the offspring. Often, the germ-line mutation results in developmental abnormalities that cause the affected embryo or foetus to be spontaneously aborted. If carried through to birth, the germ-line mutation may result in congenital disorders in the offspring with varying severity. Occasionally a gene mutation changes or enhances the function of the encoded protein, which, if circumstances suit, enhances the survival of the organism. If the mutation is consistently passed on from one generation to the next, a new allele has entered the population.

▼ **Figure 8.2**
(**a**) Mutations in somatic cells affect only the cell in which it occurred and all its daughter cells. (**b**) Mutations in germ-line cells affect all body cells of the individual who inherits them.



Types of mutations

The simplest form of mutation is a **point mutation**, in which just a single nucleotide within the original DNA sequence is affected. Differences between sequences in the nucleotides at one position are called **single nucleotide polymorphisms** (SNPs, often pronounced as 'snips'). If the point mutation occurs in a gene, the mutated gene sequence can be transcribed and translated into a protein that is the same as that encoded by the original form of the gene, or it may be altered. When the protein is altered, the mutation may have a subtle or a dramatic effect on its structure and function.

Substitution

A substitution occurs when one nucleotide is replaced by another (e.g. adenine substituted by guanine). **Substitution mutations** are a source of novel SNPs and have a number of possible effects on the translated protein.

A **synonymous mutation**, also referred to as a **silent mutation**, occurs when the substituted base results in a codon (also known as a triplet) that codes for the same amino acid as the original codon. For example, AGA and AGG both specify for the addition of an arginine amino acid in the polypeptide chain (Figure 8.3). The protein encoded by the mutated gene is therefore identical to that encoded by the original gene. Synonymous mutations are possible because a level of redundancy is built into the genetic code. Recall that the genetic code consists of 64 codons that code for 20 amino acids plus the instructions to start and stop translation. Therefore, any individual amino acid can be encoded by more than one codon.

A **missense mutation** arises when a single nucleotide substitution changes the amino acid. For example, substitution in an AGA codon to generate an AGC codon results in a serine amino acid being added to the polypeptide instead of the original arginine (Figure 8.4).

A **nonsense mutation** occurs when a single point mutation creates a new stop codon within the original gene sequence (Figure 8.5). This leads to early termination of translation of the transcribed gene sequence because the remaining sequence downstream of the new stop codon is not translated. The result is the production of an incomplete polypeptide.

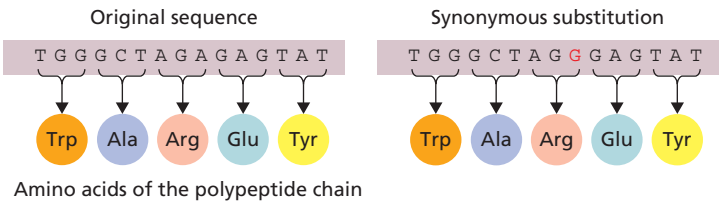


Figure 8.3 ▶ Synonymous mutation

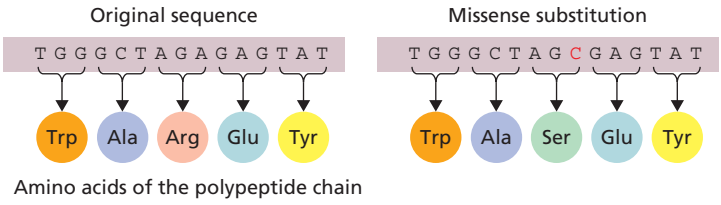


Figure 8.4 ▶ A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.

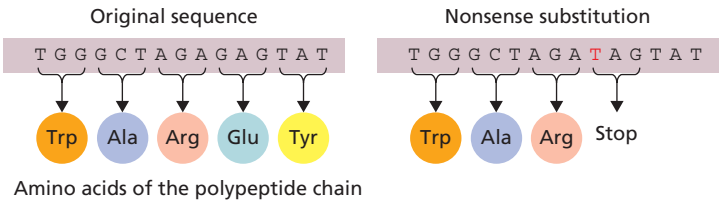
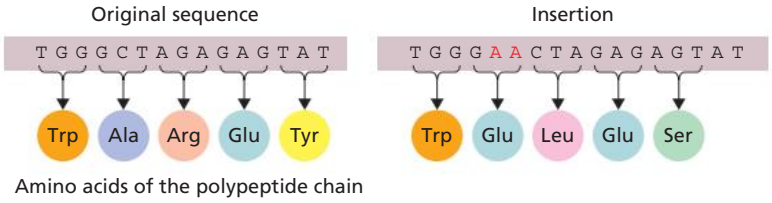


Figure 8.5 ▶ A nonsense mutation in the gene sequence results in premature termination of translation.

Insertions and deletions

As the name suggests, an **insertion mutation** is the addition of one or more nucleotides at a site within the original gene sequence. A **deletion mutation** is the loss of nucleotides from a site within the original gene. Collectively these are referred to as 'indels'. The effect of the indel is frequently a **frameshift mutation**, in which the reading frame for the corresponding amino acids has been nudged away from the original and all the codons downstream of the mutation are affected. The consequence for the translated protein is that the amino acids downstream of the mutation bear no resemblance to those of the original polypeptide (Figure 8.6). Under such circumstances, even a single nucleotide insertion or deletion can have a profound effect on the corresponding protein.



◀ **Figure 8.6**
An insertion in the gene sequence results in a frameshift mutation.

RECALL

- Mutations are changes in DNA. Factors that cause mutations are mutagens, and the potential effect of a mutation on the gene pool depends on whether the mutation occurs in somatic or germ-line cells.
- Point mutations can cause changes in a DNA sequence by either (1) substitution of a nucleotide or (2) insertion or deletion of a nucleotide.
- Substitutions can be synonymous, or cause missense or nonsense mutations.
- Insertions or deletions can cause frameshift mutations that can affect the amino acid sequence downstream, severely affecting the encoded protein.

RECAP 8.1

1 SNPs are common within genomes. Define SNP and describe what type of mutation they usually are if they are so common.
2 A frequent effect of a frameshift mutation is to produce a stop codon earlier than normal. What effect would this have on the encoded protein?
3 List the types of point mutations in the order of potential severity of their effects from least to most severe.

Variations in chromosome structure

Genetic variations can also be driven by wholesale changes to the chromosomes. Alterations to chromosomes contrast with single point mutations because they can affect many genes simultaneously. Some of the variations that occur with chromosomes, such as chromosome number, are natural in certain situations and are therefore integral to the functioning and continuity of the species. Others arise because of anomalies that occur during the formation of the gametes.

Deletions

A chromosome may undergo **double-strand breaks** at two positions and the section in between may drop out, removing all its genes with it. If the two ends then re-join,

a shorter chromosome results with a segment missing in between. This is called a chromosome deletion (Figure 8.7a) and, as it leads to an absence of certain genes, it can have a profound effect on the development of an organism. All but the shortest deletions are usually fatal and the few that survive are associated with adverse effects.

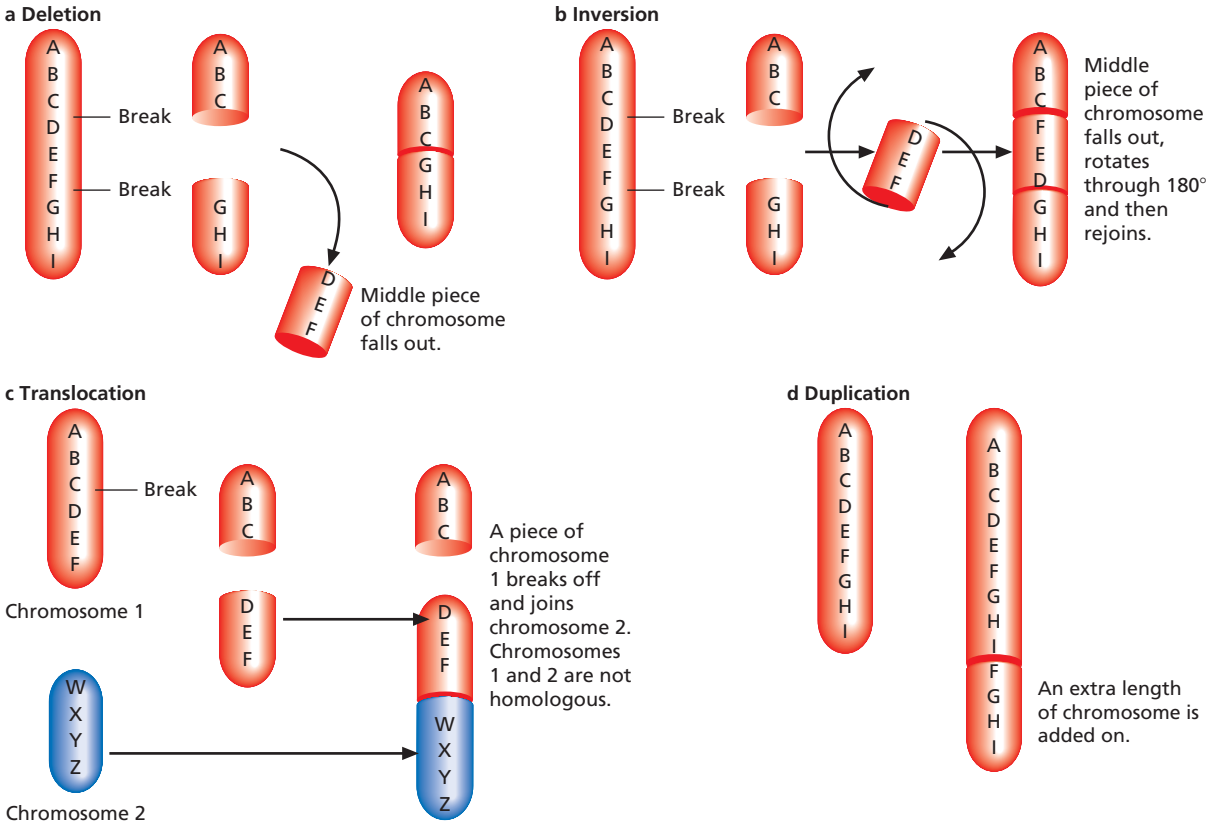


Figure 8.7 ▲
Abnormalities caused by chromosomal mutations may arise by deletion, inversion, translocation or duplication.

Inversions

Another kind of chromosomal rearrangement occurs if a chromosome breaks in two places and the segment in the middle rotates through 180° before being re-joined within the chromosome, reversing the normal sequence of genes (Figure 8.7b). This is called inversion. The effects of inversions are usually less dramatic than other types of chromosomal changes because genes have been neither gained nor lost and the genes within the inverted segment can still function normally. The inversion may, however, disrupt a gene through which it occurs or cause two different genes to become fused together. Also, if the chromosomes do not align properly for meiosis, the affected individual may have reduced fertility.

Translocations

Sometimes a section of one chromosome breaks off and reattaches to another chromosome. This is known as translocation (Figure 8.7c). An example of translocation in humans is when a segment of chromosome 8 ends up with chromosome 14, or vice versa. Normal control over the genes in that segment is lost, often resulting in a form of cancer.

Duplications

A duplication occurs when an extra copy is made of a section of chromosome and inserted either into the same chromosome or into another chromosome (Figure 8.7d). Gene sequences can be replicated many times, sometimes thousands of times. Like

other chromosomal abnormalities that change the number of copies of particular genes, duplications are frequently harmful. However, on occasions, they may be advantageous. The various genes that control the different haemoglobins produced in human red blood cells are thought to have arisen by duplications.

Variations in chromosome number

In many eukaryotic organisms, the somatic cells are **diploid (2*n*)**: the cells contain two sets of **homologous chromosomes**, one set inherited from each parent. The gametes are **haploid (*n*)**. There are consequences for organisms when the complement of chromosomes in the somatic cells varies from the usual diploid state.

Monoploidy

In many colonial insects such as ants, bees and wasps, the males of the species are **monoploid (1*n*)**. By contrast, the females, including the queen, are diploid (Figure 8.8). The males are not haploid in the sense of the gametes of regular diploid animals. Their chromosomes represent a single complete and operational set and the males function as conventional, multicellular animals. By contrast, in haploid gametes, the chromosomes represent half the complete set and are packaged in a dormant state awaiting the fertilisation event that will activate them. In the insects with monoploid males, the queen produces eggs by meiosis, whereas the males produce sperm by mitosis. Fertilisation results in diploid female offspring. The males are instead produced by **parthenogenesis**, a process by which the entire organism is regenerated from a single egg cell without the need for fertilisation.

Shutterstock.com/Lehrer

▲ **Figure 8.8** Bees maintain their colony structure with diploid females and monoploid males.

Many fungi and algae are also monoploid and, as well as insects, there are examples of monoploid fish, amphibians and reptiles. Monoploidy seems economical because only one set of chromosomes is required, so why are diploid organisms so much more common? The advantage for diploid organisms is that any defective alleles that arise can be masked by a functional allele on the corresponding chromosome. In monoploid organisms, any defective allele is the only allele available for a particular gene and the consequences are likely to be deleterious.

Polyploidy

Sometimes the cell divisions that give rise to haploid gametes fail altogether, so that half the gametes contain two copies of each chromosome (diploid, 2*n*) and the rest have none. If a diploid gamete fuses with a normal haploid gamete, the resulting individual is triploid (3*n*): it has three of each type of chromosome. If two diploid gametes fuse, a tetraploid (4*n*) individual will be produced. It is therefore possible for an organism to acquire one or more complete extra sets of chromosomes, a phenomenon called **polyploidy**.

Polyploidy is particularly common in flowering plants, ferns and green algae. Approximately half of all flowering plant species are polyploid, and many varieties of commercial fruit and cereals are generated polyploids (Figure 8.9). Polyploidy is often associated with advantageous features, such as increased size and greater hardiness, although such advantages are sometimes offset by reduced fertility. Polyploidy also occurs in fungi and in some fish and amphibian species.

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▲ **Figure 8.9** Polyploid varieties of fruit are bigger and have bigger cells than regular diploid varieties. The application of polyploidy to creating infertile fruit, such as these seedless grapes, is of considerable commercial significance.

Polyploidy is lethal in humans. In the rare situation of a pregnancy continuing to a live birth (1% of human polyploids), the newborn dies within a month.

Aneuploidy

Aneuploidy is the condition in which there is an addition or loss of one chromosome from a cell (i.e. $2n$ 1 1 or $2n$ 2 1). Occasionally more than one chromosome may be affected. Reproductive failure by miscarriage is common and it has been found that many of these miscarried embryos are aneuploids. To understand how this comes about, consider the process of meiosis. Normally in meiosis, identical chromosomes come together and then segregate into separate cells, so that the gametes finish up with only one of each pair of chromosomes. Occasionally, however, the two identical chromosomes, instead of separating, go into the same cell. This phenomenon is known as **non-disjunction**. It results in the formation of two types of gametes in equal proportions, but one type has two copies of a particular chromosome and the other type has none (Figure 8.10).

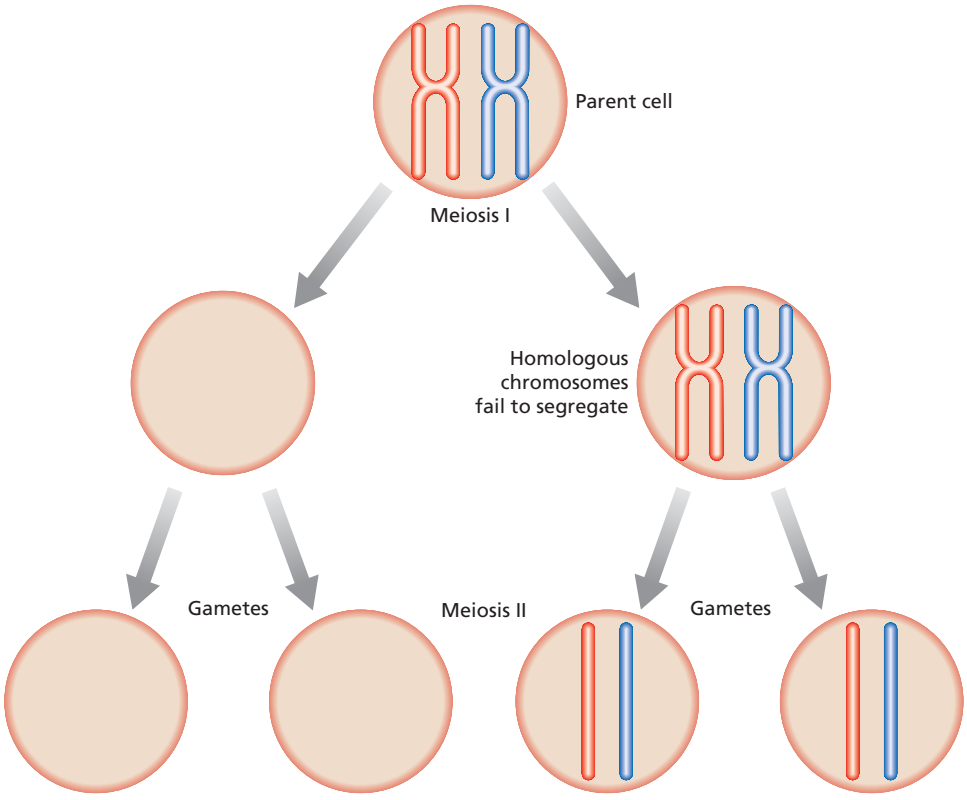


Figure 8.10 ▶ In non-disjunction, the chromosomes fail to segregate, so half the gametes contain two chromosomes of a pair each (bivalent) and the other half contain no chromosomes at all. Generally, non-disjunction only takes place with one pair of homologous chromosomes, while the rest behave normally. It can occur during either the first or the second meiotic division.

RECALL

- Mutations can arise that cause changes in chromosome structure and chromosome number.
- Deletions, inversions, translocations and duplications may involve particular segments of a chromosome, and may alter the number of alleles present in affected cells.
- Changes to chromosome structure such as inversion, deletion, duplication and translocation of chromosome segments are another cause of genetic variation.
- Most eukaryotic somatic cells are diploid. Monoploidy, polyploidy and aneuploidy are alterations in chromosome number that change the number of alleles for many different genes.

RECAP 8.2

1 Draw a diagram to show the four main types of mutations that affect whole segments of chromosomes. Which would cause the most severe effect for an organism?
2 Describe the relationships between:
   a haploid and diploid
   b monoploid and haploid
   c monoploid, diploid and polyploid
   d diploid and aneuploid.

Effects of mutations on survival

A protein's function depends on its structure. Mutations that change a protein's structure can have consequences for protein function with potential impacts on the organism's survival. Mutations can therefore also be classified according to the effect of the mutation on the protein's function and expression and whether the organism's survival is unchanged, changed for the worse, or changed for the better.

Neutral mutations

In the case of synonymous mutations, the protein product is unchanged compared with the original, so the organism's survival is unaffected by the change. This is said to be a **neutral mutation**. Missense substitutions are sometimes also neutral mutations, provided that the original amino acid is swapped with another that has similar properties. For example, in the ABCA1 gene, which codes for a protein involved in cholesterol transport, a missense substitution in a single GAA codon generates a GAC and causes the amino acid glutamic acid to be swapped for an aspartic acid. Both amino acids are negatively charged and reside on the surface of the protein where they interact with surrounding water, so the properties and function of the protein remain essentially the same.

Deleterious mutations

A living organism can be compared with a complex product of engineering, such as an aeroplane, in which the components are so intricately integrated that an indiscriminate change to any component harms the overall operation of the aircraft and makes it unfit to fly. Similarly, random mutations may disrupt the function of the encoded protein, undermining the organism's overall ability to carry out its basic processes and survive. Such mutations are referred to as **deleterious mutations**. The majority of mutations are deleterious.

Nonsense mutations are typically deleterious because they result in the production of an incomplete protein that is non-functional. However, these deleterious mutations may persist if the individual who carries them also has a copy of the normal allele that encodes the functional version of the protein. The deleterious mutation is thus masked within the phenotype of the organism. If the organism is unfortunate enough to have only non-functional alleles for a particular gene, the condition usually results in the death of the organism before they have the opportunity to reproduce and pass the alleles onto any offspring.

Beneficial mutations

Occasionally gene mutations lead to the generation of a new allele that benefits the survival of the organism. The type of **beneficial mutation** can vary: it could be a missense mutation that changes the function of the original protein, or it could be

a nonsense mutation that eliminates a protein that may have been harmful to the organism in some circumstances.

Many mutations produce recessive alleles that can be masked by the effects of the original allele, which remains the dominant allele. We each may carry several hundred mutations, most of which will never be noticed, particularly as most of us will have children with partners who are not closely related.

Conversely, recessive alleles are an important source of variation within populations. This was the case with the peppered moth population. Before the Industrial Revolution, the dominant *carbonaria* forms were extremely rare, and appear to have been maintained mainly through the occurrence of spontaneous mutations. However, during the Industrial Revolution recessive alleles coding for the white (*typica*) trait were able to survive in the population at a low level. Only the extremely rare homozygous individuals experienced the selective pressure of increased predation. In this case, the population responded quickly to a changing environment.

The evolution of sexual reproduction, with the random mixing and assortment of traits from one generation to the next through meiosis, has been very important in producing populations with variation.

RECALL

- Mutations that affect the structure or expression of a protein can have consequences for the survival of an organism.
- Based on these consequences, mutations can be classified as neutral, deleterious or beneficial.
- Most mutations are neutral or deleterious. Rarely, they are beneficial, but these beneficial mutations can be important sources of variation in a population.

RECAP 8.3

1 What type of mutation in chromosome structure may have a neutral effect on the cells it occurs in?
2 List three types of mutation that may be deleterious for an organism.
3 Give an example of a mutation that may have a beneficial effect for an organism.

Changing allele frequencies in populations

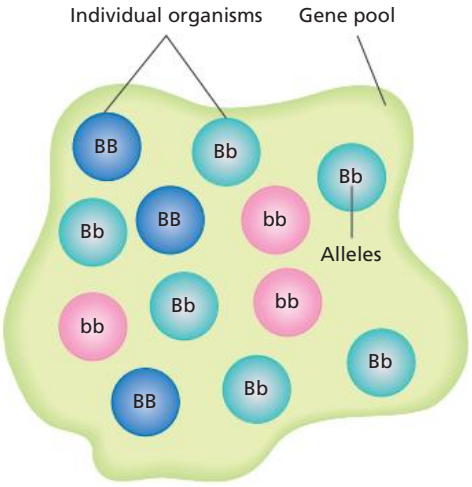
Mutations introduce new variations into a population, and so can many external influences. The collection of alleles within a population is shaped by the movement of individuals and by environmental events that can sometimes rapidly and considerably change the composition of populations. The study of allele frequencies in populations and how they change over time in response to various evolutionary processes is called **population genetics**.

Gene pools

Genes are the means of transmitting phenotypes from one generation to another. Many genes can exist in different forms as alleles, and the characteristics of individuals are determined by the alleles they inherit. It is this variation in alleles carried by different

individuals that leads to most of the variation in a population. The total collection of alleles within a population is referred to as a **gene pool** (see Figure 8.11). In biological terms, a **population** is a group of individuals of the same **species** that live in the same geographic area and readily interbreed to produce fertile offspring, so they belong to the same gene pool.

The range of variation possible in a population is restricted by the alleles available in its gene pool. For example, bearded dragons do not carry genes for wings or hard-shelled eggs or the enzymes required to synthesise chlorophyll or to digest cellulose. All bearded dragons do, however, carry genes for a tail, rudimentary teeth, scales and four legs (Figure 8.12). The many genes that have only one possible allele in a gene pool, and so do not contribute to any variation, are said to be 'fixed' in the population. Scientists believe that approximately 80–85% of our genes are fixed in this way. These genes do not make a significant contribution to evolution since there is no variety to draw on. It is the other 15–20% that can be drawn upon during evolutionary change.



▲ **Figure 8.11** The sum total of all alleles found in a population is called the gene pool.

Allele frequencies

For variation to occur in phenotypes, more than one allele of a gene must exist. Phenotypes that vary due to genetic differences are termed genetic polymorphisms ('poly' meaning multiple, 'morph' meaning form). The frequency of polymorphic alleles is not usually constant and can be affected by:

- **mutation** of an allele
- **immigration** of individuals; that is, movement into the population
- **emigration** of individuals; that is, movement out of the population
- the **reproduction rate** of various individuals in the population; that is, the number of offspring born per year to an individual.

Alamy/Juniors Bildarchiv GmbH

▲ **Figure 8.12** Bearded dragons carry genes for a tail, scales and rudimentary teeth, but do not have genes for wings.

RECALL

- Variations in populations can be very small, but they are the basis of evolution. Evolution is any change in the gene pool over time.
- Genetic mutations introduce new alleles into populations. These act as the main source of variation.
- The sum total of all alleles present in a population is called the gene pool. The frequency of an allele can change by mutation, immigration or emigration of individuals, and by how well individuals carrying the allele reproduce.

RECAP 8.4

1 Recall the relationship between genotype and phenotype.
2 Distinguish between a gene and an allele.
3 Outline why variations have to be inheritable for them to be relevant to evolutionary change.
4 Define 'population' and 'gene pool'.

Migration and gene flow

Populations, in a biological sense, are defined by their reproductive and genetic isolation. Few populations are completely isolated from each other, and generally some migration takes place both into and out of the population. **Gene flow** may occur if the migrants breed. For example, immigrants may add new alleles to the gene pool, and emigrants may completely remove some alleles or significantly change the frequency of others (Figure 8.13).

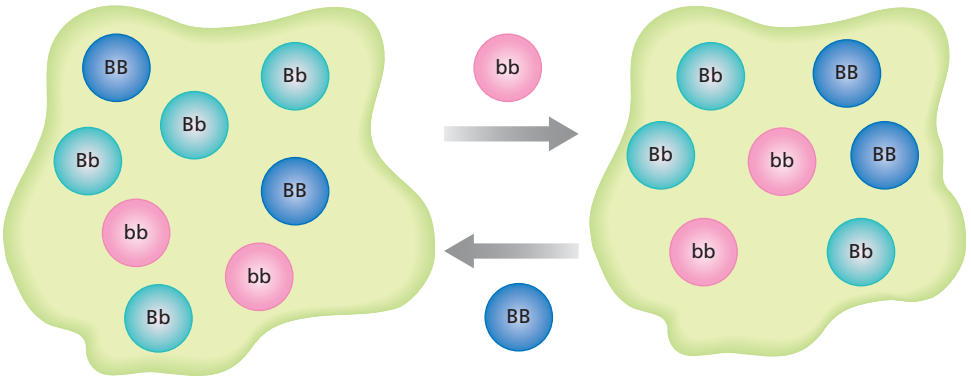


Figure 8.13 ▶
Gene flow is the transfer of alleles that results from emigration and immigration of individuals between different populations.

Humans are polymorphic for a range of blood types, including the ABO blood types. Indigenous Australians have some alleles that are present at frequencies different from that in other populations in the world. They have largely been isolated for the last 50 000 years, except for some gene flow from Asia and New Guinea in the northern regions of Australia. Most Indigenous Australians do not possess the I^B allele of the ABO blood group that results in either the B or AB blood type. The I^B allele occurs at a frequency of up to 10% in European populations and up to 20% in Asian populations. The overall frequency of the I^B allele is increasing within the Indigenous Australian population due to migration of people from Asia and Europe into Australia and the genetic flow between these populations.

Genetic drift

The term **genetic drift** applies generally to random changes in small populations. Every reproductive event involves chance. Each of us inherited half our alleles from our mother and half from our father. Which half of their alleles our respective parents passed on to us was a matter of chance. In large populations this randomness in inheritance of alleles is not noticeable overall. But if a population is small, there is a chance that some alleles present in a parental group will not be passed on at all. These alleles may be permanently lost from the gene pool. Alleles may be easy to lose, but they are virtually impossible to replace.

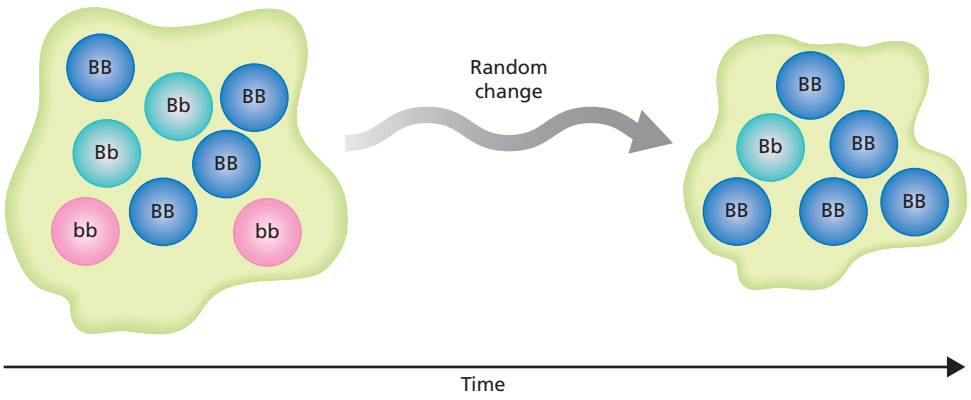


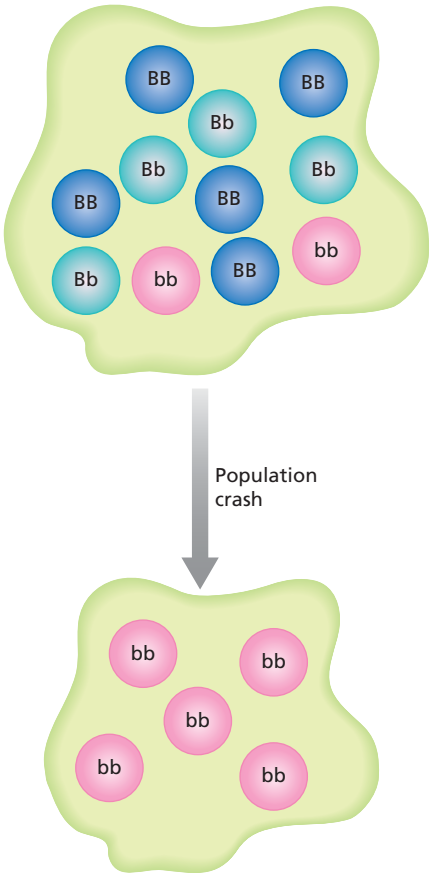
Figure 8.14 ▶
Chance events can cause the allele frequency in a population to change. This is known as genetic drift.

Genetic drift can occur in a small population or when a large population is suddenly reduced by a catastrophic event. This can give rise to a **bottleneck effect**. When a small group of individuals migrates and establishes a population in a new location, the **founder effect** may occur, as discussed below.

Bottleneck effect

Sometimes a catastrophic event or a period of adverse conditions drastically reduces the size of a population. In this scenario, certain alleles may be lost through chance (Figure 8.15). If some portion of the population survives the catastrophe, the original population gene pool cannot be recovered. The expanded population can only carry the alleles that existed in the population that survived the event. Therefore, the gene pool will now carry an indication of the bottleneck that occurred long after the population has recovered.

Cheetahs are an endangered species that have survived a genetic bottleneck. Facing a declining population, the surviving parents mated with their own offspring, and the resulting generations were left with strikingly similar alleles. One of these is a mutated allele with negative effects on fertility. Typically, a male cheetah's sperm count is low and 70% of the sperm are abnormal. Other shared alleles result in lowered resistance to disease. Infections that are seldom life-threatening to other cat species can be lethal in cheetahs. There are only around 10 000 cheetahs left in the world today.



▲ **Figure 8.15** A catastrophic decrease in population size can result in a loss of some alleles from the gene pool. This is the bottleneck effect. Deleterious genes can be preserved by chance.

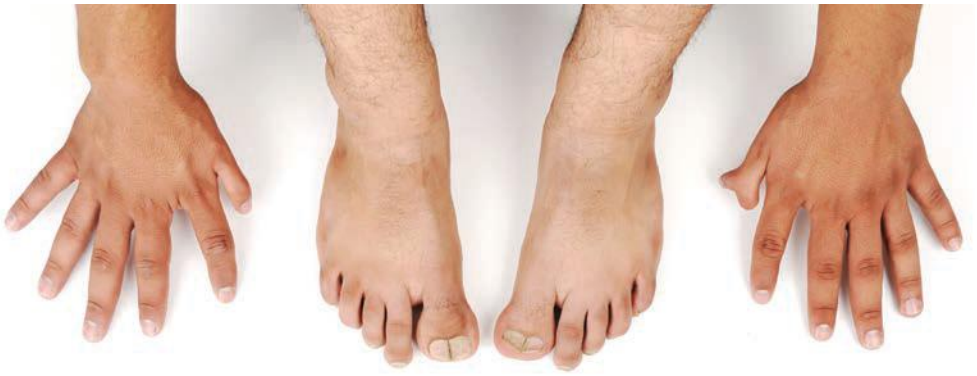
iStockphoto/DaleBHalbur

◀ **Figure 8.16** Cheetahs survived a severe bottleneck that increased the frequency of some mutated alleles.

The founder effect

The founder effect is a particular example of gene flow. A few individuals who move to a new area and become isolated from a larger population might not carry all the alleles that were present in the original population. This means that the isolated population has less genetic diversity than the original population and deleterious recessive alleles may have a higher chance of coming together than they did in the original population.

This effect has been observed in human populations when small groups of particular religious or ethnic backgrounds have settled somewhere new and mixed very little with other settlers. Around 200 people originally settled the Amish community of North America, and at least one of the settlers harboured a recessive allele for Ellis–van Creveld syndrome. This syndrome, symptoms of which include dwarfism, polydactyly (extra toes or fingers) and sometimes a hole in the heart, has been relatively common among Amish people of this region ever since.



Shutterstock.com/Zurijeta

Figure 8.17 ▶
Examples of polydactyly, one of the symptoms of Ellis–van Creveld syndrome

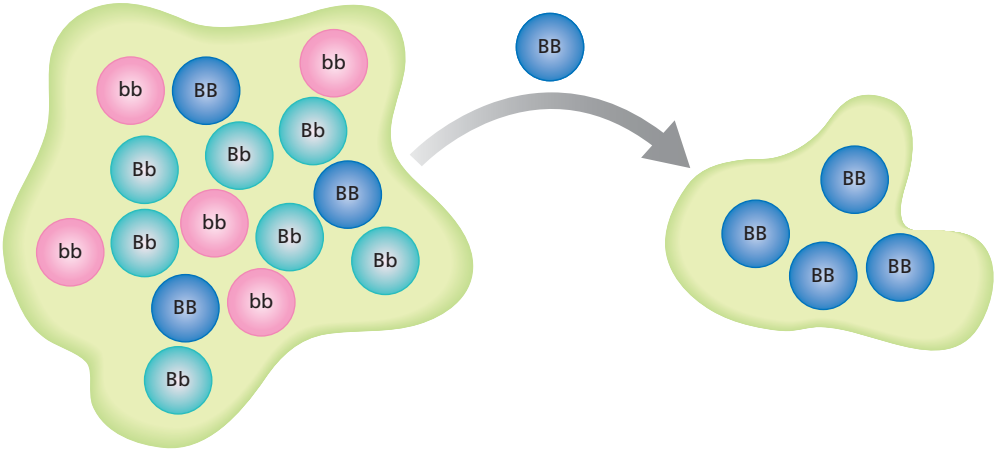


Figure 8.18 ▶
The founder effect occurs when a few individuals carry alleles to a new, isolated area and a new population is formed with allele frequencies that differ from the original population. This is also a type of gene flow.

RECALL

- Genetic drift, the founder effect and bottlenecks can lead to a change in the gene pool of small populations.
- Genetic drift describes random changes that occur in gene pools over time, due to chance effects.
- The bottleneck effect occurs when an event causes a large reduction in the gene pool of a population, causing reduced genetic diversity in the following generations.
- The founder effect occurs when a small number of individuals, together carrying a restricted number of alleles, form a new population with reduced genetic diversity compared with the original population.

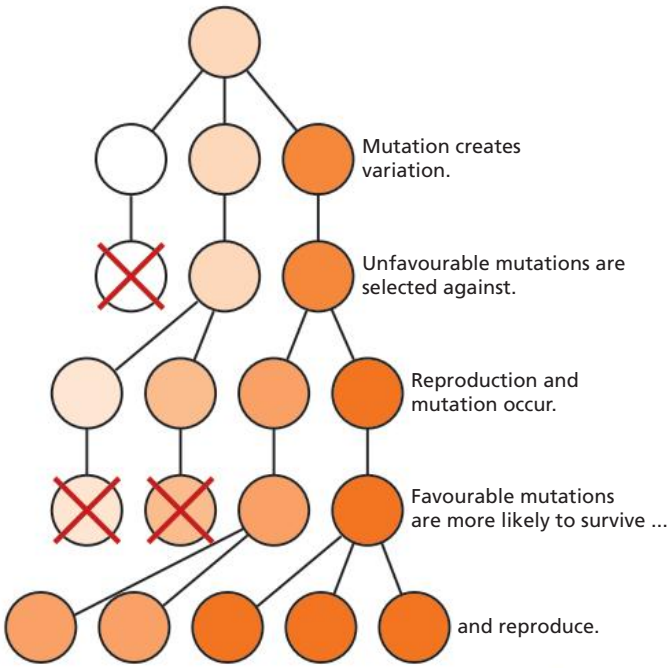
RECAP 8.5

1 Define in your own words the following terms, and describe examples of where these processes may have occurred.
   a founder effect
   b genetic drift
2 Describe the mechanisms that can lead to changes in the gene pool of a population.
3 Outline how gene flow can affect allele frequency.

Natural selection leading to adaptive evolution

In 1868 two publications were released simultaneously through the Royal Society in London. These were papers by Charles Darwin and Alfred Russel Wallace. They outlined their ideas on the evolution of life, what they referred to as **descent with modification**. This term highlights the important idea that all life that exists today has descended from shared ancestors. Their proposed mechanism by which this happened was the process of **natural selection**, which has shaped nearly every feature of living things found in the world today. Through natural selection, favourable traits are selected for, inherited and become more common in subsequent generations.

Natural selection acts on individuals to produce changes in whole populations over time. Natural selection acts on the phenotypes of individuals, so that some survive and reproduce while others do not. The capacity of an individual to survive and reproduce is sometimes referred to as its **fitness**. Natural selection is the only selection mechanism that can lead to **adaptive evolution**. This means that it is the only mechanism that leads to new species and is the mechanism that produces species that are better adapted to their environment.



▲ **Figure 8.19** A diagrammatic representation of natural selection. In this example, the darker alleles confer an advantage over lighter alleles.

The principle of natural selection leading to evolutionary change rests on a few propositions.

1 Individuals differ from one another; that is, individuals within populations show variation.
2 Many of these variations are caused by mutations in alleles and are inheritable.
3 In general, more offspring are born than can survive to maturity and reproduce. Because of this, there is a struggle for existence and only some organisms can reproduce.
4 Some individuals have traits that make them more suited to their environment than others, making them better able to reproduce and pass on their alleles to the next generation.

Selection pressures

Darwin observed the rapid changes produced by the domestication and selective breeding of several plants and animals, particularly dogs and pigeons, and considered that such changes could also occur naturally in the wild. Naturally occurring **selection pressures** would act on traits in the population, resulting in some traits becoming more common as others became less so. Selection pressures are factors that influence the survival of an individual, a population or a species. Some examples are:

- competition between species for food and territories
- predator–prey relationships
- competition within species for food or water
- competition within species for territories or nesting places
- sexual selection; that is, selection of traits that successfully attract mates.

Darwin and Malthus on the struggle for survival

In 1798, Thomas Malthus, an economist, published *An Essay on the Principle of Population*. In his essay he suggested that the human population had a natural tendency to increase at a greater rate than food supplies and other resources and this caused much human suffering: disease, famine and war. Human populations therefore faced a constant struggle to survive. There has been much discussion about the extent to which Darwin was influenced by this essay. We know that he read a later edition of Malthus' essay and he also adopted the term 'struggle' in his writings. While Malthus was mostly concerned with showing that human populations were destined to struggle in this way, Darwin's real focus was the struggle that occurred between individuals in the same population or species, to survive and reproduce, leading to evolutionary change.

Sexual selection

Sexual selection is a process linked to mating behaviour in animals. It describes a form of selection through which individuals with certain inherited characteristics or behaviours are more likely than others to obtain mates and pass on their genes. Sexual selection can produce spectacular effects, such as the enormous antlers of a moose or the long, showy tail of a male peacock.

Special characteristics such as the large tails of peacocks and lyrebirds or antlers of moose are costly to the animal that is carrying them and do not directly give them any extra survival advantage. In many cases, these attributes can be a threat to their survival. Loud and elaborate courtship displays attract predators as well as mates, and growing new antlers every year costs energy. So, what is the evolutionary advantage? One theory suggests that the females are selecting for a very obvious characteristic that correlates with other beneficial alleles. There have been some experiments carried out that suggest that this might be the case.

Sexual selection can also produce a phenomenon called **sexual dimorphism**. This term refers to situations where males and females have different appearances or size.

Some examples of sexual selection can be surprising. For example, the Soay is a breed of feral sheep (*Ovis aries*) that lives on rocky islands off the coast of Scotland. Soay sheep are well known for their agility on cliffs and for the large horns on many males. Large horn size appears to be a sexually selected characteristic and provides males with a significant advantage in securing mates. Variation in this trait appears to be controlled by a single gene. One allele (Ho^1) is linked to large horns and the other allele (Ho^P) with smaller horns.

Biologists have often hypothesised that sexual selection helps females somehow choose males that possess genes that confer a high level of fitness, but in the case of the Soay they found that males with large horns actually have less fitness overall. Rams with small horns had a better chance of surviving the harsh winters, and rams that were heterozygous, carrying one of each allele, were most successful overall in terms of

survival and reproduction. This ensured that the Ho^P allele survived in the population even though it rendered the rams less sexually fit.

The male lyrebird has another trait that is the basis of sexual selection. The lyrebird is one of the most accomplished mimics in the animal kingdom. Males sing complex songs mimicking animal and bird sounds and even mechanical sounds like chainsaws. The males with a greater repertoire achieve better reproductive success.

Alamy/David McGill

▲ **Figure 8.20**
Soay rams, showing the large horns

Getty Images/Craig Dingle

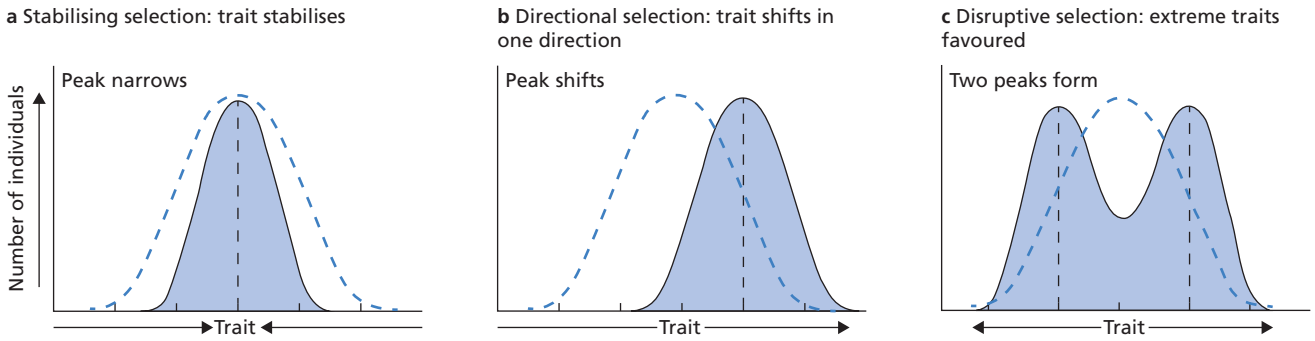
▲ **Figure 8.21**
A male lyrebird displaying its tail

Natural selection can be stabilising, directional or disruptive

Natural selection is most obvious when it is leading to changes in the gene pool of a population, causing some observable change in phenotype. The population may be gradually changing colour or becoming larger or smaller due to selective pressures in a changing environment. As long as the environment of an organism is not changing, then the selective pressures will act against deleterious alleles that cause a departure from the optimal phenotype. This is referred to as **stabilising selection** (shown in Figure 8.22).

Directional selection leads to a change in a trait over time. Changes in environment lead to selective pressures favouring organisms with new or more extreme traits.

A third mode of selection, called **disruptive selection**, operates in favour of extremes. For example, a drought may kill off a local species of shrub that produces medium-sized seeds. A species of seed-eating bird may experience disruptive selection in this situation, when there are only large seeds (or only small seeds) available to eat. Birds with intermediate-sized bills would not be as well adapted for eating either the large or the small seeds and would be selected against.



▲ **Figure 8.22**
Selection can change the distribution of phenotypes (and therefore genotypes) in three different ways. The original distribution of traits is shown with dotted lines in each graph.

RECALL

- The driving force for adaptive evolutionary change is natural selection.
- Sexual selection occurs when individual animals with certain inherited characteristics are more successful than other individuals in finding mates.
- Natural selection can be stabilising (narrows the peak representing the average of the trait in the population), directional (moves the peak), or disruptive (splits the peak).

RECAP 8.6

1 Outline the meaning of the following and give an example of each.
   a natural selection
   b sexual selection.
2 Summarise the principles of how natural selection drives evolutionary change.
3 List as many examples of selection pressures as you can.
4 Identify the role of variation in evolutionary change.

Putting it all together: the principles of evolution

The idea of adaptive evolution through natural selection is one of the most important in biology. Although Darwin and Wallace did not have a good understanding of the underlying causes of inheritance they did realise that **variable traits** must be inheritable. Subsequent understanding of the inheritance of traits, initially through the work of Mendel, fitted perfectly with their theories to produce a combined theory referred to as the **modern synthesis**. The modern synthesis of evolutionary theory, sometimes referred to as neo-Darwinism, is one of the greatest refinements of a major theory to occur in biology.

Microevolution

The significant outcome of natural selection pressure is a change in the frequency of various alleles within a population, a process called **microevolution**. Microevolution refers to any change in the gene pool of a population. The idea of microevolution puts the spotlight of evolutionary theory firmly on the genetic make-up of populations. We now see a population as a large pool of alleles that can change over time for a variety of reasons. Regardless of how this change is occurring, if the gene pool is changing over time then evolution is occurring.

Macroevolution

Major evolutionary changes above the species level are sometimes referred to as **macroevolution**. **Adaptive radiation** occurs when a single species eventually gives rise to a whole new group of organisms comprising many new species. These species have adaptations that allow them to exploit new ecological roles or **niches**. Major radiations appear to be relatively rare and often seem to depend on chance events. These often correlate with major changes in environmental conditions or catastrophic geological events causing **mass extinctions**. A mass extinction event 250 million years ago (mya) during the Permian period saw the extinction of 96% of all marine species and 76% of terrestrial vertebrates. With the loss of so many species Earth now contained many new and under-utilised ecological opportunities (niches). New speciation events occurred quickly. One of these saw the evolution of many new species from a single ancestral dinosaur over just a few million years. This example is one of several that demonstrate how the evolutionary trajectory of life on Earth is greatly affected by chance events. The range and abundance of species on Earth can be represented in a **phylogenetic tree** diagram, which can illustrate macroevolution and microevolution events (Figure 8.23).

Phylogenetics is discussed in more detail in Chapter 9.

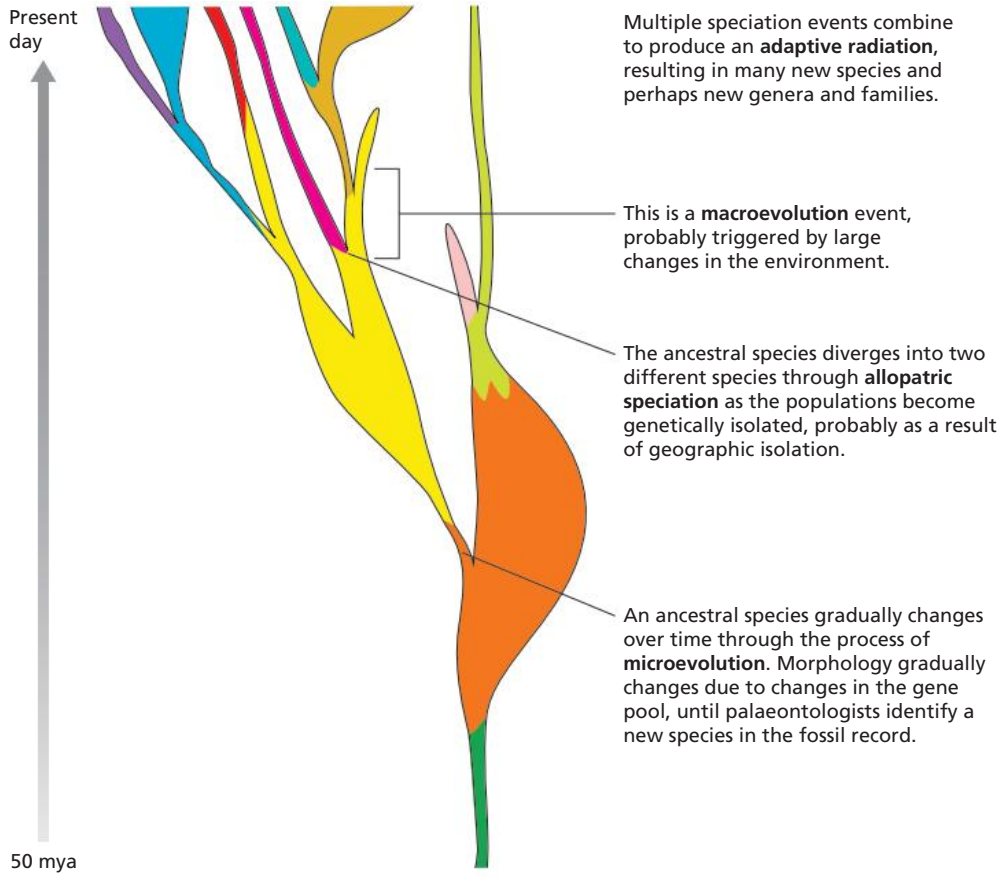


Figure 8.23
The evolution of diversity. A hypothetical phylogenetic tree showing the evolution of increasing diversity in a group of animals. Colour changes show changes in species identified in the fossil record. New species arise within single lineages as well as when species diverge. The thickness of each line represents population size.

RECALL

- Microevolution is any change in the gene pool of a population over time.
- Macroevolution is any change in groups of organisms above the species level.
- Major examples of macroevolution include adaptive radiation and mass extinction events.

RECAP 8.7

1 Define the following terms.
   a microevolution
   b macroevolution.

2 In what way does the modern synthesis of the theory of evolution differ from that proposed by Darwin and Wallace?

3 Construct a table summarising the different processes that can contribute to microevolution.

The diversity of species

The Galápagos Islands lie 1000 km west of Ecuador (South America) in the Pacific Ocean. When Darwin visited them in 1835 during his famous voyage on the *Beagle* he realised that these islands were geologically quite young. They were teeming with life but the animals and plants on the islands were of recent origin. Many of these appeared to be related to similar species on the South American mainland but were

also clearly different from them. One of the most famous groups of animals on the Galápagos Islands are the 15 or so species of giant tortoise, whose closest living relative, the Chaco tortoise, is found in mainland Argentina. Darwin wondered how the tortoises had got to the islands, and how there could be so many different species. He hypothesised that the tortoises on the Islands originally came from the mainland population but had changed over time to become better suited to the environment of the Galápagos.

Figure 8.24 ▼
(**a**) The famous Galápagos tortoises are similar to (**b**) the much smaller Chaco tortoise, *Chelonoidis chilensis*, found in South America.

Dreamstime/Rico Leffanta

Alamy/imageBROKER

See Chapter 7 for more on the fossil record.

Before Darwin's theory of evolution by natural selection, there was a general belief that species were **immutable**, or unchanging; that each species had been put on Earth in its current form and could not change over time. Indeed, our current understanding of evolution tells us that sometimes natural selection produces very little change.

In general, though, the fossil record shows that not only do species change, but that these changes can be dramatic. It also shows that a single species can diverge to produce several new species. The ancestral type of tortoise from the South American mainland had somehow split to create new species of giant tortoise on the Galápagos Islands. How this occurred was a key aspect of Darwin's theory. Darwin wondered how species arose only to disappear and be replaced by new animals and plants. We call this **speciation**.

Scientists have hypothesised that there may be more than be 8 million different species on Earth, but this is difficult to estimate accurately because only around 1.2 million species have been identified and classified so far. How new species have evolved in such large numbers is a key part of the theory of evolution.

There are three broad processes that work together in the evolution of this great diversity.

1 Natural selection favours phenotypes that make the population better adapted to its environment. Populations change over time as their gene pools accumulate small changes in response to natural selection. This is called microevolution.
2 Eventually a population accumulates so many changes that a new species can be identified. This process can lead to speciation, the multiplication of species.
3 Sometimes a rapid series of speciation events leads to the development of a whole collection of new species, or even genera, families, or higher classification groups. This is referred to as macroevolution.

Evolution is also marked by another powerful process: extinction. Most species that have ever evolved are now extinct. The broad sweep of evolution is exactly how Darwin imagined it: the constant appearance and disappearance of species over vast periods of time.

A key idea required to make sense of these processes is the concept of what defines a species, and how a species can be identified in the present time and in the fossil record.

The biological species concept

▲ **Figure 8.25** Ancestral kangaroos 25 mya would have looked different from modern kangaroos but are connected with them through an unbroken line of descent.

Species can be identified in a variety of ways. In 1940, Ernst Mayer proposed that species are groups of actual, or potentially, interbreeding natural populations that are reproductively isolated from other such populations. This is called the **biological species concept**. According to this model individuals from different species are unable to produce viable offspring under natural conditions. The biological species concept is the most widely used in evolutionary biology. It relates directly to the concept of a species as a genetically isolated group, which can only interbreed within itself. In this sense a species is represented by a totally isolated gene pool.

Sometimes, the only evidence that a species existed is in the fossil record. When dealing with fossils only, the **morphological species concept** can be applied. This concept identifies different species based on their physical and physiological characteristics but is limited to what can be observed in the fossil record. For example, kangaroos are perhaps Australia's most recognised marsupials and they are well represented in the fossil record. Twenty-five mya the ancestors of modern kangaroos lived in rainforests and fed on fruit. Kangaroo species of today are connected to these distant ancestors through an unbroken line of descent.

RECALL

- The biological species model defines a species as a reproductively isolated group of organisms.
- Species can be identified through consistent differences in morphological, physiological, or other traits as well as genetic differences.

RECAP 8.8

1 What is the biological species concept?

2 Recall the morphological species concept and state when it is used.

Mechanisms of speciation

Speciation occurs when a single population becomes two separate populations that are unable to interbreed as a result of changes that produce physical, biological or behavioural barriers. This separation, termed reproductive isolation, results in the gene pool of the original species being divided. Selection pressures act on the separated populations to cause microevolution, which can begin to change them in different ways. Over time their allele frequencies may become so different that the individuals are no longer able to interbreed even if they are reunited, and we come to regard them as two distinct species.

For example, small species such as frogs can cover long distances if enough time is available. Thus, during a period of hundreds of thousands of years, frogs can 'pond hop' hundreds of kilometres, which means that they can colonise new habitats and

exploit new breeding sites. It seems that Victorian frogs colonised Tasmania in this way during the succession of recent ice ages. They did not evolve into new species until the subpopulations became isolated, in this case by the rising sea waters of an interglacial period.

Reproductive isolating mechanisms

Isolating mechanisms separate two groups and prevent them from producing fertile, viable offspring – that is, offspring that survive and can themselves reproduce (Figure 8.26). These mechanisms can operate before reproduction has occurred or after reproduction. Genetic isolation (where populations become so genetically different that they can no longer interbreed) can occur before or after physical isolation. In either case, once isolation has occurred, the two groups can acquire different phenotypes, as natural selection works on the members of the two groups so they become adapted to their new, different environments.

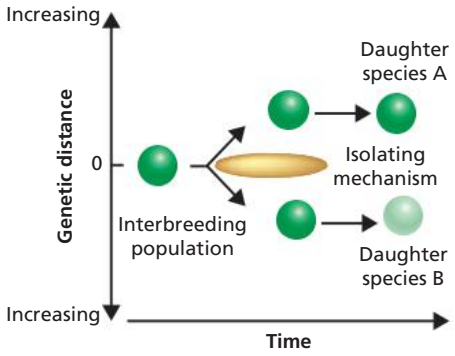


Figure 8.26 ▲
An isolating mechanism can prevent two subgroups of a species from breeding, until they are so genetically diverse that they form two new species. After a period of time they are no longer able to interbreed, even if the populations come back together.

Pre-reproductive isolating mechanisms

Some isolating mechanisms prevent organisms from being able to interact to reproduce. **Pre-reproductive isolating mechanisms** include the following.

- Geographic mechanisms: individuals are separated by geographic features, such as seas, mountains, distance or habitat
- Temporal (time) mechanisms: individuals breed during different seasons of the year or different times of the day
- Behavioural mechanisms: individuals have different courtship patterns
- Morphological mechanisms: individuals have different reproductive structures – that is, genitalia of different size, shape or location – so that mating is physically impossible.

The effectiveness of a geographic barrier as an isolating mechanism depends on the size and mobility of the individuals concerned. For example, small organisms may be easily transported across ocean barriers by being carried by other animals. Parts of plants, such as seeds and stems, can float; small rodents can cling to floating vegetation carried by tides; and winds may carry insects over bodies of water.

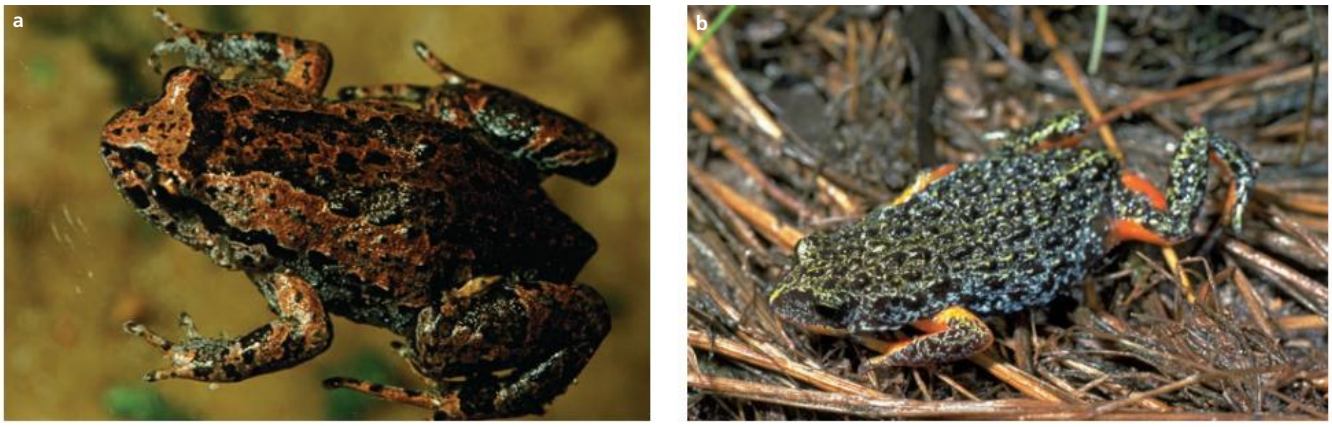
Insects, in particular, can have very precise timing systems that determine when mating occurs. Periodical cicadas have one of the longest insect life cycles known. In North America there are several species of periodical cicadas (genus *Magicicada*). Recent studies have focused on several species: some that hatch out every 17 years and others that hatch every 13 years. It is possible that the unusually lengthy life cycle acts to prevent different populations interbreeding and producing **hybrid** offspring.

Another example of a pre-productive isolating mechanism can be seen in frogs. The mating calls of frogs may sound very similar to us but to other frogs they sound vastly

iStockphoto/traveler1116

Figure 8.27 ▶
Magicicada, a periodical cicada endemic to the northern United States

different. Frogs usually reproduce only with members of their own species so their call acts as a pre-reproductive isolating mechanism. In many cases, frogs have undergone speciation because their mating calls ensure that they mate only with their own species.



▲ **Figure 8.28** Two Victorian frogs, (**a**) *Geocrina victoriana* and (**b**) *Pseudophryne semimarmorata*, breed in the same habitat at the same time but are prevented from interbreeding by alternating their calls. If they do mate, their tadpoles do not develop.

Post-reproductive isolating mechanisms

If a frog does accidentally mate with a frog from another species, they will not produce fertile, viable offspring because the parents' chromosomes cannot line up successfully during meiosis and no zygotes are formed.

Methods such as this are called **post-reproductive isolating mechanisms**. They do not prevent mating from occurring, but they do prevent young from being produced. These genetic post-reproductive isolating mechanisms include the following.

- Gamete mortality: the gametes do not survive.
- Zygote mortality: the zygote forms but does not survive.
- Hybrid sterility: adult offspring are formed but are infertile because they are unable to produce viable gametes, usually as a result of a different number or structure of chromosomes from each species.

In general, hybrid sterility acts as a post-reproductive isolating mechanism in animals but not in plants. Many plants can interbreed; for example, polyploidy, or multiple sets of chromosomes, is common in eucalypts. Species of coffee plants with 22, 44, 66 and 88 chromosomes are known; this suggests an ancestral plant with a haploid number of 11 and a diploid number of 22.

RECALL

- Speciation occurs when two populations of a species become isolated and accumulate differences in their gene pools such that they can no longer interbreed to produce viable fertile offspring.
- Isolating mechanisms include pre-reproductive and post reproductive mechanisms.
- The key to the formation of new species involves reproductive isolation combined with selection pressures, leading to a disruption of the flow of genes.

RECAP 8.9

1 List four examples of pre-reproductive isolating mechanisms.

2 List three examples of post-reproductive isolating mechanisms.

3 Define 'hybrid organism'.

Allopatric speciation

In **allopatric speciation** (from the ancient Greek 'allos' other and 'patra' homeland), gene flow is disrupted as populations become physically separated through geographical isolation. The populations diverge. This may be because of different selection pressures on the two populations, or it may be due to other random processes such as genetic drift (see page 268). The isolation may happen on a very small scale such as when a river or stream changes course and divides a population of small animals that cannot cross it. On a somewhat larger scale, deserts may expand, cutting off populations that cannot live under desert conditions. Allopatric speciation is the most common form of speciation and, in terms of evolutionary time scales, species can be easily and rapidly separated by:

- water, for terrestrial organisms
- land, for aquatic organisms
- mountains
- continental drift
- rising sea levels
- climate change.

Islands are home to many examples of allopatric speciation. On the Galápagos Islands Darwin noticed a flightless cormorant. This species most likely originated from a small population of ancestral flying species that reached the islands from the South American mainland. The two populations would have been physically isolated by the 1000 km of ocean between the islands and the South American mainland. There would have been no gene flow between the two populations. The islands were totally free of predators. Reduced predation changed the selective pressures acting on this cormorant population. There were still selection pressures for efficient movement underwater, but there was less pressure for efficient flight. This led to a reduction in the size of the wings in the cormorant population, to a morphology that was well suited to movement under water but which no longer allowed flight. This led to allopatric speciation.

The more recent arrival of feral dogs and cats on the islands has once again led to a change in selection pressures on this animal. This has led to dramatic reductions in the cormorant population, which is now less well adapted to the new predation pressures because it cannot fly. It is now recognised as an endangered species. Comparative morphological and genetic studies have only recently identified which mainland species (all flighted) the Galápagos cormorant is most closely related to (Figure 8.29).

Sympatric speciation

Allopatric speciation seems to be the main mechanism producing new species throughout evolutionary history, but sometimes species diverge without any obvious physical or geographical isolation. **Sympatric speciation** refers to the evolution of two or more new species from a single population within the same place. How could new species arise without physical separation? It might be that groups within a single population feed on different things, or choose mates based on different characteristics. They may also choose to mate at different times. The genetic separation may occur due to the various pre-reproductive and post-reproductive processes discussed on pages 278 and 279. There are not as many clear examples of this type of speciation but a few are quite striking, as in the case of *Magicicada* (Figure 8.27).

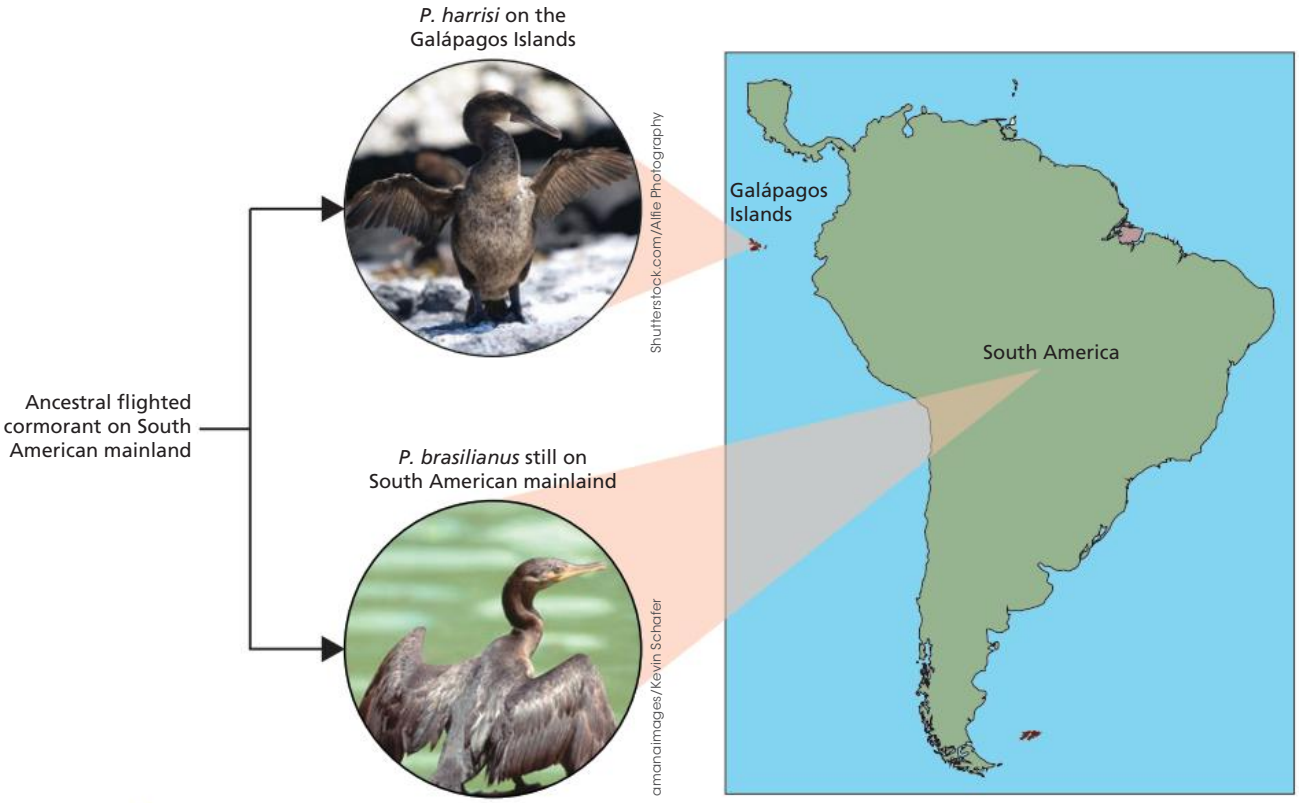


Figure 8.29 ▲
The flightless cormorant (*Phalacrocorax harrisi*) of the Galápagos Islands diverged from flighted cormorants on the mainland through allopatric speciation. *P. harrisi* is most closely related to cormorants such as the neotropic cormorant (*P. brasilianus*), which is widespread throughout tropical regions of South and North America.

RECALL

- Most speciation events seem to occur as a result of populations becoming physically separated through geographical isolation leading to the disruption of the gene flow. This process is called allopatric speciation.
- Sympatric speciation refers to the evolution of two or more new species from a single population within the same place.

RECAP 8.10

1 What effects do isolating mechanisms have on a population?
2 Give five examples of factors that may result in allopatric speciation.
3 Describe an example of where allopatric speciation has occurred.

ACTIVITY 8.1

SPECIATION AND CONSERVATION: THE EASTERN BARRED BANDICOOT

Populations with reduced diversity face increased risk of extinction, so conservation efforts are usually focused on maintaining genetic diversity. When large-scale extinctions occur, not all species are lost, and some seem to be at more risk than others. Rapid extinction events can lead to the loss of larger organisms rather than smaller ones. Large populations can be more resilient than small populations, probably because the population has a more diverse gene pool. That is, it holds a greater reserve of different alleles to draw on as the pressures from natural selection change.

amanaimages/Steve Kaufman

Figure 8.30 ▲
The eastern barred bandicoot (*Perameles gunnii*)

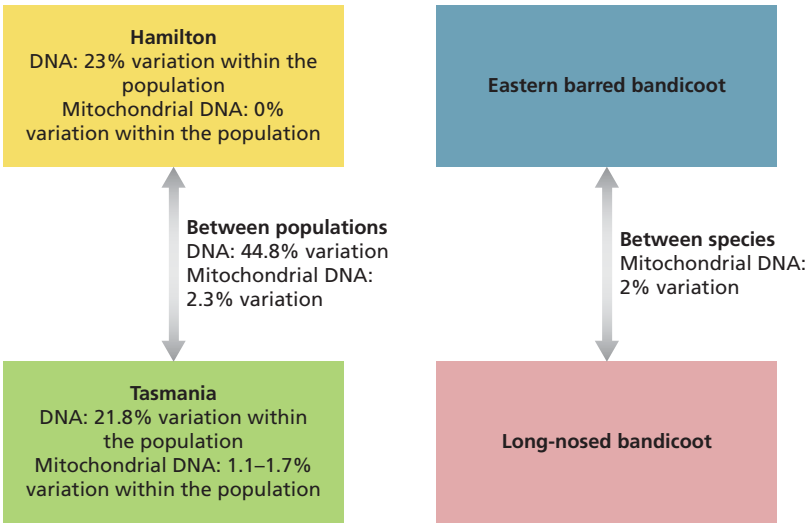
The eastern barred bandicoot (*P. gunnii*) belongs to the marsupial family Peramelidae. It is small (body about 300 mm, tail 200 mm), grey-brown in colour, with four pale stripes or 'bars' on its hindquarters. It has three claws on the front feet, which it uses for digging, while the back feet are long, similar to those of a kangaroo.

Populations of the eastern barred bandicoot were once common over a wide area of south-western Victoria. Numbers were reduced dramatically in the 1900s and now the eastern barred bandicoot is isolated to a small area around Hamilton, numbering less than 200. This resulted from a change in environmental conditions (e.g. clearing of woodlands, growing exotic pasture grasses, grazing by domestic stock, introduction of rabbits and foxes), which severely reduced its available habitat in Victoria. Numbers of the eastern barred bandicoot throughout most of Tasmania, however, are still healthy.

Conservation plans for the eastern barred bandicoot depend heavily on how populations are classified. A **subspecies** is a level of classification below species, referring to races of a species that are fairly permanently geographically isolated from each other and may in future diverge to become two different species. Because of the relatively healthy bandicoot populations in Tasmania it is not regarded as an endangered species. If the Victorian population were identified as a different species, or subspecies, then it could be recognised independently for conservation purposes.

A number of studies have been conducted on the Victorian and Tasmanian populations in an attempt to protect the Victorian population. The bandicoots were trapped, small blood samples were taken and the animals were released immediately into the same areas. The blood was snap-frozen and later a DNA fingerprint was taken by analysing genomic variable nucleotide tandem repeats (VNTRs). The average percentage difference in VNTRs within the populations around Hamilton was found to be about 23%, and for those in Tasmania it was 21.8%. The average percentage difference between the Hamilton and Tasmanian populations was 44.8%.

Figure 8.31 ▶
DNA variability in different populations of eastern barred bandicoot. A 2% variation is the average difference between subspecies and closely related species of mammals.



Further testing was done using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis. This revealed a 0% nucleotide variation within the Tasmanian populations and a 1.1–1.7% variation for the Victorian populations. The percentage variation between the Victorian and Tasmanian populations was 2.3%. Variation of 2% is the average difference between subspecies of mammals.

There is no doubt that the two populations have diverged to some extent due to geographical isolation. But are the two populations separate subspecies? The answer to these questions is vital to how the conservation of these two populations of eastern barred bandicoot is managed. Biologists currently use a variety of species concepts, all of which are based on the theory of evolution.

The biological species concept defines a species as a reproductive community of populations that occupies a specific niche in nature. The identification of species often uses data from genetic analysis. **DNA fingerprinting** is predominantly used to determine which groups are related – that is, share a gene pool – and which do not. A species defined according to this concept would be the smallest group of organisms that share a common ancestor not shared by any other organism.

The Australian Government, through the Department of the Environment, lists two subspecies of *P. gunnii*. The following is an excerpt from the listing for the eastern barred bandicoot.

> Scientific name: *Perameles gunnii* Victorian subspecies
> Common name: Eastern Barred Bandicoot (Mainland)
> The genetic diversity, as measured by the variable number of tandem repeat markers and mitochondrial DNA restriction fragment length polymorphisms, among specimens from Hamilton, Victoria, was greater than that found in widespread populations of the Tasmanian subspecies (*Perameles gunnii gunnii*). The justification for considering the mainland form to be distinct is based in part on morphological comparisons of island and mainland forms, and that mtDNA data indicated separation 270000–620000 years ago.

Aim

To investigate speciation in the eastern barred bandicoot and relate this to conservation approaches

Questions

1. What species definition could be used to justify classifying the two populations as separate subspecies?
2. Does the recognition of two separate subspecies appear to be well accepted by the Australian Government at this stage?
3. What does the DNA evidence suggest about how the populations became separated? To what extent does this example illustrate the concept of allopatric speciation?
4. In your opinion, would the small genetic variability found in the eastern barred bandicoot populations affect their survival? Explain.
5. Explain why the identification of the two possible subspecies of bandicoot is important for their conservation.

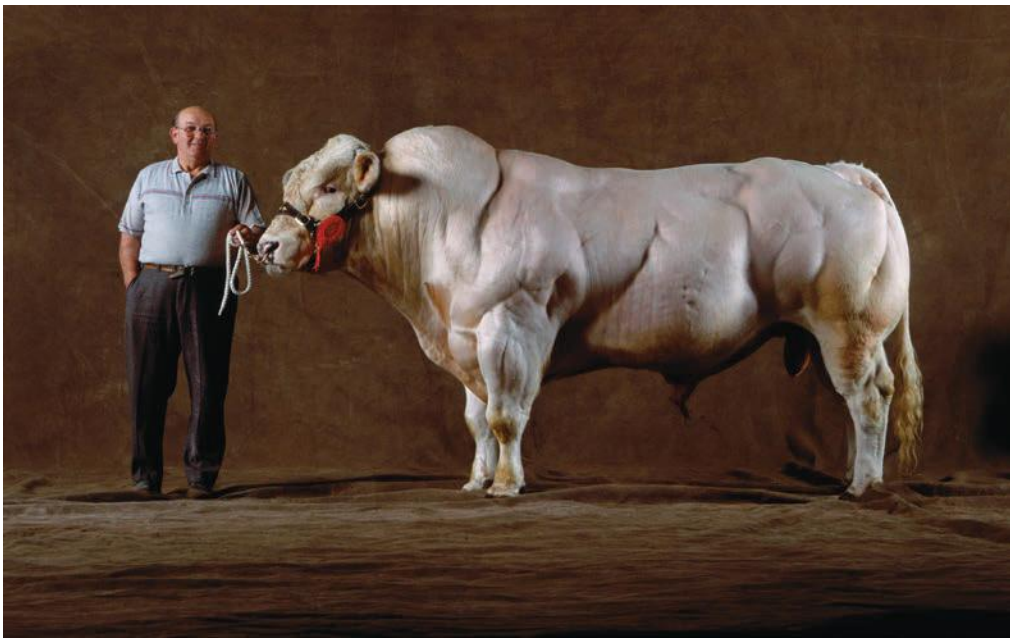
Artificial selection: animal and plant breeding

Both Darwin and Wallace understood the importance of inheritable variation for any sort of selection to work, but their understanding was very limited at that time. If all individuals within a population were identical then selection would have no effect.

Darwin drew long comparisons with breeding programs for domestic animals, including dogs and pigeons. The processes for breeding different strains of dogs or different varieties of pigeons (Darwin was an avid pigeon fancier) were well understood. Parental stock with certain desirable traits are selected and mated, and it was understood that these traits were often passed on to the offspring. Over time the new traits could be established in later populations. This process is called **artificial selection**, also called **selective breeding**, and relies upon human intervention to determine which traits are selected for.

Many of the familiar forms of domesticated plants and animals have arisen as a result of selective breeding. This process relies on human intervention to determine which animals are allowed to breed, removing alleles that produce undesirable traits from the gene pool and increasing the frequency of alleles that produce desirable traits.

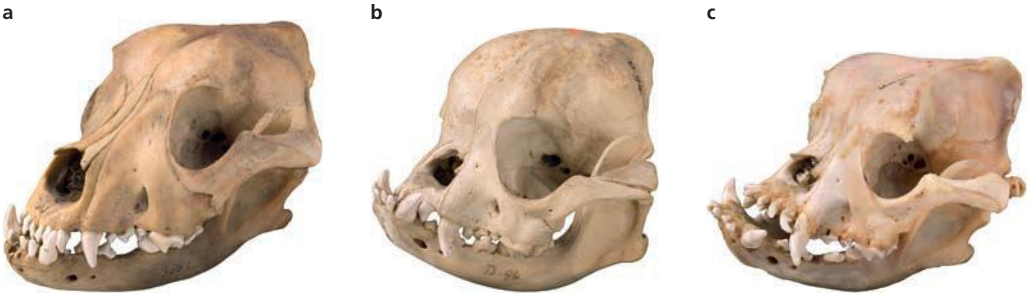
Which traits are considered desirable in an organism depends on the use of the organism and the preference of the breeder. The Belgian Blue breed of cattle contains an allele that promotes abnormal muscle growth (Figure 8.32). Farmers have only allowed the cows and bulls with the highest muscle mass to breed, producing more profitable offspring.



amanaimages/Yann Arthus-Bertrand

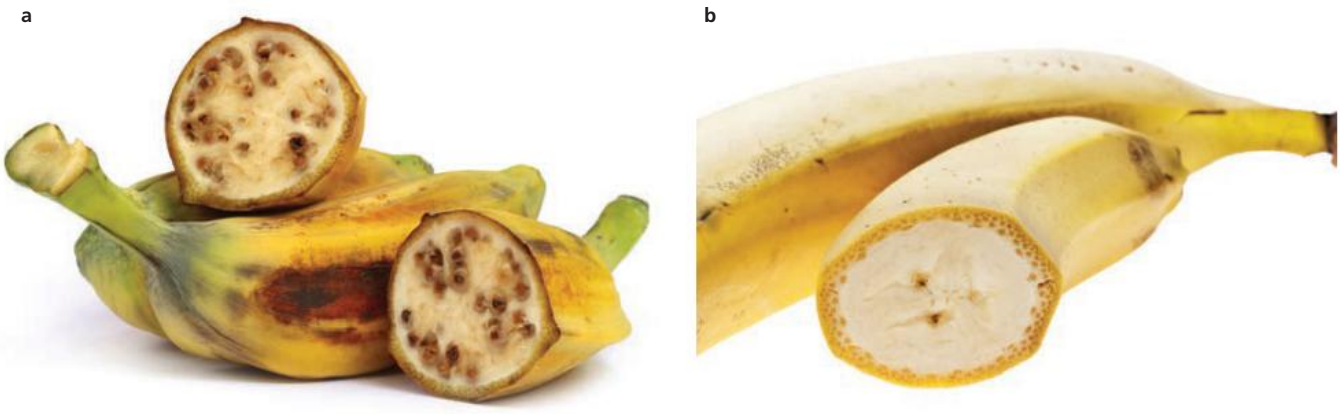
Figure 8.32 ▶
Belgian Blue cattle have been selectively bred to fix an allele for abnormal muscle growth that appeared in the nineteenth century.

Dog breeds have been subjected to intensive selective breeding programs, producing forms that are aesthetically pleasing to breeders but which sometimes come with a number of considerable health problems for the dogs. This is because their acquired form may not be the best for optimal functioning, and because selective breeding processes may cause deleterious recessive alleles to become homozygous. Once alleles have been lost from a population through natural selection or selective breeding, the traits they encode are permanently lost with them.



The Natural History Museum Picture Library

Figure 8.33 ▶
Skulls of English bulldogs showing the effects of selective breeding: (**a**) the original English bulldog, with a functional skull in 1860, (**b**) 1867 and (**c**) 1906, showing a very exaggerated skull. The skull has evolved dramatically in a short span of time.



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Shutterstock.com/Igor Sokolov (breeze)

▲ **Figure 8.34**
(**a**) Wild bananas and (**b**) selectively bred commercial bananas

Many of the food crops that are now commercially and domestically grown have been selectively bred to favour traits that make them better foods or better products for the producers.

RECALL

- Selective breeding, or artificial selection, occurs when humans selectively breed organisms with desired traits.
- In selective breeding, the frequency of alleles encoding desired traits increases and the frequency of other alleles at the locus decreases, and this reduces genetic diversity.

RECAP 8.11

1 Explain the effects of selective breeding on the gene pool of a population.

2 Give an example of selective breeding other than those described here.

CONCEPT SUMMARY

Genetic variation

Evolution depends on variation in the DNA sequence between individuals. Individuals in any population express a range of different phenotypes. This is because members of a population have variation in genotypes that causes variation in their phenotypes.

This genetic variation is therefore inheritable. Mutations are the source of new variation. They are caused by mutagens and are inheritable if they occur in the DNA in germ-line cells.

Types of mutations

Point mutations (SNPs)

- Substitution
  - Synonymous
  - Missense
  - Nonsense
- Insertion
- Deletion
- Frameshift

Chromosome structure (block mutations)

- Deletion
- Inversion
- Translocation
- Duplication

Chromosome number

- Monoploidy
- Polyploidy
- Aneuploidy
- Non-disjunction

Effects on survival

- Neutral
- Deleterious
- Beneficial

Changing allele frequencies in populations

- Gene pool: The total of all alleles found in a population (a collection of individuals of the same species that can readily interbreed). Many alleles in a gene pool are fixed.
- Allele frequencies: Not constant, and can be affected by mutation of an allele; immigration and emigration of individuals (movement into and out of the population); and the reproduction rate of an individual.
- Gene flow: Transfer of alleles that results from emigration and immigration of individuals between populations, illustrated by the ABO blood group example in Indigenous Australians.
- Genetic drift: When chance events cause the allele frequency in a population to change.
- Bottleneck effect: When a catastrophic decrease in population size results in the loss of some alleles from the gene pool. Cheetahs are an example.
- The founder effect: When a few individuals move to a new area and become isolated from a larger population, they might not carry all the alleles present in the original population. The new population is formed with different allele frequencies to the original population. An example is Ellis–van Creveld syndrome, found in North America's Amish community.

Natural selection

Natural selection acts on the phenotypes of individuals, so that some survive and reproduce while others do not, depending on their reproductive fitness. This results in changes in populations over time.

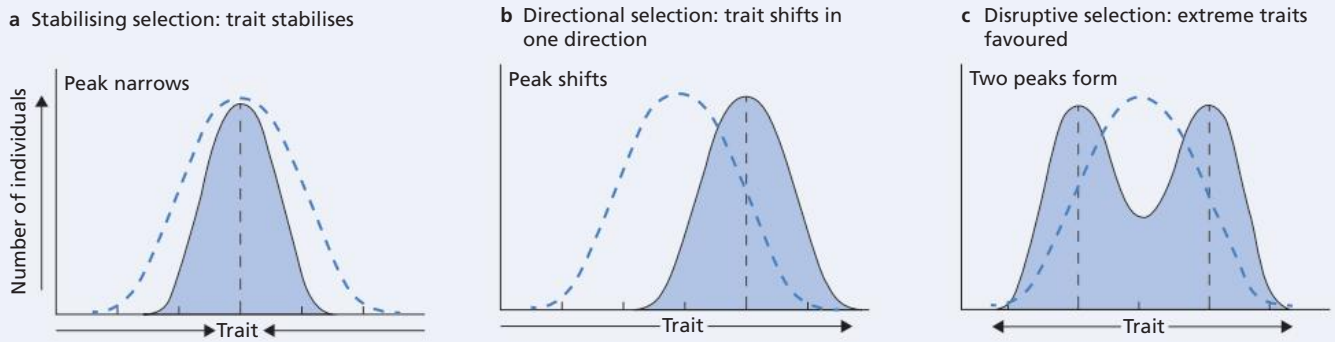
Principles

1. Individuals within populations show variation.
2. Many of these variations are caused by mutations in alleles and are inheritable.
3. Generally, more offspring are born than can survive to maturity and reproduce, so there is a struggle for existence and only some organisms can reproduce.
4. Some individuals have traits that make them more suited to their environment than others, making them better able to reproduce and pass on their alleles to the next generation.

Selection pressures

- Competition between species for food and territories
- Predator–prey relationships
- Competition within species for food or water
- Competition within species for territories or nesting places
- Sexual selection; that is, selection of traits that successfully attract mates. This can result in sexual dimorphism.

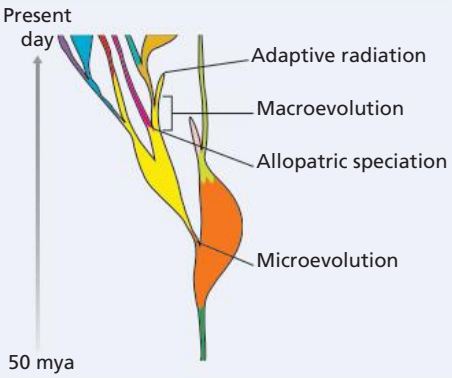
NATURAL SELECTION CAN BE STABILISING, DIRECTIONAL OR DISRUPTIVE.



The principles of evolution

The modern synthesis of evolutionary theory (or neo-Darwinism) is a refinement of Darwin and Wallace's theory of evolution that is based on the understanding of the inheritance of traits, initially through Mendel's work.

- Microevolution describes changes in the gene pool of a population.
- Macroevolution describes major evolutionary changes above the species level. It includes adaptive radiations and mass extinctions.



Speciation

- Biological species concept: Species are groups of populations that can naturally interbreed to produce viable offspring; that is, they are in the same gene pool.
- Morphological species concept: Identification of species is based on physical and physiological characteristics preserved in the fossil record.

Mechanisms of speciation

Speciation occurs when a single population becomes two separate populations that are unable to interbreed as a result of changes that produce physical, biological or behavioural barriers. The gene pool is divided by isolating mechanisms.

Reproductive isolating mechanisms

Isolating mechanisms separate two groups and prevent them from producing fertile, viable offspring.

Pre-reproductive isolating mechanisms

- Geographic
- Temporal
- Behavioural
- Morphological

Post-reproductive isolating mechanisms

- Gamete mortality
- Zygote mortality
- Hybrid sterility

- Allopatric speciation: A population becomes physically separated by geographical isolation, which disrupts gene flow.
- Sympatric speciation: Species diverge without any obvious physical or geographical isolation.
- Artificial selection: Humans selectively breed organisms with desired traits, increasing the frequency of alleles encoding desired traits and decreasing the frequency of other alleles, reducing genetic diversity in the gene pool.

CHAPTER GLOSSARY

adaptive evolution changes in a population of organisms that make that population better adapted to its environment over time

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

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

allopatric speciation speciation that occurs as a result of physical or geographic isolation

aneuploidy describes a genome that varies from the conventional by the loss or addition of one or just a few chromosomes

artificial selection the breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

beneficial mutation a mutation that increases an organism's chances of survival and reproduction

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population and its genetic diversity

deleterious mutation a mutation that decreases an organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

descent with modification Darwin's terminology indicating that life today has descended and evolved from common ancestors that were generally different from their modern descendants

diploid (2*n*) describes a cell or organism that has a genome comprising two copies of each chromosome, represented by 2*n*

directional selection a form of selection that selects against one of two extremes and leads to a change in a trait over time

disruptive selection a form of selection that operates in favour of extremes and against intermediate forms

DNA fingerprinting also called DNA profiling; comparison of individuals or groups based on patterns of non-coding, repeating base sequences in the genome

double-strand break a mutation involving breaks in the sugar-phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

fitness the capacity of an individual to survive and produce viable offspring

founder effect a type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

frameshift mutation a mutation that dislocates the translational reading frame

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

genetic drift a change in the gene pool of a population as a result of chance; usually occurs in small populations

genotype a specific combination of alleles for a particular gene locus belonging to an individual

germ-line the cell line in eukaryotic organisms from which the gametes are derived

haploid (*n*) describes a cell or organism that has a genome that contains one copy of each chromosome, represented by *n*

homologous chromosomes a pair of chromosomes that have the same size, shape and genes at the same locations

hybrid offspring of parents from two different species; some hybrids are also fertile and can produce further offspring

immutable unchanging; the idea (now considered incorrect) that species did not change over time

inheritable capable of being passed on to the next generation

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

isolating mechanism a mechanism that prevents organisms from mating or producing viable offspring

macroevolution the evolution of new groups of organisms comprising many related species through multiple speciation events; includes adaptive radiations

mass extinction extinction of many species over a relatively short (geological) period of time

microevolution any change in the gene pool of a single population over a short time

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missense mutation a gene mutation that results in one amino acid being replaced by another amino acid in the encoded protein

modern synthesis the theory of evolution incorporating our understanding of how traits are inherited

monoploid (1*n*) describes a cell or organism that has a functional genome consisting of one copy of each chromosome, represented by 1*n*

morphological species concept to define a species using measurable anatomical criteria and characteristics

mutagen an agent capable of inducing mutations

mutation occurs when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

natural selection the process whereby individuals with certain inheritable traits survive and reproduce more successfully than other individuals, leading to evolutionary change in the population

neutral mutation a mutation that has no effect on an organism's chances of survival and reproduction

niche an organism's habitat; or way of life or function of an organism in its environment

non-disjunction the failure of sister chromatids in mitosis or homologues in meiosis to separate and go to opposite poles

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

parthenogenesis a process by which the entire organism is regenerated from a single egg cell without the need for fertilisation

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

point mutation a mutation that affects a single base-pair position within a gene

polyploidy condition of a cell or organism with a genome comprising three or more copies of each chromosome, represented by 3*n*, 4*n*, 5*n*, 6*n* etc.

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time in response to various evolutionary processes

post-reproductive isolating mechanism a mechanism that prevents fertilisation occurring, or an embryo developing into viable offspring if fertilisation does occur

pre-reproductive isolating mechanism a mechanism that prevents organisms from being able to interact to reproduce

selection pressures factors that influence the survival of an individual within a population

selective breeding see **artificial selection**

sexual dimorphism the situation where males and females of a species have different morphologies, often in shape or size

silent mutation see **synonymous mutation**

single nucleotide polymorphism (SNP) a nucleotide difference that occurs at a given position in the genomes of two or more individuals

somatic describes a body cell that will not pass its genes on to the next generation

speciation the evolution of one or more new species from an ancestral species

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same

spontaneous mutation a mutation occurring in the absence of exposure to mutagens

stabilising selection natural selection that tends to advantage organisms similar to their parents; this usually occurs when the environment is very stable and unchanging and selects against extremes of phenotype

subspecies distinct populations of a species, which can interbreed but usually do not due to geographical isolation

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

sympatric speciation speciation that occurs without physical or geographic isolation

synonymous mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a 'silent' mutation

variable traits traits that vary in the population due to differences in alleles carried by different individuals

CHAPTER REVIEW QUESTIONS

Remembering

1 Define the following terms:
   - a gene pool
   - b allele frequency
   - c genetic drift.

2 Draw a diagram to outline the founder effect.

Understanding

3 Classify the following mutations as neutral, deleterious, or beneficial to an organism's chances of survival.
   - a An indel in the human *Hexosaminidase A* gene results in improper neural development.
   - b A mutation in the beta-lactamase gene of the bacterium *Escherichia coli* generates a new version of the enzyme that detoxifies the antibiotic ampicillin.
   - c A nonsense mutation in the human *SURF1* gene encodes a protein crucial for formation of a key metabolic enzyme.
   - d A synonymous mutation in the codon for an amino acid occurs at the active site of bovine salivary amylase.
   - e Various mutations in a gene for the enzyme alcohol dehydrogenase result in different versions of the functional enzyme.
   - f A mutation that extends expression of a human lactase gene enables lactose digestion into adulthood.

4 Draw an annotated diagram of a diploid cell with four chromosomes undergoing meiosis and show two ways that non-disjunction can occur. Indicate the kind of chromosome anomalies that can arise in a zygote formed by fertilisation with each of the resulting gametes and one normal gamete.

5 Draw an annotated diagram of two chromosomes showing that one of them has experienced two double-strand breaks. Draw the possible chromosomal rearrangements that might occur when the fragments of the broken chromosome are re-joined.

6 Discuss why parental age might be a factor in the increasing incidence of mutation in the offspring.

7 Defend or refute the statement 'Aneuploidy is always deleterious' and explain your reasoning.

8 The images in Figure 8.35 show segments of chromosome with genes numbered along their lengths. Identify the mutation that has occurred to produce each of these structural rearrangements from the original segment.

9 Discuss the relationship between SNPs, substitutions, synonymous, missense and nonsense mutations.

10 Mimicry is a common phenomenon in natural systems. The mimic seeks to take on the appearance of another organism. The organism being mimicked, called the model, is harmful, distasteful or unpalatable to predators. Predators learn to avoid the model and therefore the mimic. It is assumed that the origins of mimicry lie in random, spontaneous gene mutations, recombinations and chromosome alterations that result in colour, structure or pattern change.
   - a Explain the possible advantages of mimicry.
   - b Describe the type of evolution involved in mimicry.
   - c Explain what would you expect the ratio of models to mimics to be in natural systems.
   - d Describe how the disappearance of the model might affect the mimic.

Applying

11 What might account for the fact that most forms of aneuploidy are rarely, if ever, observed in humans?

12 Explain the term 'bottleneck' and what effect a bottleneck may have had on the human gene pool.

13 Draw a diagram to summarise the natural selection that occurred among the peppered moths of Great Britain as described in this chapter.

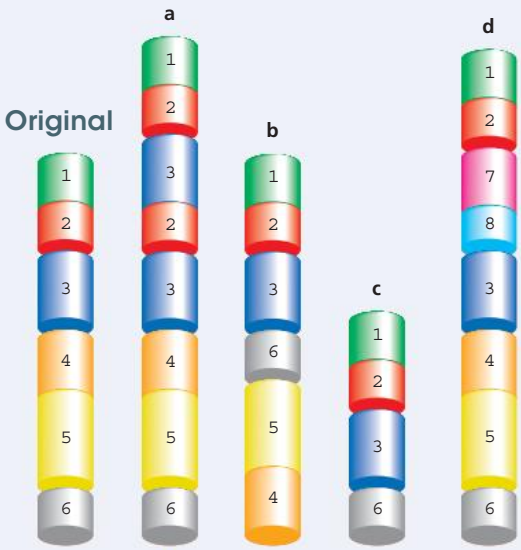

Figure 8.35 ▲
Chromosomal mutations

14 Provide an example of how an understanding of changing gene pools is important to understanding evolutionary change.

15 The founder effect leads to evolutionary change but is not 'adaptive'. Explain what this means.

16 You are related to your first cousins because you share two recent 'common ancestors' (your grandparents). The theory of evolution states that all organisms on Earth today have also arisen from a single common ancestor. How are these two usages of this term similar and how are they different?

17 Species are defined as a group of organisms that can interbreed and produce fertile offspring. Subspecies are defined as distinct populations of a species that can interbreed and produce fertile offspring with other members of the species but, due to isolating factors, they do not interbreed in nature. Many subspecies exist today among all organisms. Identify and explain the evolutionary pattern that is occurring in this example.

18 Copy and complete the following table using information provided in Table 8.1. Note that more than one type of mutation may describe the effect on the protein.

Genetic mutation	Amino acid	Type of genetic mutation	Effect on protein
GTCCCA ↓ GTCCCT	Valine-Proline ↓ Valine-Proline	Substitution	Synonymous
TCAATA ↓ TAATA	Serine-Isoleucine ↓		
AGAGGT ↓ AGATGT	Arginine-Glycine ↓		
GCAAGA ↓ GAAAGA	Alanine-Arginine ↓		
CAGTAC ↓ CACGTAC	Glutamine-Tyrosine ↓		

Table 8.1 Properties, names and DNA codons for each of the 20 amino acids

Characteristics	Name	DNA codons
Small, hydrophobic	Glycine	GGT, GGC, GGA, GGG
	Alanine	GCT, GCC, GCA, GCG
	Valine	GTT, GTC, GTA, GTG
	Leucine	TTA, TTG, CTT, CTC, CTA, CTG
	Isoleucine	ATT, ATC, ATA
Cyclic	Proline	CCT, CCC, CCA, CCG
Bulky, hydrophobic	Phenylalanine	TTT, TTC
	Tyrosine	TAT, TAC
	Tryptophan	TGG
Sulfur-containing, hydrophobic	Methionine (START)	ATG
	Cysteine	TGT, TGC

(*Continued*)

Characteristics	Name	DNA codons
Hydrophilic	Serine	TCT, TCC, TCA, TCG, AGT, AGC
	Threonine	ACT, ACC, ACA, ACG
	Asparagine	AAT, AAC
	Glutamine	CAA, CAG
Positively charged, hydrophilic	Aspartic acid	GAT, GAC,
	Glutamic acid	GAA, GAG
Negatively charged, hydrophilic	Histidine	CAT, CAC
	Lysine	AAA, AAG
	Arginine	CGT, CGC, CGA, CGG, AGA, AGG
	STOP	TAA, TAG, TGA

Analysing

19 List all the codons that could result from a synonymous mutation of GGG. What observation can you make about which of the three nucleotides in the codon is most prone to being mutated?

20 An exceptionally large plant with enlarged fruit grows among a natural population. Discuss what genetic change might have occurred in this individual and describe how you could test it to find out.

21 Apply the definition of microevolution to discuss whether modern humans are still evolving.

22 Herbert Spencer used the phrase 'survival of the fittest' to describe Darwin's concept of natural selection. Outline the ways in which this term could be misleading.

23 In North America, species of fruit fly of the genus *Rhagoletis* are confined to different species of apple trees and hawthorn bushes.

a Describe how this could lead to speciation.

b Would this be allopatric speciation? Explain.

24 Construct a diagram that illustrates how recessive traits that are deleterious can survive in a population.

25 Identify the key difference between Darwin's original conception of adaptive evolution through natural selection and what is referred to as the 'modern synthesis of the theory of evolution'.

26 Explain why processes such as genetic drift, the founder effect and sexual selection are not regarded as examples of adaptive selection.

27 When a mutation occurs in a large population it has very little effect on the population as a whole. Explain why mutations are still vital to the process of evolutionary change despite this small effect.

28 Artificial breeding of horses and cattle is not an example of natural selection but does lead to change in populations. Explain why Darwin still felt that artificial breeding was relevant to understanding evolution through natural selection.

29 Over the last 30 years many new pre-human fossils have been found, but scientists often find it difficult to agree whether they should be identified as new species or not. Account for this limitation in terms of our current understanding of the species concept.

Evaluating

30 Trypsin and chymotrypsin are proteases (enzymes that digest proteins) with strikingly similar structures, but they preferentially split proteins at the site of different amino acids. The enzymes are coded by different genes; however, scientists propose that the two genes arose from a common ancestral gene. Discuss, with annotated diagrams where appropriate, what mutations may have occurred to generate the two different genes from the same original gene.

31 Imagine a situation in which the offspring of dark-skinned parents has inherited a mutated form of a gene that confers light skin pigmentation. Predict whether this mutation would be neutral, beneficial or deleterious if the individual is located in the Arctic Circle as compared with equatorial Africa, and explain your reasoning. Discuss how, if at all, your interpretation of 'neutral', 'beneficial' and 'deleterious' is influenced by the individual's environment.

Creating

32 Design a diagram that clearly summarises the different mechanisms leading to evolutionary change. Your table or diagram should indicate which mechanisms contribute to evolutionary changes that lead populations to become better adapted to changing environments.

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CHAPTER 9

FROM GENES TO SPECIES: MOLECULAR EVOLUTION

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

KEY KNOWLEDGE

Determining relatedness between species

- molecular homology as evidence of relatedness between species including DNA and amino acid sequences, mtDNA (the molecular clock) and the DNA hybridisation technique
- the use of phylogenetic trees to show relatedness between species
- the evolution of novel phenotypes arising from chance events within genomes, specifically sets of genes that regulate developmental processes and lead to changes in the expression of a few master genes found across the animal phyla, as demonstrated by the expression of gene BMP4 in beak formation of the Galapagos finches and jaw formation of cichlid fish in Africa.

KEY SCIENCE SKILLS

Analyse and evaluate data, methods and scientific models

- organise, present and interpret data using schematic diagrams and flow charts, tables, bar charts, line graphs, ratios, percentages and calculations of mean

Draw evidence-based conclusions

- draw conclusions consistent with evidence and relevant to the question under investigation

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Figure 9.1 ▲
The koala and wombat are each other's closest living cousins, but how did two such different species evolve from a common ancestor?

Survival is the outcome of a dynamic interaction between proteins, their functions in an organism and the circumstances in which the organism must live and reproduce. Changes to the protein, the function or the circumstances could result in evolution or extinction. Underpinning changes to proteins are mutations in the genes that code for them. Mutations can be as subtle as a single nucleotide substitution, or as severe as the rearrangement of entire chromosomes. Mutations can occur in structural genes, with effects restricted to a single protein. Mutations can also occur in regulatory genes. Because these genes control the expression of a suite of structural genes, these mutations can have an impact on an organism's development. With isolation, sufficient time and environments conducive to survival, mutational changes accumulate in populations, leading to new species.

Mutations inherited by individuals can be tracked back to their parents and to their grandparents and so on. This implies mutations can be used to trace a pattern of inheritance back through many unseen generations. This rationale enables scientists to construct hypotheses about evolutionary relationships based on the mutations observed in living species today. In this chapter, we explore mutations, the effects they have on proteins and organisms and their application to reconstructing evolution.

Gene and protein evolution

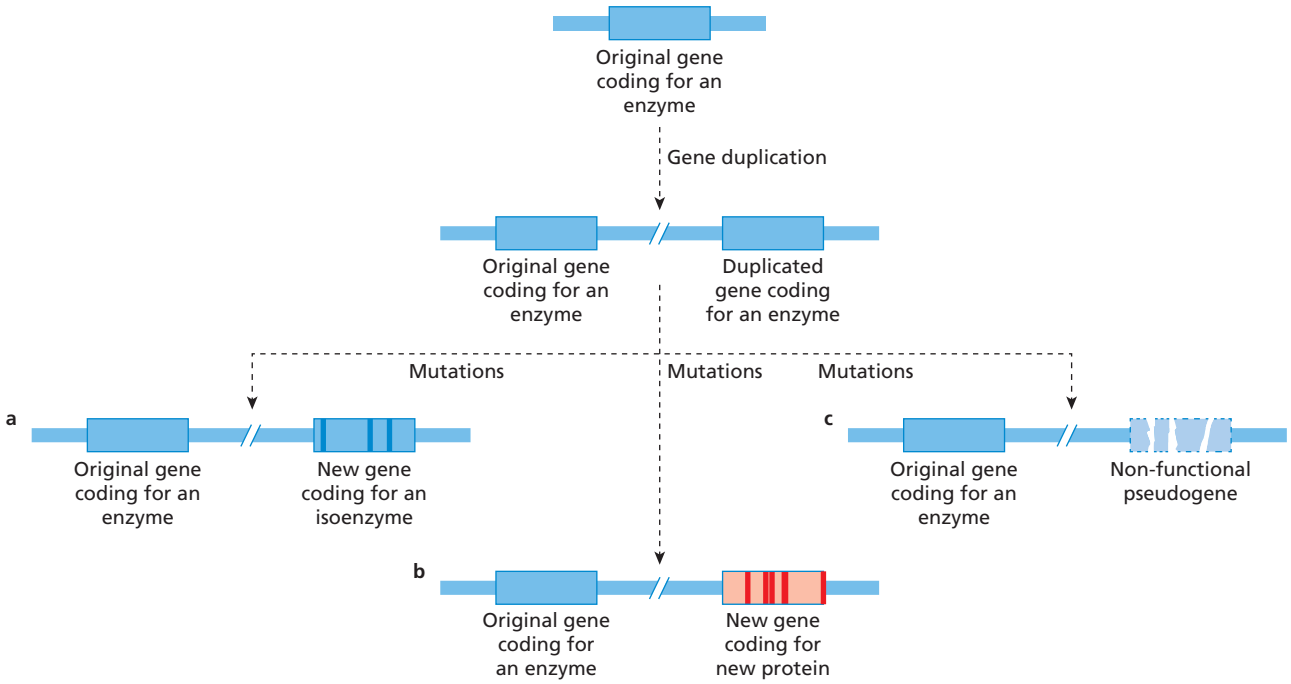
A mutation in DNA may affect the encoded protein in different ways, all of which may ultimately impact on the survival of the offspring. Neutral mutations do not appreciably change the protein's structure or function so they do not affect the offspring's chances of survival. Deleterious mutations are those that result in a protein with reduced or lost function, harming the offspring's chances of survival. Beneficial mutations are those which result in a protein with enhanced function, or possibly a new function, which may improve the offspring's chances of survival. During the course of evolution, deleterious mutations tend to be eliminated from the population, whereas neutral mutations and beneficial mutations persist as they are transmitted from generation to generation. Over a long period of time, the prevalence of beneficial mutations tends to increase in a population as a result of natural selection.

See Chapter 8 for a full discussion of different types of mutations and the effects they have on an organism's survival, and for more on natural selection and gene pools.

Generating genes for novel proteins

New genes coding for proteins evolve primarily through a process of **gene duplication** and subsequent mutation. This contrasts with the evolution of new alleles, which arise by mutation in an existing gene. Gene duplication often occurs when a chromosomal segment carrying the gene, or part of the gene, is duplicated by mutation. Gene duplication may also occur when an individual inherits three or more copies of the complete set of homologous chromosomes. Many plants, for example, are polyploid. Following gene duplication, one of the gene copies usually retains its original function. The fate of the other copy, however, can vary (Figure 9.2).

See Chapter 8 for more on polyploidy.



▲ **Figure 9.2** Gene duplication and subsequent mutation during evolution results in different fates for the duplicated gene. (**a**) Synonymous or silent mutations result in multiple genes for isoenzymes. (**b**) Beneficial mutations may result in the evolution of a new gene for a protein with a novel function. (**c**) Mutations in a duplicated gene that is unnecessary result in a pseudogene.

Generating genes for isoenzymes

Both gene copies may be retained during evolution but become expressed differently. They may be expressed at different developmental stages of a growing organism. Alternatively, they may be expressed at the same time but in different tissues in a multicellular organism.

Most vertebrate animals, for example, have multiple versions of the metabolic enzyme lactate dehydrogenase. Each version of the enzyme is a tetramer made up of four subunits assembled from a combination of three polypeptides (Figure 9.3). Each polypeptide is coded by a distinct gene at a different **locus** of the genome: *LDHA*, *LDHB* and *LDHC*. One form of the enzyme comprises exclusively the *LDHA* polypeptide and is expressed in skeletal muscle. A second is composed exclusively of the *LDHB* polypeptide and is expressed in highly aerobic tissues, such as the heart and the brain. A third, composed exclusively of *LDHC* polypeptide, occurs in various tissues such as the spermatozoa of birds and mammals, the eye lenses of birds and crocodiles, and in the liver of many fish species. Other versions of the enzyme are formed by mixed combinations of the three polypeptides and these are found in various tissues in different animals.

These different forms of lactate dehydrogenase are described as **isoenzymes** because they carry out the same reaction but are the products of different genes with different DNA sequences. The multiple gene copies are interpreted as having arisen by gene duplication and subsequent mutation. During the course of evolution, the different genes have developed unique expression patterns in different tissues. As a consequence, the different forms of the enzyme have become finely tuned to suit the conditions of the tissues in which they are made.

Generating genes for proteins with novel functions

A duplicated gene may acquire mutations resulting in it coding for a protein with a completely new function. If the new protein provides a selective advantage for the organisms that have it, the corresponding gene may become common and eventually fixed in the gene pool.

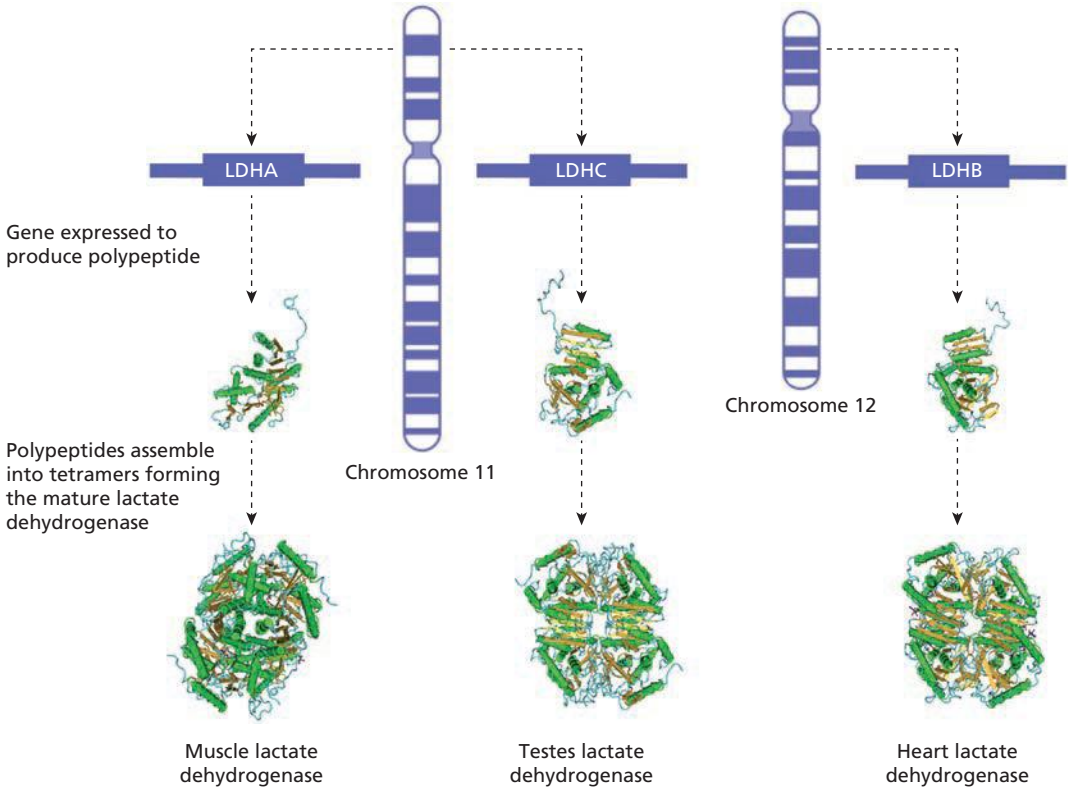


Figure 9.3 ▶
Three separate genes (*LDHA*, *LDHB* and *LDHC*) code for lactate dehydrogenase in humans. The polypeptides assemble into mature proteins comprising four identical subunits. Each form of lactate dehydrogenase has different properties, and each corresponding gene is expressed in different tissues.

All mammals, for example, have genes for hormones belonging to the oxytocin/vasopressin family of chemical signalling peptides. The hormones are small cyclic peptides comprising nine amino acids (Figure 9.4). The ring is formed by a disulfide bridge that links cysteines at positions 1 and 6. The key distinction between them is the identity of the amino acid at position 8. This amino acid is hydrophobic in oxytocin and positively charged in vasopressin. The difference reflects the way the hormones bind to their unique receptors.

In mammals, the genes for oxytocin and vasopressin are very similar and are located near each other on the same chromosome. They are on chromosome 20 in humans. The two genes sit tail-to-tail separated by a short segment of DNA, and are transcribed

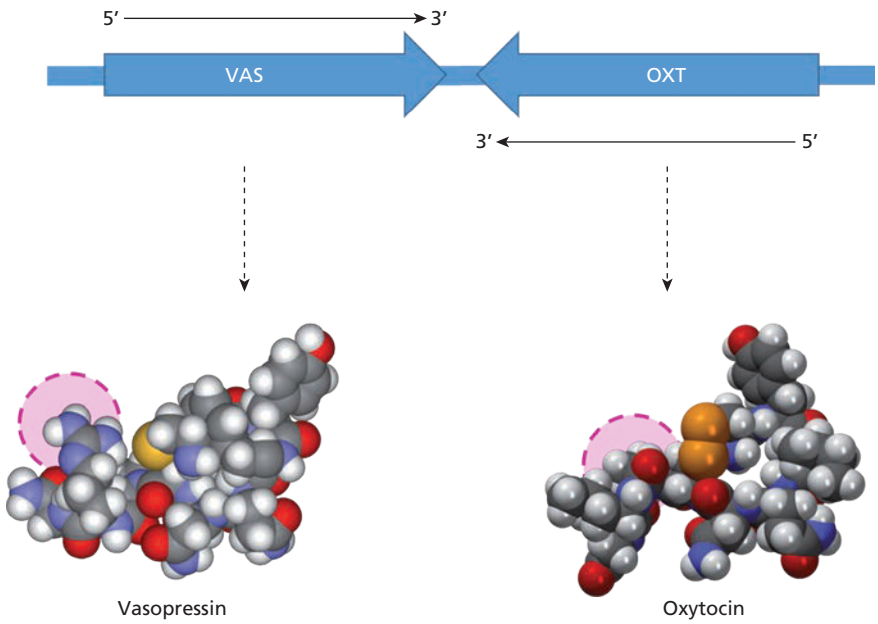


Figure 9.4 ▶
Arrangement of the vasopressin (VAS) and oxytocin (OXT) genes in mammals. Expression of the genes and subsequent processing of the proteins releases the active peptide hormones. Disulfide bridges are shown in bronze. Highlighted in pink is the distinctive amino acid at position 8, which is positively charged in vasopressin and hydrophobic in oxytocin.

in opposite directions (Figure 9.4). The two genes are interpreted to be derived from a common ancestral gene situated on a chromosomal segment that was duplicated and inverted during evolution. Although very similar, mutations in the genes have given rise to two distinct hormones with markedly different functions. Oxytocin mediates positive feedback responses, such as sexual development, initiating labour during childbirth, and lactation. Vasopressin (also known as antidiuretic hormone) is involved in homeostatic mechanisms requiring negative feedback, including regulating water balance and blood vessel constriction. Both hormones are also associated with eliciting psychological states, including anxiety, sexual intimacy and social bonding.

See Chapter 4 for more about chemical signalling and feedback loops.

Although there are small variations in different animal groups, the structures of the two hormones are relatively constant throughout the animal kingdom. This observation highlights the critical roles the two hormones play in animal biology. Their genes evolved at least 500 million years ago and have been retained all the way through the course of animal evolution.

Generating pseudogenes

A duplicated gene is sometimes superfluous for survival so its evolution is free of selection pressure. As a result, over successive generations, the duplicated gene accumulates random mutations that ultimately disrupt the reading frame of the gene. Over time, it becomes a non-functional **pseudogene**. The pseudogene broadly resembles the original gene in sequence and structure but is not transcribed or not translated into polypeptide.

For example, one way animals regulate their feeding behaviour is through taste receptors attuned to sense foods that are nutritious or potentially toxic. Most land mammals have functional genes encoding receptors for five basic tastes: sweet, salty, umami (savoury), sour and bitter. By contrast, in marine mammals, such as bottlenose dolphins (Figure 9.5), toothed whales and baleen whales, the complete suite of known genes for sweet, umami, sour and bitter taste receptors are all pseudogenes. Most of these genes have nonsense mutations: they have acquired premature stop codons that prevent them from yielding a mature polypeptide. The widespread loss of taste receptors indicates it occurred in the common ancestor of these marine mammals over 36 million years ago. There was no evolutionary pressure to conserve these genes after the move from a terrestrial to a marine environment dominated by a high salt concentration, as well as a dietary switch from plants to meat and feeding by swallowing the prey whole.

Getty Images/Jeff Rotman

▲ **Figure 9.5** A bottlenose dolphin feeding in its native habitat has little need of taste receptors.

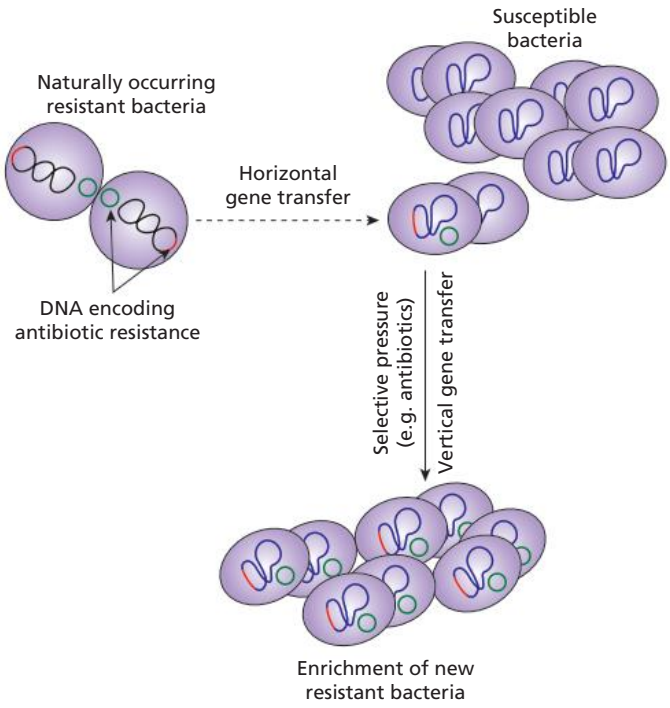
The origin of pseudogenes can be inferred because they share similar DNA sequences with the functional genes from which they were derived.

Acquiring new genes by horizontal gene transfer

Genes for proteins with novel properties can also be procured by **horizontal gene transfer**. This is the process by which an organism obtains a new gene directly from another organism, even of another species, rather than by mutation or inheritance from a parent.

Prokaryotes frequently acquire new genes by a variety of horizontal transfer mechanisms. These include taking up and incorporating naked pieces of DNA from their environment, acquiring new genes through infection by a virus (bacteriophage), or acquiring new genes directly from another bacterium through a process of cell-to-cell transfer called **conjugation**. Conjugation enables genes that enhance survival to spread through a population of bacteria in a relatively short span of time. It is believed to be one reason for the emergence of new strains of multi-drug resistant bacteria (Figure 9.6).

Figure 9.6 ▶
New species and strains of antibiotic-resistant bacteria are sometimes generated by horizontal gene transfer.



RECALL

- New genes may be acquired by gene duplication or by horizontal gene transfer.
- Duplicated genes may evolve to become genes for isoenzymes, genes for proteins with novel functions, or non-functional pseudogenes.

RECAP 9.1

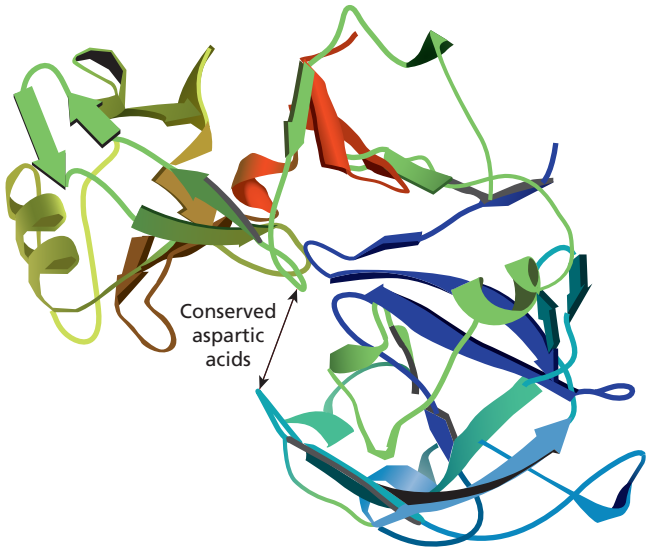
1 Contrast how a new allele and a new gene may be introduced into a population.
2 Contrast the acquisition of a new gene by horizontal gene transfer with that by gene duplication.
3 Describe the kinds of mutations that might result in a duplicated gene becoming:
   a a gene coding for a protein with novel function
   b a pseudogene.
4 Draw an annotated diagram that shows how the genes for vasopressin and oxytocin may have evolved. Start with a single gene for vasopressin in the animal ancestor. Your diagram should document mutations and effects on the encoded proteins.

Mutations affect protein structure and function

A protein's function ultimately depends on its shape, which is determined by the protein's primary structure. Although mutations affect protein primary structure directly, it is how they influence protein folding that ultimately impacts on an organism's survival.

Indispensable amino acids are conserved in evolution

Some regions of proteins' three-dimensional structure are much more sensitive to alteration than others. Changes to the amino acids lining the active site of an enzyme can profoundly influence its function. Even a single missense substitution that leads to the loss of an enzyme's function is likely to be deleterious. The critical amino acids involved in binding substrates or carrying out reactions in the active site of enzymes are often **conserved**. These amino acids are retained during the course of evolution, whereas other amino acids of the protein may become substituted. For example, the **amino acid sequences** and three-dimensional structures in the active site of a family of enzymes called the aspartyl proteases are consistent from fungi to mammals (Figure 9.7), even though the details of the rest of the proteins vary considerably. The two aspartic acids in the active sites of these enzymes are completely conserved across all eukaryotes.



Adapted from 1PSO The crystal structure of human pepsin and its complex with pepstatin, Fujinaga, M., Chernaia, M.M., Tarasova, N., Mosimann, S.C., James, M.N.G., RCSB PDB 1995

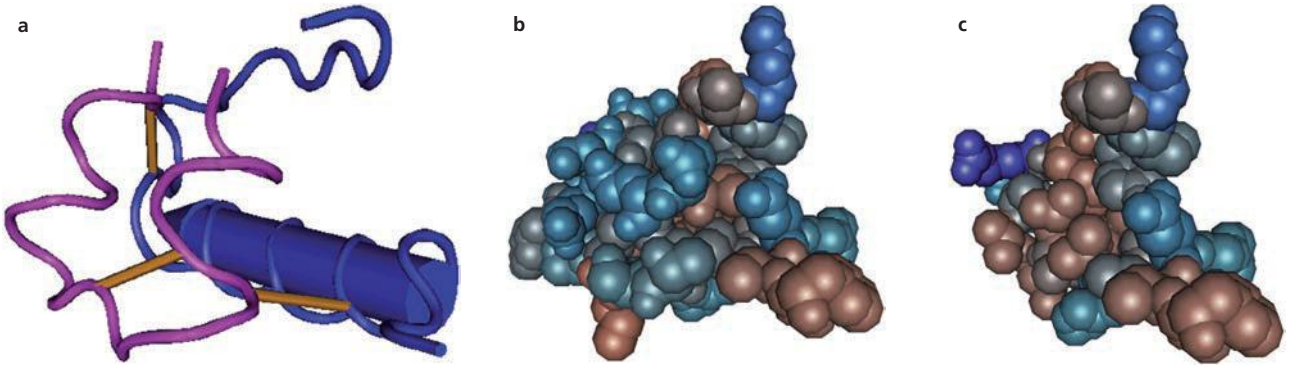
▲ Figure 9.7
Human pepsin, an example of an aspartyl protease, showing the conserved aspartic acid residues in the active site

Substitution of surface and core amino acids

Amino acids exposed on the surface of soluble proteins are mainly hydrophilic or charged (Figure 9.8). They serve to maintain the protein's 'skin' by projecting into and interacting with the surrounding water. A missense substitution that replaces one hydrophilic amino acid on a protein's surface for another is unlikely to disrupt the protein's structure or function. Proteins are therefore relatively tolerant of such substitutions during the course of evolution. As a consequence, the surface amino acids of corresponding proteins from different organisms may vary considerably.

Similar principles apply to the hydrophobic cores of proteins. During evolution, proteins accommodate the substitution of one hydrophobic amino acid for another. However, the protein core is more vulnerable than the surface to changes in amino acid size because the core amino acids must pack closely together. Substituting a small hydrophobic amino acid for a large one, or vice versa, can distort polypeptide folding in the centre of the protein. Some proteins are very sensitive to such substitutions. For example, in diverse vertebrate animals from hagfishes to mammals the core hydrophobic amino acids of the hormone insulin are highly conserved compared with the surface amino acids (Figure 9.8). Packing of the core amino acids affects insulin's volume, which in turn affects its capacity to fill the binding site of its receptor.

▼ Figure 9.8
Insulin from pig (*Sus scrofa*) consists of two polypeptide chains. (**a**) Ribbon diagram showing the A chain (pink) and B chain (blue) of insulin. Disulfide bridges between the two chains are shown in yellow. The B chain contains an alpha-helix. (**b**) A space-filling representation of the same molecule with hydrophilic amino acids coloured blue and hydrophobic amino acids coloured brown. Most of the hydrophilic amino acids are on the surface. (**c**) The space-filling representation of only the B chain showing the tightly packed hydrophobic core



RECALL

- Mutations can alter a protein's shape, properties or function.
- The more critical the role of an amino acid is to a protein's function, the more likely it is to be conserved during evolution.

RECAP 9.2

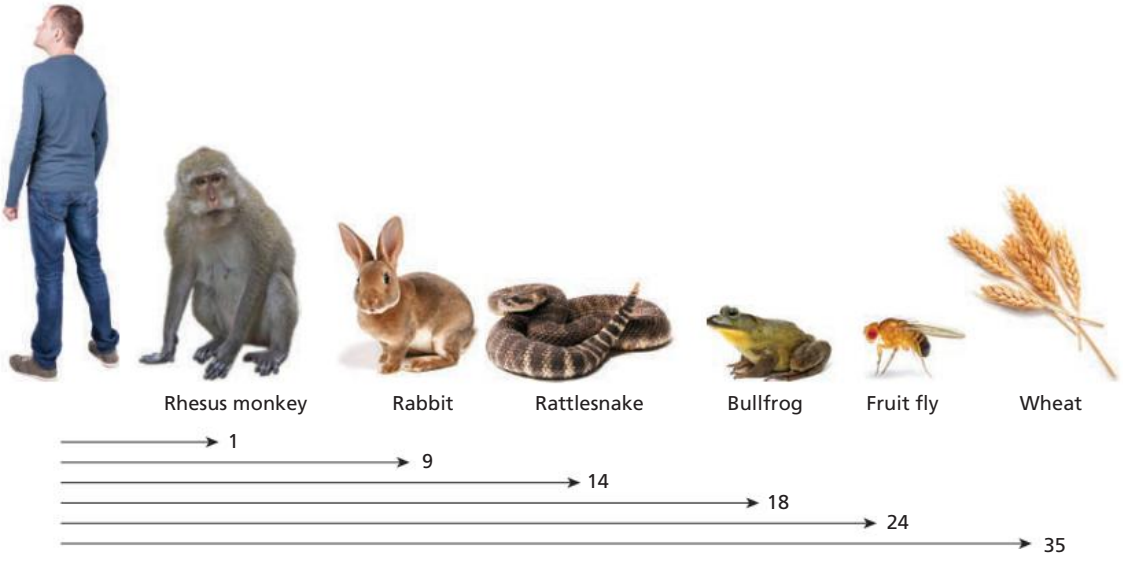
1 What is meant by a 'conserved' amino acid?
2 Why do amino acids in the active sites of enzymes tend to be highly conserved during evolution?
3 Why are amino acids on the surface of a protein relatively less conserved?
4 What factors influence conservation of amino acids in the core of a protein?

Molecular homology as evidence of relatedness between species

See Chapter 10 for more about primates and the classification of humans.

Almost as soon as the amino acid sequences of proteins were being determined in the 1960s, it became clear that proteins could be used to infer evolutionary relatedness. For any given protein, the number of amino acids differing between a pair of species provided a guide for the **evolutionary distance** between the two species. To appreciate this, let's explore the mitochondrial protein cytochrome c. Consider the organisms shown in Figure 9.9. The cytochrome c sequences have been determined for each of these species. The number of amino acid differences between the cytochrome c of the human and that of each of the other species is presented. The data show that the number of differences increases in order from the rhesus monkey (another primate), to the rabbit (another mammal), the rattlesnake (a reptile), the bullfrog (an amphibian), the fruit fly (an insect), and finally wheat (a plant). Interpreted this way, the number of amino acid differences in the cytochrome c sequence reflects the evolutionary distance between the human and each of the other organisms. The human is most closely related to the rhesus monkey and least related to wheat.

Figure 9.9 ▼
The number of amino acid differences between the cytochrome c of humans and that of other organisms



The concept of molecular homology

You will already be familiar with the concept of homology from the study of comparative anatomy. In that context, homology refers to the similarity in patterns of anatomical structures between different organisms. The more similar the structural pattern, the closer the evolutionary relationship between the organisms. Molecular biologists have adopted the concept of homology and applied it to molecules. The term **molecular homology** refers to the similarity of patterns in the **nucleotide sequences** of DNA or the amino acid sequences of polypeptides from different organisms as evidence for a common evolutionary origin. Genes or polypeptides from different species that exhibit molecular homology are described as **homologous**. Homologous genes from different organisms are referred to specifically as **homologues**.

See Chapter 7 for more on the application of homology in anatomy and morphology.

DNA and proteins suit studies of molecular homology

There are two key reasons why DNA and proteins lend themselves conveniently to the study of evolution. The first relates to the structure of the biomacromolecules themselves. DNA and proteins are unbranched polymers. They are very long, linear molecules composed of a limited number of possible building blocks. A **DNA sequence** is the order of the four possible nucleotides in a segment of DNA. A **polypeptide sequence**, the primary structure of a protein, is the linear order of the twenty possible amino acids in the polypeptide. This makes it straightforward to compare the sequences of DNA or proteins from different organisms. Similarities in sequences can be identified readily, and differences, corresponding to changes over time, are easily calculated.

The second reason relates to technological developments in the field of molecular biology. These reflect advances in both the efficiency of chemical analysis of the molecules and the speed and power of computer processing. One outcome of this is automated, high-throughput sequencing to generate large amounts of DNA sequence data. Complementing this is the enhanced capacity to manage and explore the large amounts of data produced. A relatively new branch of science called **bioinformatics** has emerged from the application of computer science to storing, retrieving and analysing large volumes of biological data. Bioinformatics is an interdisciplinary field combining mathematics, computer science, engineering, chemistry and biology. There are many different aspects to the field of bioinformatics. For our purposes in this chapter, bioinformatics is used to determine the closeness of the relationship between different species.

See Chapter 11 for more about automated DNA sequencing.

A model to explain molecular homology

To appreciate why molecular homology can be used to reconstruct evolutionary relationships, let's consider how **speciation** results in sequence variation between species. Consider the situation where there is a single interbreeding population (Figure 9.10, 5 mya). Assume there is a gene with a nucleotide sequence that is characteristic for the population. In reality, there may be a very few individual variations (alleles) in this **gene sequence** among members of the population, but if we examine the gene sequence from any member of the population selected at random it is broadly representative of the whole population. In this scenario, a span of geological time has elapsed and the original population has diverged to become two reproductively isolated populations (Figure 9.10, 4 mya). The divergence may be because a geographical barrier has formed to divide the original population, or because of physiological or behavioural differences that segregate members of the original population.

See Chapter 8 to learn more about natural selection and genetic drift.

After more time has passed, mutations have occurred in the gene sequence in each population (Figure 9.10, 3 mya). Within each interbreeding population, specific

See Chapter 7 for more on divergent evolution.

Chapter 8 contains examples of selective pressures and an explanation of genetic drift.

mutations may be naturally selected and become representative for that population. Other mutations may become fixed in each population by genetic drift. If the populations separated recently in geological time, the representative sequences of the two populations still appear relatively similar. The longer the two populations are separated, however, the more unique mutations accumulate in the sequences of each population (Figure 9.10, 2–1 mya). After a comparatively long period of geological time, the representative sequences of the two populations become increasingly different. The two populations today may be sufficiently different that they are two distinct species (Figure 9.10, the present).

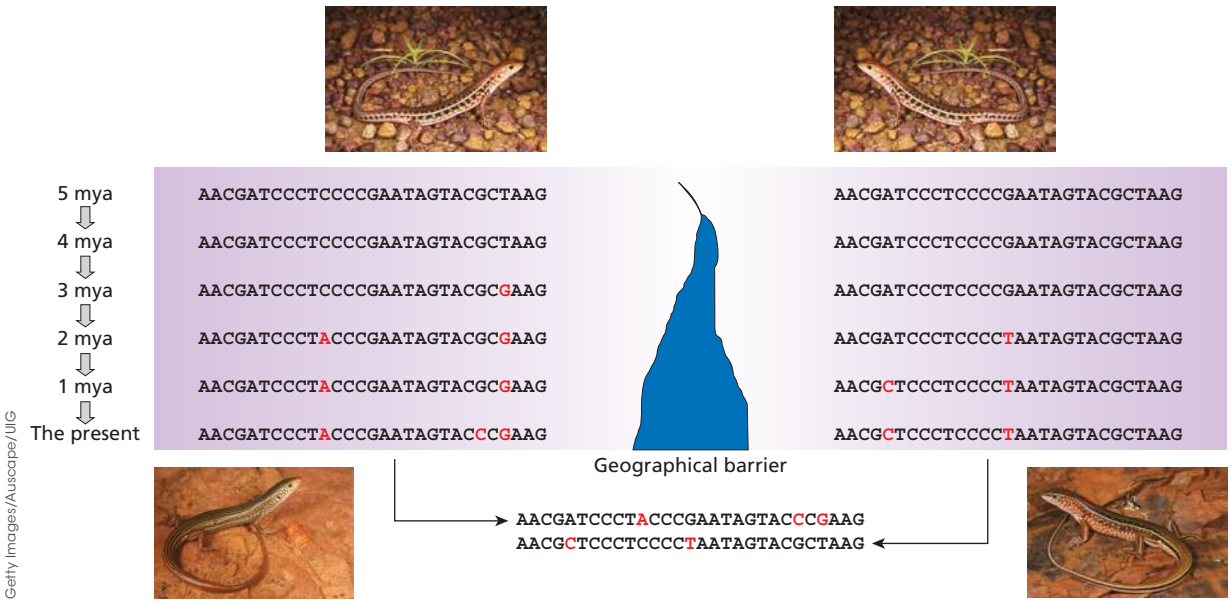


Figure 9.10 ▲
A representative DNA sequence in a population of skinks from 5 million years ago (5 mya) to the present. After the original population becomes divided by a geographical barrier (such as a body of water), independent mutations accumulate in the DNA sequences of each isolated population over time. Today, the two independent populations are separate species. The representative DNA sequences of the two species are aligned to show their homology (similarities in the sequence). The differences between the two sequences provide a measure of the evolutionary distance between the species.

The example illustrates two important concepts in evolution. The first is that of **divergent evolution**, in which a single ancestral species diverges, or splits, to eventually become two or more descendant species. Two **lineages**, or two separate lines of descent, emerge from the point in time when the split occurs. Over time, the descendant lineages evolve different features and become recognisably distinct from one another as separate species. It is possible to infer speciation events that have occurred in the past by comparing DNA sequences of different species that are alive today.

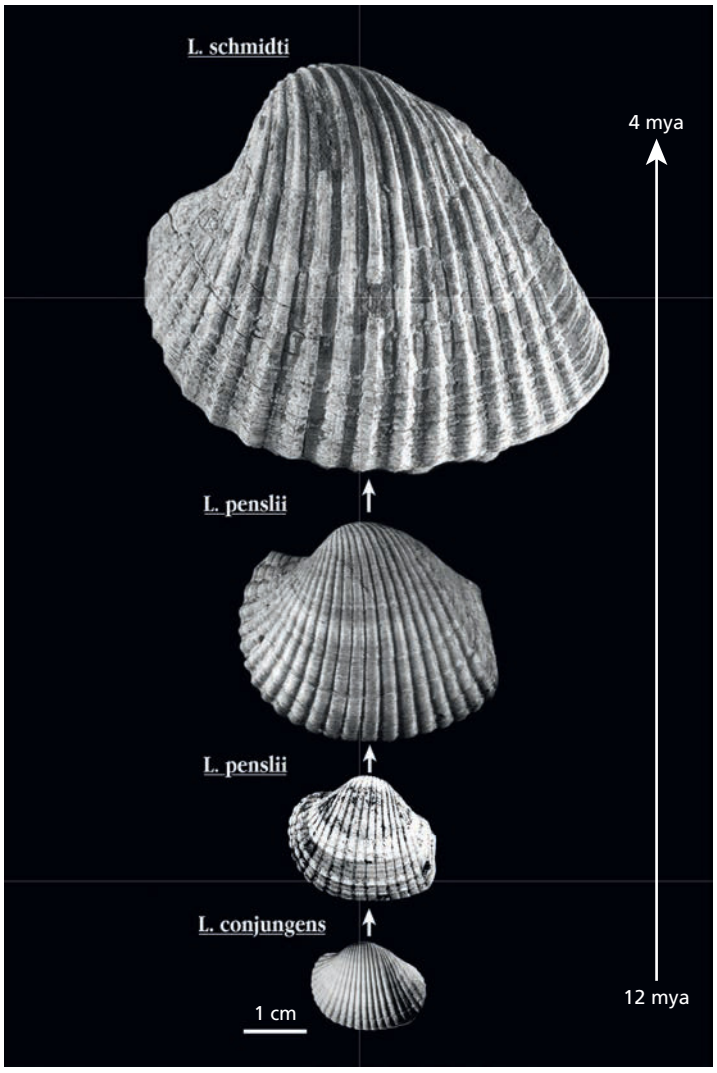
The second is the concept of **phyletic evolution**, in which there is continuous change over time in a single line of descent. Phyletic evolution is sometimes observed in the fossil record as gradual morphological or anatomical changes that have occurred in a single evolutionary lineage (Figure 9.11). Through a combination of natural selection and genetic drift, new species emerge and replace older ones, but the lineage never splits. Molecular homology makes use of the fact that phyletic evolution occurs in the DNA sequences of all species. The ongoing changes in DNA sequences provide a kind of 'ruler' for measuring relatedness between species.

Sequence alignments as a tool for investigating molecular homology

The DNA sequences in the present-day species illustrated in Figure 9.10 are homologues. They are related to each other by descent from a common ancestral DNA sequence. If we make a **sequence alignment** of the homologues today we can identify the original

pattern in the gene by the similarities (homology) in the nucleotide sequences (Figure 9.10). Comparing this alignment with the mechanism depicted in Figure 9.10 highlights three key observations.

1 The nucleotides that are identical in both sequences are presumably ancestral. That is, these nucleotides were in the gene of their common ancestor and both descendant species have inherited those nucleotides. These nucleotides are conserved nucleotides. They were preserved during the course of evolution and are retained in the sequences of the descendant species.
2 The number of differences between the gene sequences of the two species – the independent mutations in each sequence – provide a measure of how related the two species are. This is the measure of evolutionary distance. Generally, for a given gene, the higher the proportion of differences in the sequence, the less related the two species are.
3 The degree of difference in the sequences gives an indication of the time of divergence from the last common ancestor. Broadly speaking, for a given gene, the more differences there are between the two sequences, the more time has elapsed since the species last shared a common ancestor. This last point leads to a concept described as the 'molecular clock,' which will be discussed in detail later.



courtesy of Dr. Imre Magyar and Prof. Dana Geary

◀ Figure 9.11
Fossil shells from the ancient lake bed of Lake Pannon in Hungary dating from 12 mya to 4 mya. The fossils show phyletic evolution in bivalve molluscs of genus *Lymnocardium*.

RECALL

- The evolutionary distance between two species is estimated by the number of amino acids differing in a homologous polypeptide, or the number of nucleotides differing in a homologous gene, between the two species.
- Molecular homology is explained by patterns of divergent evolution from a common ancestor and phyletic evolution within each branching lineage.

RECAP 9.3

1 Define the term 'molecular homology'.
2 Give two reasons why DNA and proteins are suited to studies of molecular homology.
3 Describe and contrast phyletic and divergent evolution.
4 What three things does a sequence alignment of two homologues reveal?

See Chapter 3 for more on endosymbiosis and the origin of mitochondria.

Mitochondrial DNA: a versatile subject for investigating molecular homology

The mitochondrial genome is contained entirely on a double-stranded, circular chromosome (Figure 9.12) and possesses a suite of genes for many of the proteins involved in cellular respiration. In order to express these genes inside the organelle, the mitochondrial genome also possesses genes for ribosomal RNA (rRNA) and transfer RNA (tRNA) molecules. Polypeptide synthesis is achieved by ribosomes within the mitochondrion.

Mitochondrial DNA (mtDNA) is often favoured for evolutionary studies because it is found in essentially all eukaryotic organisms, it is abundant, it has sufficiently variable DNA sequences, and its inheritance is easily traced.

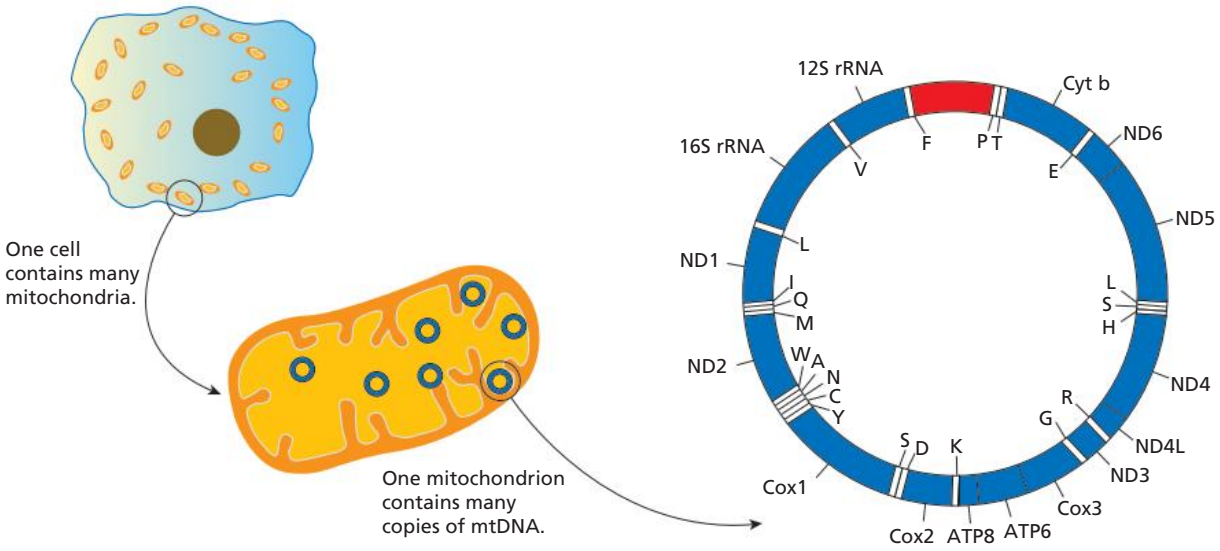
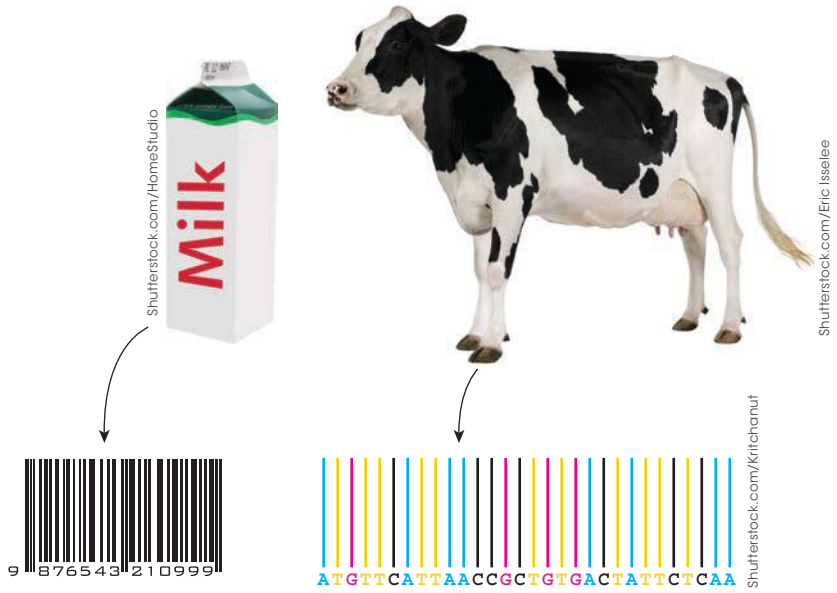


Figure 9.12 ▼ Eukaryotic cells contain many copies of the mitochondrial genome. Genes for mitochondrial proteins and rRNA are labelled on the outside of the chromosome; genes for mitochondrial tRNA are labelled with single letters on the inside.

Mitochondrial DNA is convenient for identifying and comparing eukaryotic species

The mitochondrion is a universal feature of all eukaryotic organisms because they rely on it for energy production. Mitochondrial genes can be sequenced and compared between a wide range of organisms, including different animals, plants, fungi and protists. The mtDNA is therefore a practical target for generating and comparing DNA sequences of diverse organisms.

Certain genes of the mitochondrial genome are commonly used as sources of DNA barcodes. A **DNA barcode** is a nucleotide sequence derived from a representative gene that uniquely identifies a particular species, subspecies or variety of organism (Figure 9.13). The analogy is drawn here with the barcodes used for product identification on commercial goods. DNA barcodes can be generated from a large number of small samples in a short amount of time. They are especially useful if only a fragment of the organism is available, or if the organism is disfigured or otherwise unrecognisable. The application of DNA barcodes therefore extends beyond evolutionary studies. DNA barcodes enable diverse species to be identified efficiently for ecological surveys or for cataloguing prey in a predator's gut or faeces. They are also used to authenticate the identity of marketed goods or quarantined materials. For example, in 2013, DNA barcoding revealed that many European processed foods that listed minced beef as its principal ingredient actually contained horse meat.



◀ Figure 9.13
The milk carton has a barcode that uniquely identifies the product. In a similar way, the nucleotide sequence of a cow's mitochondrial gene acts as a barcode to identify the species.

Eukaryotic cells contain many copies of mitochondrial DNA

Each cell harbours many identical copies of the mitochondrial chromosome. This is because there are many copies of the chromosome inside each mitochondrion, as well as many mitochondria inside each cell (Figure 9.12). A given eukaryotic cell will contain only a diploid set (two copies) of each nuclear chromosome but thousands of copies of the mitochondrial chromosome. Furthermore, the mtDNA is a very compact genome. In humans, for example, the mitochondrial chromosome comprises ~16 600 base pairs coding for just 37 genes (Figure 9.12). This contrasts with the nuclear genome, which comprises ~3.1 billion base pairs encoding between 21 000 and 25 000 genes. The very high number of relatively small mtDNA molecules makes it comparatively easy to extract and manipulate for sequencing. It also means that, compared with nuclear DNA, there is a better chance of recovering an intact mitochondrial genome than a nuclear genome

from fossil specimens. These attributes of mtDNA favour its application for studying the evolutionary relationships of recently extinct organisms up to 100 000 years old.

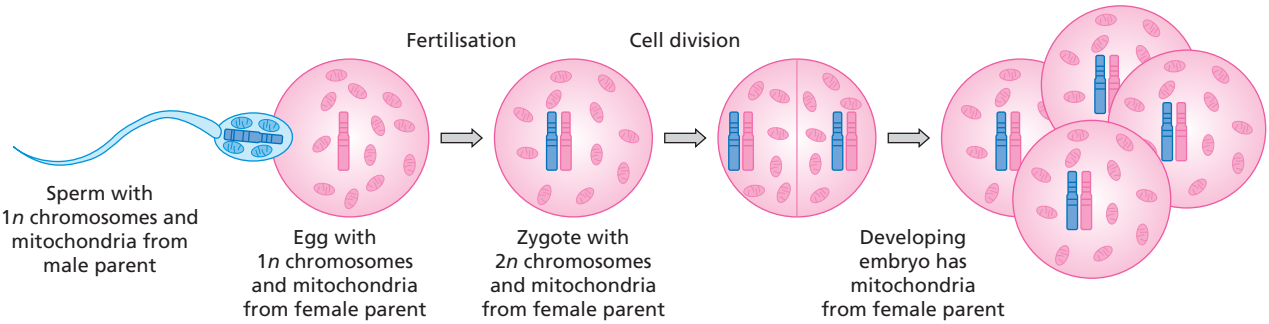
Mitochondrial DNA exhibits high mutation rates

The mtDNA endures a higher rate of mutation than nuclear DNA. This is presumably due to the highly oxidising environment inside the mitochondrion. Corrosive forms of oxygen, called oxygen radicals, are produced as a by-product of the electron transport chain. The oxygen radicals can damage the mtDNA before they are eliminated. As a result of the higher mutation rate, mtDNA sequences can show substantial variation among individuals of the same species, as well as between different species. This aspect of mtDNA favours its application for studying relatedness among populations or subspecies of a single species.

Mitochondrial DNA is maternally inherited

Nuclear DNA is inherited equally from both parents, but mitochondrial chromosomes are inherited independently of nuclear chromosomes. The mtDNA is **maternally inherited**. Offspring inherit their mtDNA only from their mother because essentially all the cytoplasm in the fertilised zygote is derived from the egg and not the sperm. If any mitochondria from the sperm penetrate the egg at fertilisation, they are recognised as foreign and targeted for destruction (Figure 9.14). This ensures the zygote contains an essentially uniform population of mitochondria derived from the female parent. Furthermore, mtDNA does not undergo independent assortment or crossing over in the way nuclear chromosomes do during meiosis. Consequently, while the ancestral history for a mutation in nuclear DNA is quickly obscured or lost after just a few generations of recombination and random fertilisation, the ancestry of mtDNA variation can be simply and continuously traced through the female line of inheritance, from mother to offspring, or vice versa (Figure 9.15). This aspect of mtDNA favours its use for identifying individuals long deceased, as well as for exploring patterns of ancestry and migration within and between populations.

Figure 9.14 ▼
Following fertilisation, any remnant mitochondria from the sperm are destroyed, so the zygote and subsequent embryonic cells contain mitochondria from the female parent only.



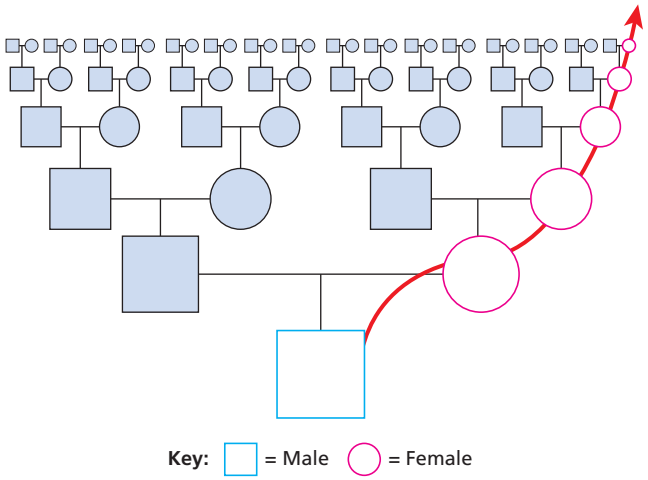


Figure 9.15 ▶
Inheritance of mitochondrial DNA can be traced simply through the female line of descent.

The molecular clock

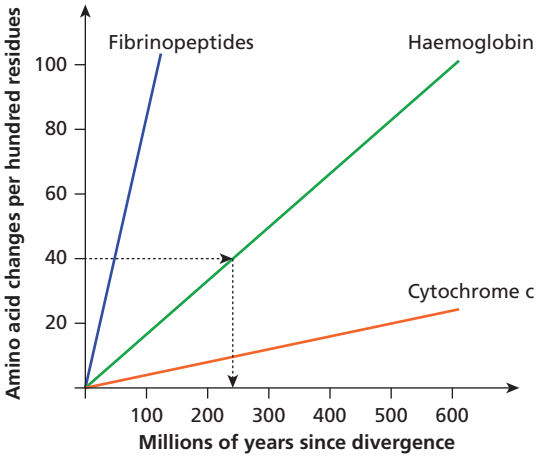
In the 1960s scientists first determining the amino acid sequences of proteins from various organisms discovered a trend. The number of differences between the sequences of two species was roughly proportional to the time that fossil evidence indicated had elapsed since they diverged. The trend could be used to determine the rate of amino acid substitution in a given polypeptide over time (Figure 9.16). This rate could in turn be used to estimate the time of divergence between two species based on the amino acid sequences of their polypeptides. This line of reasoning underpins the concept of the molecular clock.

The **molecular clock** refers to the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage. The rate of the molecular clock describes the number of substitutions that occur in a specified polypeptide or gene over a defined period of time. In principle, the 'ticking' of the molecular clock is used to estimate the time since two species diverged from their last common ancestor. The timing of the divergence is calculated from the number of differences in their corresponding sequences (dotted arrows in Figure 9.16). The molecular clock therefore enables time to be inferred from the outcomes of divergent and phyletic evolution.

Factors affecting the rate of the molecular clock

The molecular clock presumes that the rate of substitutions remains constant; but as Figure 9.16 indicates, the rate clearly differs from one gene to another. This is because some genes are generally more sensitive to mutations than others.

Even for a given gene, the rate varies depending upon species-specific differences. Differences occur, for example, between large and small animal species. Compared with elephants, mice have higher metabolic rates, live shorter lives, reproduce more frequently and produce many more offspring. A high metabolic rate is associated with greater production of oxygen radicals, which increase the rate of DNA mutation. Shorter generational times and larger numbers of offspring increase the opportunities for introducing mutations into the population. All these factors increase the rate of the molecular clock in the mouse lineage compared with that of the elephant lineage.



▲ **Figure 9.16** Rates of the molecular clock for three different proteins from different organisms: fibrinopeptides, haemoglobin and cytochrome c. The dotted arrows show how the number of amino acid differences in haemoglobin from two organisms (in this case, 40 per hundred residues) can be used to estimate the time since they diverged from a common ancestor (~250 mya).

The rate of the molecular clock also varies as the organisms' circumstances fluctuate over time. For example, the impact of natural selection on a population varies as environments change. The rate may also vary with changes in population size because the effect of genetic drift increases as the population decreases.

These factors challenge the assumption of a stable rate for the molecular clock. Ultimately, molecular clock rates must be calibrated against independent evidence for the time of species' divergence. Divergence times are often derived from the fossil record.

RECALL

- Mitochondrial DNA is a versatile subject for evolutionary studies because it is universal in eukaryotic organisms, abundant in most cells, relatively variable, and maternally inherited.
- The rate of a molecular clock is set by the number of substitutions in a homologous polypeptide, or the number of substitutions in a homologous gene, over time.

RECAP 9.4

1 Describe the concept of a DNA barcode.
2 Compare mitochondrial DNA with nuclear DNA and explain why features of mitochondrial DNA especially suit evolutionary studies of extinct organisms.
3 Explain how it is that mitochondrial DNA is maternally inherited and what advantage this confers to studies of evolution.
4 Is the molecular clock uniform for all genes and all species? Explain.

Walking in elephants' footsteps: reconstructing evolution from nucleotide sequences

The example modelled in Figure 9.10 provides a rationale for the application of molecular homology. Molecular homology is based on the fact that DNA sequences keep changing over time. The longer two species have been separated, the more time there has been for changes to accumulate in their respective DNA sequences. Consequently, the corresponding DNA sequences of two species that separated recently, which we consider to be closely related, will be more similar than those of two species that diverged a relatively long time ago.

How can DNA sequences of organisms alive today be used to reconstruct evolution? Let's consider the example of three elephant species. These are the Asian elephant, the African savannah elephant and the African forest elephant. These three elephants are found in different locations and vary according to their size and morphology (Figure 9.17a and b). Now, let us compare a 100-nucleotide segment of the mitochondrial ND4 gene from each of the three elephant species (Figure 9.17c). The gene sequences

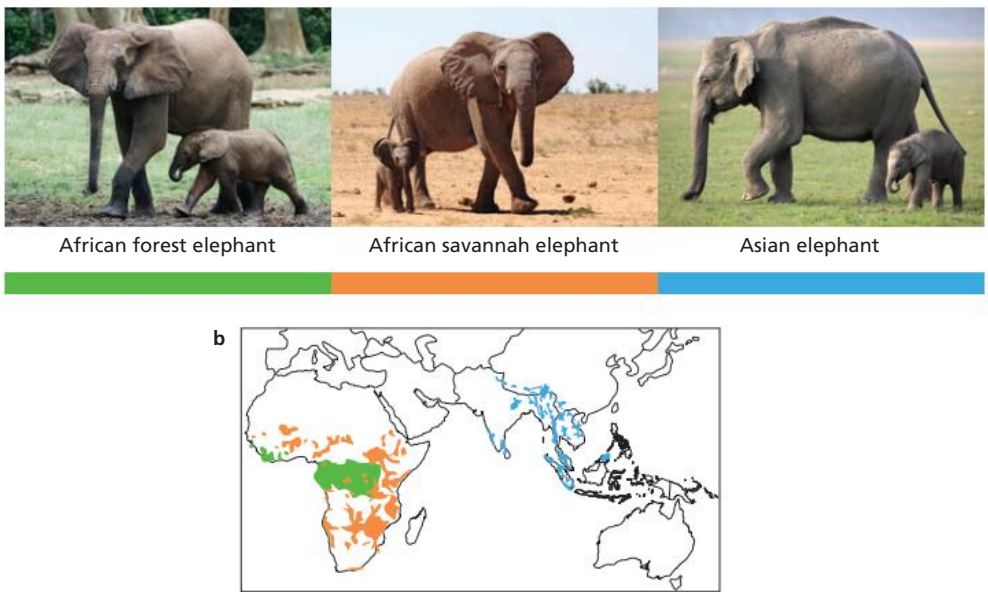


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Figure 9.17 ▶ **(a)** Three elephant species and **(b)** their corresponding global ranges. **(c)** A sequence alignment of 100 nucleotides from the mitochondrial ND4 gene of each elephant species. Asterisks indicate conserved nucleotides in the sequences of the three species.

from the three species can be aligned by their conserved nucleotides – the nucleotides that are identical in each gene. While most of the nucleotides are conserved, there are some nucleotides that differ between each sequence. These differences have arisen by mutation in one or another of the sequences.

If each pair of sequences is compared, it is possible to determine how similar the sequences are. For example, a **pairwise comparison** of the sequence of the African forest elephant with that of the African savannah elephant shows that 97 of the 100 nucleotides are identical (Table 9.1). Based on the total length of the sequence used for the comparison, the degree of sequence conservation for the DNA segment can be calculated.

Table 9.1 Pairwise comparisons of DNA sequences between three elephant species

Pairwise comparison	Number of nucleotides in the DNA segment	Number of conserved nucleotides	Number of nucleotide differences	% sequence conservation
African forest elephant with African savannah elephant	100	97	3	97%
Asian elephant with African forest elephant	100	94	6	94%
Asian elephant with African savannah elephant	100	93	7	93%

$$\%\ \text{sequence conservation} = \frac{\text{conserved nucleotides}}{\text{total nucleotides}} \times 100\%$$

For the African forest and African savannah elephants:

$$\%\ \text{sequence conservation} = \frac{97}{100} \times 100\% = 97\%$$

The DNA sequences of the African forest and African savannah elephants are 97% conserved. Pairwise comparison of the DNA sequence of the Asian elephant with those from either the African forest or African savannah elephant gives lower levels of sequence conservation: 94% and 93%, respectively (Table 9.1). The DNA sequences from the two African species therefore share more nucleotides with each other than either does with the Asian elephant. This observation suggests the two African species shared a more recent common ancestor than either did with the Asian elephant. In evolutionary terms, the data indicate the two African species are more closely related. This reasoning is based on the concept of evolutionary distance and is described as the **distance method** for inferring evolutionary relationships.

The example of the three elephant species can be re-framed in a slightly different way. Rather than asking how conserved the DNA sequences are, it is possible to ask how different the DNA sequences of each species pair are. Counting the differences between each pair of sequences yields the complementary set of results shown in Table 9.1. In essence, these differences provide a measure of the evolutionary distance between each species. The more nucleotide differences, the greater the evolutionary distance. The data show that the Asian elephant is the most distantly related of the three species.

Assembling phylogenetic trees from DNA sequences

Phylogeny is the study of the evolutionary history of an organism or a group of organisms. Phylogeny considers the lines of descent from ancestral organisms and the relationships among descendant organisms. A convenient way to visually represent phylogeny is with a **phylogenetic tree**. The evolutionary relationships inferred from the data in Table 9.1 can be represented in a phylogenetic tree. Phylogenetic trees can be described as unrooted or rooted. Each is demonstrated below.

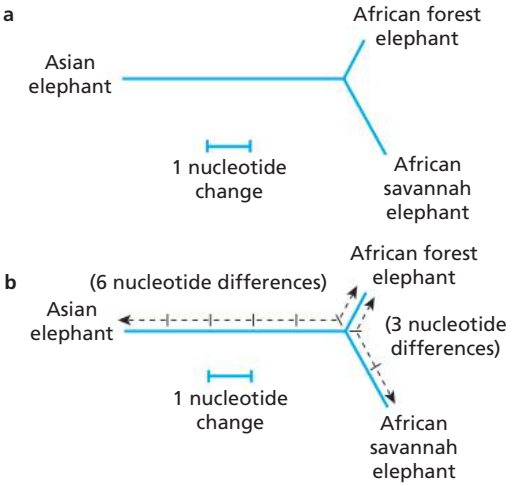


Figure 9.18 ▼
(**a**) Unrooted tree depicting relatedness between three elephant species (**b**) Demonstration of how the scale is used to measure the evolutionary distance between the African forest and African savannah elephants (three nucleotides) or the Asian and African forest elephants (six nucleotides)

Unrooted phylogenetic trees

In an **unrooted tree**, the direction of evolution is not indicated. The tree is used simply to depict relatedness between different organisms. The relative evolutionary distance between the organisms is represented by the lengths of the branches and is measured as the number of nucleotide differences between each respective DNA sequence. The unrooted tree in Figure 9.18a is inferred from the data in Table 9.1. At a glance, it is clear that the two African elephant species cluster together. This implies they are the two most closely related species. The Asian elephant is the least related and is positioned on a branch tip that is furthest from the other two.

The scale in the diagram of the unrooted tree represents one nucleotide change. If a path is traced along the branches from the tip labelled with the African forest elephant to the tip labelled with the African savannah elephant, the distance is three times the length of the scale, or three nucleotide changes (Figure 9.18b). The distance between the tips labelled with the Asian elephant and the African forest elephant is six times the length of the scale, or six nucleotide changes (Figure 9.18b). The differences between the DNA sequences therefore provide a measure of the evolutionary distance between the species.

RECALL

- The distance method for reconstructing evolution is based on the concept of evolutionary distance and is inferred by pairwise comparisons between homologous polypeptides or homologous genes.
- Unrooted phylogenetic trees depict the evolutionary distance between different organisms.

RECAP 9.5

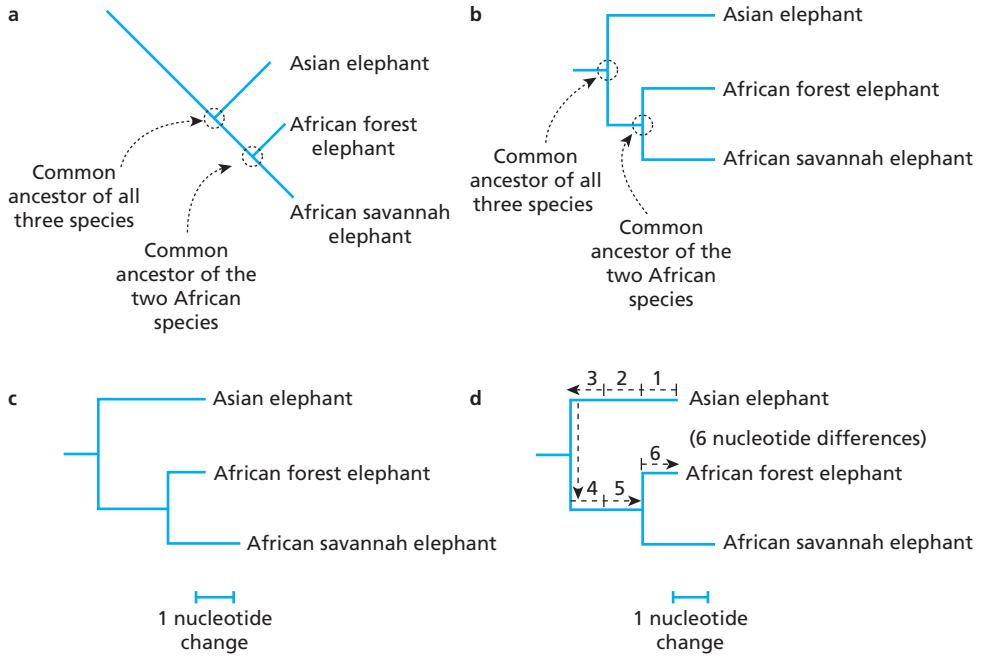
1 Explain how sequence conservation can be used to estimate how closely or distantly related species are.

2 Use the alignment presented in Figure 9.10 to estimate how conserved the reptile sequences are.

3 Describe how the evolutionary distance between two species can be interpreted from an unrooted phylogenetic tree.

Rooted phylogenetic trees

Rooted trees are phylogenetic trees that depict a history of ancestry. In a rooted tree (Figure 9.19), the tips are labelled with the organisms under study. Each **node**, or branch point, represents the last common ancestor of the organisms whose lineages emerge from it. The root is the ancestral lineage leading to all the descendants in the tree. The most basal node is the common ancestor of all the organisms in the tree. In these rooted trees, the two African elephant species are the most closely related because they share the most recent common ancestor.



◀ **Figure 9.19** Rooted phylogenetic trees depicting the evolutionary relationships of three elephant species. (**a**, **b**) Two representations of the same cladogram. The nodes are circled and each indicates a common ancestor to the lineages branching from it. (**c**) A phylogram with horizontal branch lengths representing the number of nucleotide changes occurring during evolution of the lineage. (**d**) Demonstration of how the scale of the phylogram is used to measure the evolutionary distance between the Asian and African forest elephants (six nucleotides)

The rooted phylogenetic tree also implies the passage of time. The tips represent the present. To trace back to the root is to journey back in time. The most ancient lineages branch near the base of the tree and the most recently derived ones branch closer to the tips. In the trees shown in Figure 9.19, the lineages leading to the Asian elephant and to the African elephants diverged first. The lineage leading to the two African species diverged more recently.

There are two types of rooted trees – cladograms and phylograms. A **cladogram** (Figure 9.19 a and b) represents a hypothesis for the evolutionary history leading to the descendant species. It is characterised by clades, where each **clade** is a branch of the cladogram that comprises an ancestor and all its descendants. In each of Figures 9.19a and 9.19b, two clades can be identified. One clade comprises all three elephant species and their common ancestor. The other comprises the two African elephant species and their common ancestor.

A **phylogram** is a scaled, or quantified, version of the rooted tree. In a phylogram (Figure 9.19c), the lengths of the horizontal branches represent the number of nucleotide changes that have occurred during the evolution of the lineage. The scale bar represents a single nucleotide change. Evolutionary relatedness between species is measured by the lengths of the horizontal branches (Figure 9.19d). The nodes – the points of divergence from a common ancestor – are counted as zero length. The path traced along the horizontal branches from the Asian elephant to the African forest elephant is a total of six times longer than the scale of one nucleotide. The difference between the sequences of the two species is therefore six nucleotides. As seen with unrooted trees, the

differences between DNA sequences of two organisms is a measure of the evolutionary distance between the organisms.

In the era of modern bioinformatics, computer algorithms are used to process large sets of DNA or polypeptide sequences. Algorithms such as BLAST (for Basic Local Alignment Search Tool) enable an unknown sequence from an organism to be identified against a collection of known sequences from other organisms. Other algorithms, such as Clustal, generate sequence alignments and draw phylogenetic trees based on homologous sequences.

Classifying relatedness

Since the time of Linnaeus, biologists have used a wide variety of features to categorise organisms. The goal is to group organisms according to the degree to which they share similarities. This is the basis of **taxonomy**, the system of scientific conventions for naming and classifying organisms.

Systematics is the branch of biology that categorises organisms according to their phylogeny. In modern biology, taxonomy serves as a tool of systematics for formalising hypotheses about evolutionary relationships. Cladograms dictate how taxonomic groups are arranged and composed. Navigating through the taxonomic ranks from phylum, class, order, family to genus and species, one proceeds from the ancestors towards ever more closely related descendants. In essence, systematics provides a guide to the progress of evolution over time. The higher ranks, such as phyla or classes, represent very ancient divergences, whereas the lower ones, such as genera and species, represent relatively recent divergences between organisms.

In systematics, the goal is to define each taxonomic group as a clade within the cladogram. A taxonomic group is described as **monophyletic** if all the species in that group are descended from the same common ancestor. A clade is therefore the representation of a monophyletic group. If the taxonomic group is missing one or more descendants, it is invalid and is described as **paraphyletic**. In the example shown in Figure 9.20a, the family Elephantidae is monophyletic. All three elephant species included in the family are descendants of the one common ancestor (blue circle). On the other hand, the taxonomic family Elephantidae is paraphyletic if one of the descendant species is not included (Figure 9.20b).

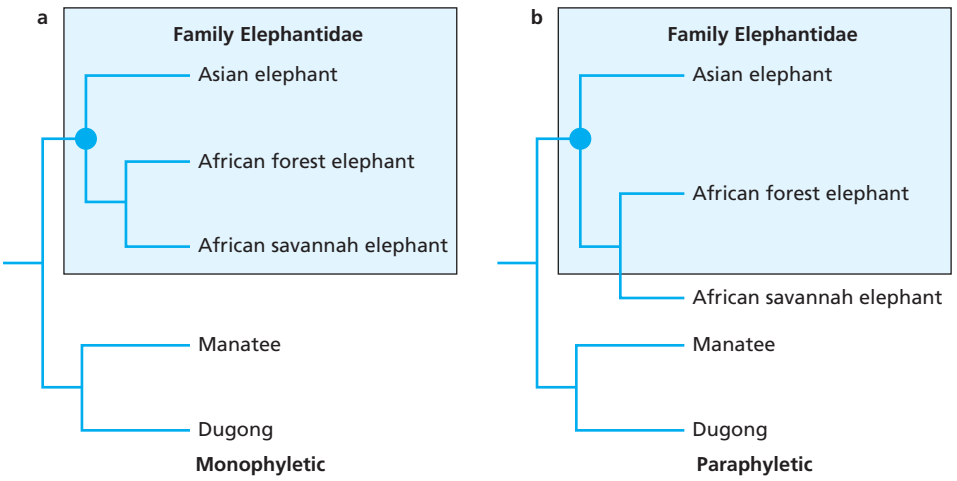
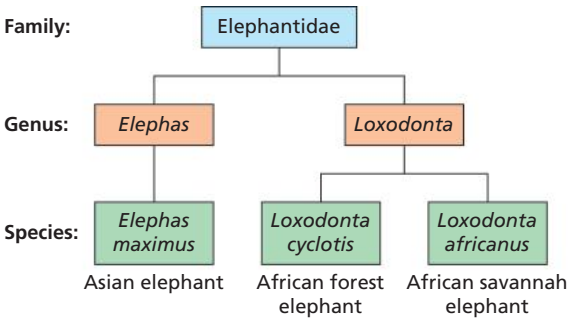


Figure 9.20 ▶ The taxonomic family Elephantidae as (**a**) a monophyletic group and (**b**) a paraphyletic group

In the example of the elephant species (Figure 9.21), all three are members of the same monophyletic family (Elephantidae), so Figure 9.20a is the correct representation of the family and Figure 9.20b is not. Reflecting the evidence from molecular homology, the Asian elephant is classified in one genus (*Elephas*), whereas the two African species are classified in another (*Loxodonta*). The placement of the two African species in the

same genus formally recognises them as more closely related to each other than either is to the Asian elephant. The genus *Loxodonta* is therefore also monophyletic.

Systematics is based on an interpretation of the available evidence, so schemes may be modified as new evidence emerges or as existing evidence is re-examined and re-interpreted. Traditionally based on anatomy, type of body covering (e.g. cuticle, scales, feathers, fur) and reproductive characteristics, systematics has been updated to take account of a broad range of evidence, including embryology, cellular substructure and modern molecular data.



▲ **Figure 9.21** The systematics of elephant species

RECALL

- Rooted phylogenetic trees can be used to represent the descent of species from common ancestors, as well as the evolutionary distance between species.
- Systematics is concerned with categorising organisms based on patterns of evolution and uses taxonomy to formally frame hypotheses about evolutionary relationships.
- Cladograms dictate the arrangement of taxonomic groups, with the aim of classifying monophyletic groups and avoiding paraphyletic groups.

RECAP 9.6

1. How do rooted and unrooted phylogenetic trees differ in presentation and in the type of information they convey?
2. What does a node represent in a rooted phylogenetic tree?
3. Describe a cladogram and a phylogram. Distinguish between them.
4. What is the difference between a monophyletic and a paraphyletic taxonomic group?

EXPERIMENT 9.1

DNA FIT FOR A MAMMOTH TASK

The largest living land animals in the world today are the elephants, members of the order Proboscidea. The proboscideans were clearly far more numerous and more diverse in the geological past than they are today. There are many dozens of species represented in the fossil record over some 60 million years but nearly all are now extinct. Among the more famous examples of extinct proboscideans are the mammoths (genus *Mammuthus*) and the mastodons (genus *Mammut*).

The woolly mammoth (*Mammuthus primigenius*) and the American mastodon (*Mummut americanum*, Figure 9.22) occupied the cold arctic and sub-arctic regions of the world. As a result, a number of deceased representatives of these extinct species were preserved in permafrost. A few specimens have been excavated and ancient DNA was extracted from their fur. Although still relatively degraded, scientists have managed to sequence the entire mitochondrial genomes (~16 500 bp) of the woolly mammoth and the American mastodon.

The DNA sequences of organisms can be analysed and used to build phylogenetic trees.

Wikimedia/Danthemann9758

Figure 9.22 ▲ Two extinct members of the order Proboscidea: (left) the woolly mammoth, and (right) the American mastodon

This approach has provided insights into evolutionary relationships between living species. Sequencing ancient DNA means that phylogenetic trees can be used to explore evolutionary relationships between living and extinct species.

Aim

To investigate the evolutionary relationships between the woolly mammoth, the American mastodon and members of living proboscideans using molecular homology

Observations and predictions

Mammoths appear in the fossil record ~4 million years ago and were widespread throughout Europe, Asia and North America. They persisted until ~4000 years ago, disappearing as the climate warmed after the last ice age. There were around 12 mammoth species, including the woolly mammoth (*Mammuthus primigenius,* Figure 9.22).

The fossils of mastodons (genus *Mammut*) date back at least 40 million years, with discoveries in Africa, Europe and North America. Like woolly mammoths, mastodons were covered by thick, shaggy fur, but mastodons were smaller and stockier and their skulls were larger and flatter than those of mammoths. The most recent species is the American mastodon, *Mummut americanum* (Figure 9.22), which died out ~10000 years ago.

Fossils suggest modern elephants originated in Africa ~4 million years ago and dispersed from there. African savannah elephants (*Loxodonta africana*) inhabit the sub-Saharan regions of Africa as far south as South Africa. They are larger than the Asian elephants (*Elephas maximus*), which occur from India in the west to Myanmar in the east. The ranges of elephants are rapidly shrinking and fragmenting due to habitat loss. Elephant species are threatened, with the Asian elephant listed as endangered.

In the Proboscidea, the distinctive tusks evolved from the 'adult' incisors, rather than from canines, as in other tusked mammals like walruses and wild boars. Teeth are one diagnostic feature used to distinguish between the many different living and extinct members of the order.

Mastodons got their name because their molars have 6–8 cone-shaped cusps that resemble nipples (mastodont = [Greek] 'nipple tooth', Figure 9.23b). African elephants also have raised diamond-shaped extensions on their molars. By analogy with modern African elephants, mastodons are believed to have been browsers that ate leaves and twigs.

In contrast, mammoths had teeth with thin, parallel ridges (Figure 9.23a) that resemble those of modern Asian elephants. It is inferred that mammoths, like Asian elephants, were grazers that preferentially ate grass.

What pattern of evolution might have given rise to the woolly mammoth, the American mastodon and the African and Asian elephants? Consider the discussion above and answer the following.

a

Alamy/Sabena Jane Blackbird

b

amanaimages/Andreas Pulwey/F1 Online

Figure 9.23 ▲
Molar teeth of (**a**) the woolly mammoth and (**b**) the American mastodon

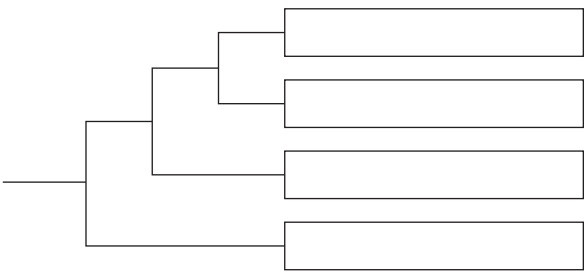


Figure 9.24 ▲
Hypothesis for the evolution of four species of the order Proboscidea

1. Suggest two physical (morphological) features that characterise animals of the order Proboscidea.
2. Which one of the four proboscideans might have evolved earliest? What evidence supports your answer?
3. Which of the four proboscideans are extinct today?
4. Divide the four proboscideans into two most similar pairs (pair 1 and pair 2) based on their fur covering.
5. Divide the four proboscideans into two most similar pairs (pair 1 and pair 2) based on their teeth morphology.
6. Consider the evidence and propose your own hypothesis for proboscidean evolution, which gave rise to the four species. Fill in the four species in the evolutionary tree (Figure 9.24).

Analysis and results

We will examine the DNA sequences from two living and two extinct proboscidean specimens (Table 9.2).

Table 9.2 Probiscidean specimens used in this study

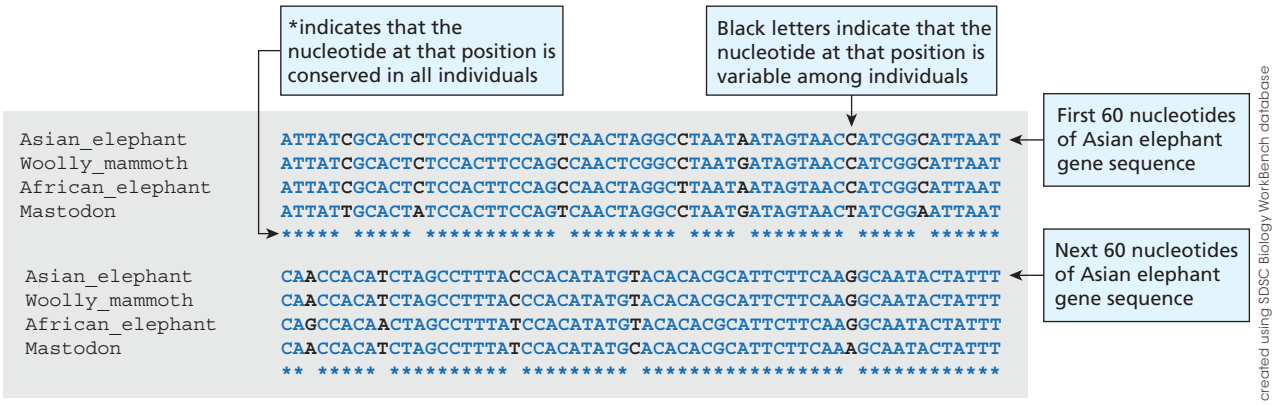
Common name	Scientific name	Sample age
Woolly mammoth	*Mammuthus primigenius*	~17 000 years
American mastodon	*Mammut americanum*	~90000 years
African (savannah) elephant	*Loxodonta africana*	Present day
Asian elephant	*Elephas maximus*	Present day

1 Figure 9.25 shows a sequence alignment of a segment of the mitochondrial ND5 gene from the four proboscidean species. Use the sequence alignment to complete Table 9.3.

2 Explain how you would determine the two most closely related species from the data in Table 9.3.

3 Complete the cladogram presented in Figure 9.26.

- **a** Identify the two most closely related species based on the data in Table 9.3. Enter their names into the appropriate boxes of the cladogram.
- **b** Identify the next most closely related species and enter its name into the appropriate box of the cladogram.
- **c** Identify the least related species and enter its name into the appropriate box of the cladogram.



▲ **Figure 9.25** Alignment of 120 nucleotides of the ND5 gene from four probiscidean species. The alignment is chunked into two blocks of 60 nucleotides each. Conserved nucleotides are shown in blue and variable nucleotides are black.

Table 9.3 Summary of pairwise comparisons between each of the four proboscidean species

Pairs	Number of nucleotide differences	% sequence conservation
Asian elephant, Woolly mammoth		
Asian elephant, African (savannah) elephant		
Asian elephant, American mastodon		
Woolly mammoth, African (savannah) elephant		
Woolly mammoth, American mastodon		
African (savannah) elephant, American mastodon		

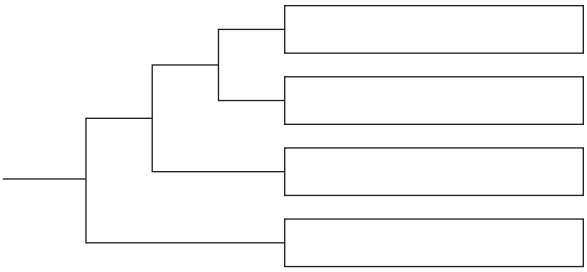


Figure 9.26 ▲
Cladogram generated from a 120-nucleotide segment of the mitochondrial ND5 gene from four species of the order Proboscidea

Discussion

Examine the cladogram you completed in Figure 9.26 and respond to the following questions.

1. Which one of the four species diverged earliest? Explain how you interpreted this from the cladogram.
2. Which species is most closely related to the Asian elephant?
3. Would a taxonomic family Elephantidae, containing only the African and Asian elephants, be a monophyletic or paraphyletic group? Explain.
4. Does the cladogram generated from the ND5 gene sequences (Figure 9.26) support or reject the hypothesis you proposed in Figure 9.24? Explain.
5. Which character – fur covering or teeth morphology – provides evolutionary inferences that agree best with the ND5 gene? Explain.
6. Is molecular homology more, less or equally useful to the character you identified in Question 5 for inferring evolutionary relationships among proboscideans? Give reasons to justify your response.

Conclusion

Write a brief conclusion stating the outcomes of your analysis and summarising your evaluation of the value of molecular homology for inferring evolutionary relationships.

Evolutionary developmental biology

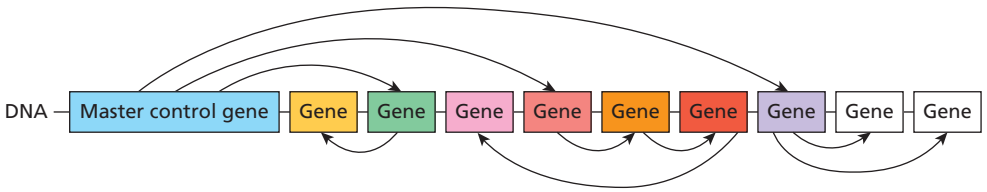
See Chapter 2 for more on structural and regulatory genes.

The human genome is 99% similar to that of the mouse in terms of the number and types of genes. How can such small differences in genomes account for the stark differences in morphology and behaviour of the two organisms?

Much of what we have explored in this chapter so far examines mutations in **structural genes** and their effects on individual proteins. However, mutations sometimes affect **regulatory genes**. The protein product of a regulatory gene serves to switch expression of structural genes on or off. Mutations also occur in regulatory sequences and other non-coding regions of DNA and these may modify the availability, timing, location or quantity of genes for expression.

Some regulatory genes, described as **master control genes**, initiate the developmental program for a particular body plan or tissue type during embryonic development. They do so by regulating whole sets of other genes for cell development either directly, or indirectly through a cascade of gene expression (Figure 9.27). In animals, a limited number of master control genes, sometimes called 'toolkit' genes, control formation of the body axes (front, rear, top and bottom), segmentation (head, thorax, abdomen), appendages (antennae, limbs), the number of body parts, and the identity and development of organs and tissues. Studies of model organisms ranging from fruit flies to humans show that essentially all animals have a common set of perhaps a few hundred master control genes. What differs between animal species is the timing, the specific locations and the degree of expression of the master control genes.

Figure 9.27 ▶
During embryonic development, the hierarchy of gene activation begins with initiation by a master control gene.

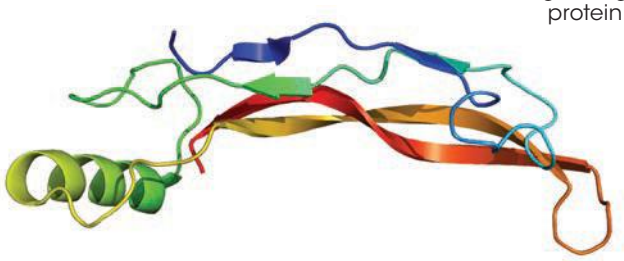


A mutation affecting a master control gene has consequences for the expression of the set of genes it controls. Modifying the expression patterns of an entire suite of genes can alter the embryonic development of an organism, ultimately bringing about changes in its morphology. Mutation events of this magnitude accelerate speciation. This is the focus of **evolutionary developmental biology**, the study of the developmental processes of different species to determine how the species are related and the genetic basis for how their developmental patterns evolved.

Master control gene *Bmp4*

We now turn to the functional role of one master control gene, *Bmp4*, and the insights it has provided to the field of evolutionary developmental biology. The product of the *Bmp4* gene is bone morphogenetic protein 4 (BMP4, Figure 9.28). The protein BMP4 is a type of **transforming growth factor**. It is a secreted signalling protein with a role in stimulating cells to divide and differentiate. By binding to cell surface receptors, BMP4 initiates a signalling cascade that activates expression of all the genes required for the cell's differentiation program. It also serves to block expression of genes that may interfere with the intended developmental program.

▼ **Figure 9.28**
BMP4, the product of the *Bmp4* gene, is a secreted signalling protein.



Structure of the BMP4 protein. Based on PyMOL rendering of PDB 1reu (RCSB PDB)

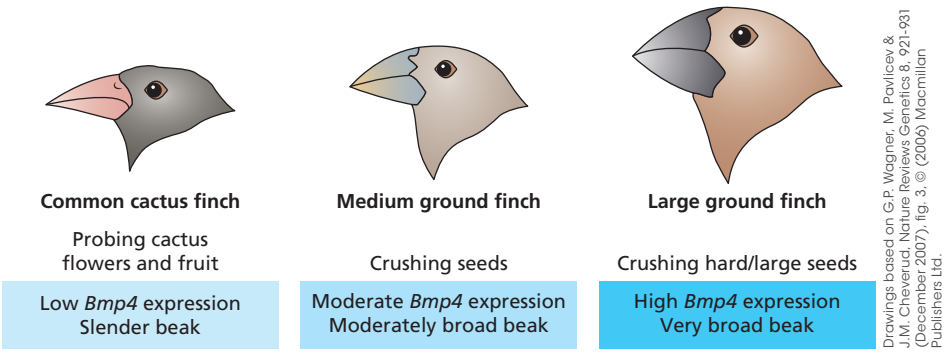
We begin our exploration of *Bmp4* by returning to the historical cradle of the theory of evolution: the Galápagos Islands and Charles Darwin's finches.

Galápagos finches and molecular genetics

Best known as a paradigm example of speciation by natural selection, Darwin's finches are 14 songbird species first collected on the Galápagos and Cocos islands by Charles Darwin and his shipmates during the voyage of the *Beagle* in 1835. The birds are between 10 and 20 cm in height and show variations in beak size and shape associated with how they obtain their food (Figure 9.29). Ground finch species have broad and deep beaks used to crush seeds. Cactus finches have long, narrow beaks to extend into the cactus flowers to feed on the pollen and nectar, or to pick the seeds out of cactus fruits. The sharpest, most slender beaks belong to the sharp beaked finch, which captures insect prey.

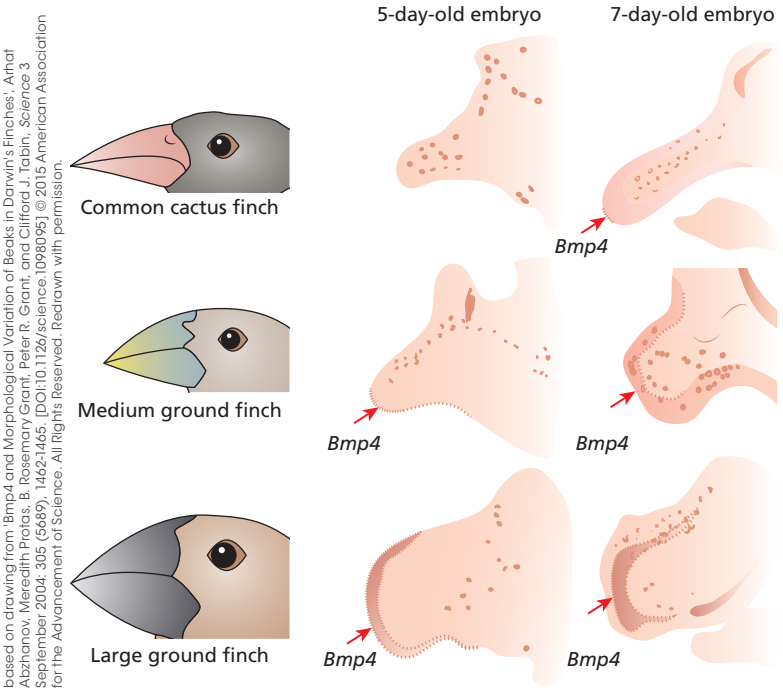
The beak shapes are determined genetically and are evident at hatching. Beak formation is controlled by cells located in a bulge of the craniofacial region of the chick embryo. Early development of the beak is marked by BMP4, the product of the *Bmp4* gene. The timing and amount of *Bmp4* gene expression in the embryo determines the size and shape of the future beak (Figure 9.30). For example, the common cactus finch (*Geospiza scandens*) has a slender beak. For this species, expression of *Bmp4* is comparatively weak and occurs relatively late during development. By contrast, the large ground finch (*Geospiza magnirostris*) has the most prominent beak. This species exhibits the earliest, most abundant and most sustained expression of *Bmp4* during development. Stronger, more persistent expression of the *Bmp4* gene apparently leads to continued enlargement of the beak. Overall, the ground finches with the more robust beaks express *Bmp4* in their craniofacial bulges at earlier stages and at higher levels than the cactus finch species with narrower beaks. Differences in the beak shapes of finch species reflect species-specific patterns of *Bmp4* expression in the embryo (Figures 9.29 and 9.30).

We are now in a position to make the conceptual link between mutations in a single regulatory gene, such as *Bmp4*, and Darwin's observations of finch evolution. The *Bmp4* gene regulates a suite of genes responsible for forming the beak. Mutations that modified the expression pattern of *Bmp4* would have resulted in new alleles entering the ancestral finch population. The new alleles resulted in alternative phenotypes for beak shape. The alleles



Drawings based on G.P. Wagner, M. Pavlicev & J.M. Cheverud, Nature Reviews Genetics 8, 921-931 (December 2007), fig. 3. © (2006) Macmillan Publishers Ltd.

Figure 9.29 ▶
Darwin's finches show various beak shapes aligned with the type of food they consume. The beak shape is determined to a large extent by expression of the *Bmp4* gene during embryonic development.



based on drawing from 'Bmp4 and Morphological Variation of Beaks in Darwin's Finches', Arhat Abzhanov, Meredith Protas, B. Rosemary Grant, Peter R. Grant, and Clifford J. Tabin, *Science* 3 September 2004: 305 (5689), 1462-1465. [DOI:10.1126/science.1098095] © 2016 American Association for the Advancement of Science. All Rights Reserved. Redrawn with permission.

Figure 9.30 ▶
Comparison of *Bmp4* gene expression in 5- and 7-day-old embryos of three finch species. The red arrows indicate detection of gene expression with probes for *Bmp4* mRNA (shaded regions).

See Chapter 10, Nelson Biology VCE Units 1 & 2 for more on Mendelian inheritance.

were transmitted by Mendelian inheritance from one generation to the next. Competition for food among finches enabled natural selection to play a part in speciation. In a population of finches with diverse beak shapes, some beaks were better suited than others to obtain nutrition from the available food sources. If the primary food source was hard, tough seeds, finches with the broadest, deepest beaks for crushing those seeds were most likely to survive. If the main food source was the nectar of cactus flowers, finches with long, narrow beaks fared best. The surviving finches were most likely to leave offspring, many of which inherited their parents' advantageous alleles for *Bmp4*. Over many generations, diverse ecological niches filtered the best suited beak shapes on each of the Galápagos Islands until the corresponding alleles were fixed in the resident finch populations. Today we see those finch populations as distinct species with distinct beak shapes.

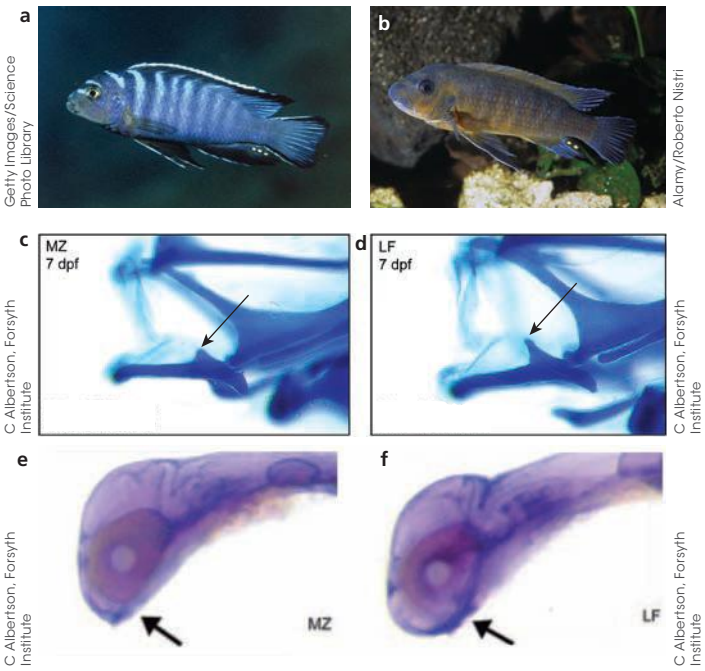
Bmp4 gene expression and cichlid fishes

Cichlids are a family of over 1600 tropical, freshwater fish found in rivers and lakes throughout the world. Cichlid fish species display a wide variety of sizes, body shapes and behaviour, and occupy a wide range of ecological niches. Notably, at least 770 species of cichlid fish are known from lakes Victoria, Malawi and Tanganyika in the East African Rift Valley. Compared with African riverine cichlids, the lake species are vastly more numerous and morphologically more diverse. The cichlid fish of these African lakes

have, however, evolved in a surprisingly short span of time. They are estimated to be about 700 000 years old in lakes Malawi and Victoria and less than 12 400 years old in Lake Tanganyika.

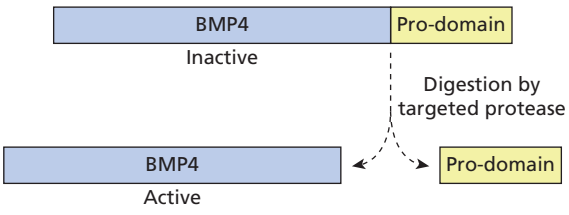
The mouth structure of fish species is adapted to the animal's feeding strategy. Among African cichlid fish, two species that typify different feeding styles are *Metriaclima zebra* and *Labeotropheus fuelleborni* (Figure 9.31). *M. zebra* (Figure 9.31a) has a protruding lower jaw with a comparatively weak bite adapted for sucking plankton from the water column. By contrast, *L. fuelleborni* (Figure 9.31b) has a reduced lower jaw that facilitates a stronger bite for gnawing algae off rock surfaces. Differences in development of the lower jaw are evident in the larvae of these fish species by the seventh day after fertilisation. The lower jaw displays a protrusion called 'Meckel's cartilage' (indicated by arrow, Figures 9.31c,d). When fully formed, this protrusion attaches to muscles that contract to shut the jaw. Meckel's cartilage is larger in *L. fuelleborni* and it will ultimately anchor more muscle, giving this fish its stronger bite.

The differences in jaw structure correspond to differing expression patterns of the *Bmp4* gene in the two species. The *Bmp4* gene is expressed in an arc of cells that will ultimately form the lower jaw. In *M. zebra*, expression of the *Bmp4* gene is restricted to the tip of the arc (Figure 9.31e). In *L. fuelleborni*, it is distributed throughout the arc (Figure 9.31f). As with Darwin's finches, increased expression of *Bmp4* is associated with stronger biting mouth parts in cichlid fish.



◀ Figure 9.31
Adult and larvae of two African cichlid species. (**a**) Adult and (**c**, **e**) larva of *Metriaclima zebra* (MZ); (**b**) adult and (**d**, **f**) larva of *Labeotropheus fuelleborni* (LF). The arrows in (**c**) and (**d**) indicate Meckel's cartilage of the developing lower jaw. (7 dpf = 7 days post-fertilisation) The arrows in (**e**) and (**f**) indicate the sites of *Bmp4* gene expression (highlighted in blue).

When amino acid sequences of the protein BMP4 are compared among cichlid fish, those of African lake species are five times more variable than those of distant riverine species. The greater variability suggests that the amino acids in BMP4 changed at an accelerated rate in African lake species. BMP4 is produced as a large inactive protein capped by an additional segment called the 'pro-domain'. Protease digestion detaches the pro-domain, releasing the mature, active form of the BMP4 protein (Figure 9.32). The efficiency of digestion, as well as the life span of the mature BMP4, is determined by the pro-domain. In cichlid fish, amino acid variations in BMP4 are restricted to the pro-domain region and none occur in the functional



▲ Figure 9.32
Active BMP4 is released by slicing off the pro-domain.

BMP4 portion. Mutations in the *Bmp4* gene therefore have not affected the master control function of the BMP4 protein. Instead, they alter the way the active BMP4 protein is regulated, modifying its location, timing and persistence.

Lessons from *Bmp4*

The *Bmp4* gene provides a unique insight into evolution. It complements the classical view of evolution driven by accumulated mutations in many genes. *Bmp4* demonstrates that explosive rates of adaptive radiation can be achieved by modifying just a single gene. Altering a master control gene that coordinates the expression of many other genes results in significant morphological change. Modifying the timing, the intensity and the location of a master control gene creates countless possibilities for novel body structures. Morphological diversity among species is therefore accomplished with the minimum of change to the genome.

RECALL

- Master control genes initiate developmental programs during embryonic development by regulating whole sets of other genes.
- BMP4, the product of the *Bmp4* gene, is a secreted signalling protein that switches on expression of many genes required for cellular differentiation.
- Differences in beak size of Galápagos finches and in jaw sizes of cichlid fish correspond to differences in expression patterns for BMP4, rather than any change in the function of BMP4.

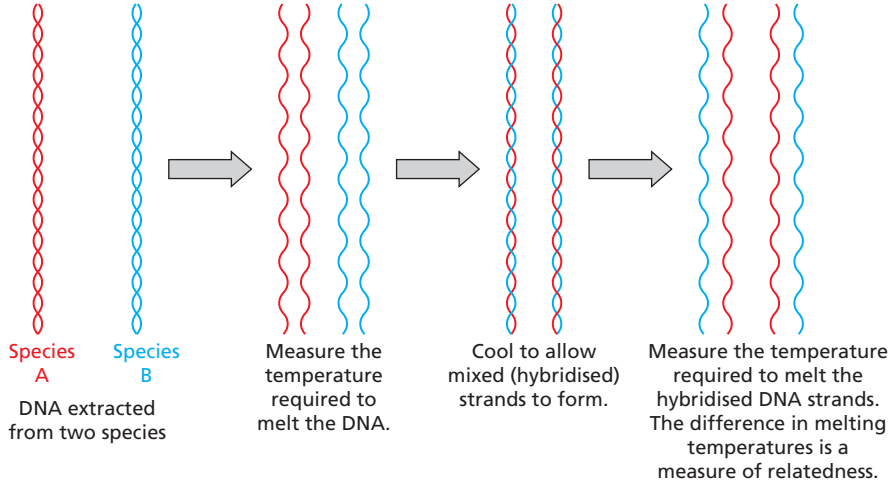
RECAP 9.7

1. In what ways can the patterns of expression for a master control gene differ between different species?
2. Describe how expression of the master control gene *Bmp4* influences beak development in Galápagos finches.
3. Relate the process of evolution by natural selection to expression patterns of *Bmp4* in Galápagos finches.
4. Describe how expression of the master control gene *Bmp4* affects jaw development in cichlid fishes.

DNA–DNA hybridisation

Extensive speciation often proceeds with only modest changes to the genome. Genes and tracts of functional DNA in genomes therefore tend to be conserved during evolution. Extending beyond homology of individual genes, it is pertinent to assess the homology of whole genomes. **DNA–DNA hybridisation** is a method for measuring the homology between two samples of DNA. Applied to genomic DNA from two species, it is used to determine the degree of relatedness between the genomes of those species.

The technique exploits the capacity for hydrogen bonds between complementary strands of DNA to break upon heating and reform upon cooling (Figure 9.33). To facilitate the experiment, the genomic DNA is also cut into manageable fragments. First, the **melting temperature** of the DNA from the principal species to be investigated is measured. This is the temperature at which 50% of the double-stranded DNA has separated into single strands. Next, this DNA is then combined with the DNA from another species with which it is to be compared. The combined sample is heated to ensure the double-stranded DNA is completely melted. Upon cooling,

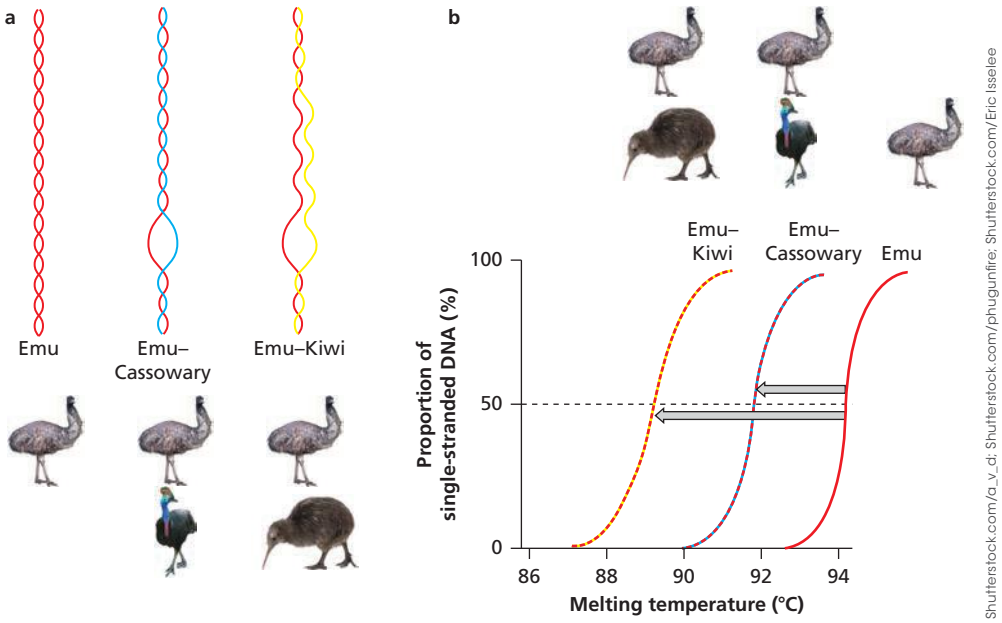


◀ Figure 9.33
The steps in a DNA–DNA hybridisation experiment

complementary single strands from both species **hybridise** to form mixed double strands. Finally, the melting temperature of the hybridised DNA is measured. The difference in melting temperatures between the original DNA and the hybridised DNA provides a measure of the similarity of the two species' DNA. The smaller the difference, the more similar the DNA from the two species.

Interpreting relatedness and reconstructing evolution

The premise underlying the technique is that the more closely related two species are, the more similar their genomes will be. In principle, regions of identical sequences in the genomes of both species hybridise perfectly (Figure 9.33). Melting these sequences requires as much heat as either species' purified, unmixed DNA. However, regions of DNA that have undergone substantial mutation in one lineage or another result in discordant sequences between the two genomes. Upon hybridisation, these regions form improper double strands weakened by mismatched base pairs. These mismatched regions are already dissociated to some degree so less heat is required to melt, or separate, the strands (Figure 9.34a). The greater the level of mismatch, the lower the melting temperature of the hybridised strands (Figure 9.34b).



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◀ Figure 9.34
The relationship between the extent of hybridisation between the DNA of two species and the melting temperature of the hybridised DNA: (**a**) The DNA of the emu hybridises more extensively with that of the cassowary than it does with the DNA of the kiwi. (**b**) The hybridised DNA of the emu and the cassowary melts at a higher temperature than the hybridised DNA of the emu and kiwi. The melting temperatures of the hybridised DNA indicate that the emu is more closely related to the cassowary than to the kiwi.

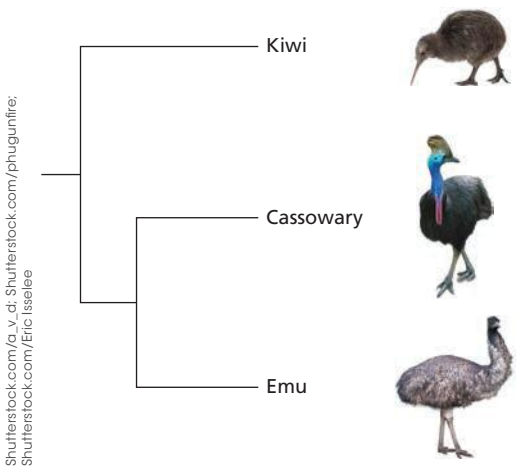


Figure 9.35 ▲
A cladogram based on the results of the DNA–DNA hybridisation experiment shown in Figure 9.34

DNA–DNA hybridisation data give a proxy measure of relatedness between two species. Pairwise comparisons between species provide a single value that summarises the differences between their genomic DNA. In this way, the technique emulates distance methods for constructing evolutionary relationships. As with the pairwise comparisons discussed earlier, DNA–DNA hybridisation values can be configured into a phylogenetic tree (Figure 9.35).

Comparative genomics

DNA–DNA hybridisation measures differences between whole genomes, but comparative genomics drills down to the detail. The field of **comparative genomics** is concerned with comparing DNA sequences, gene arrangements and chromosome structure between different species. The first genomes sequenced were those of bacteria species, published in 1995. This was followed by those of the first eukaryote, yeast (*Saccharomyces cerevisiae*, 1996), the nematode worm (*Caenorhabditis elegans*, 1998), the fruit fly (*Drosophila melanogaster*, 2000) and, not least, the draft human genome (2001). Within a few more years, the number of published genomes expanded rapidly. In the past decade, advances in DNA sequencing and bioinformatics have provided unparalleled opportunities to sequence and compare genomes.

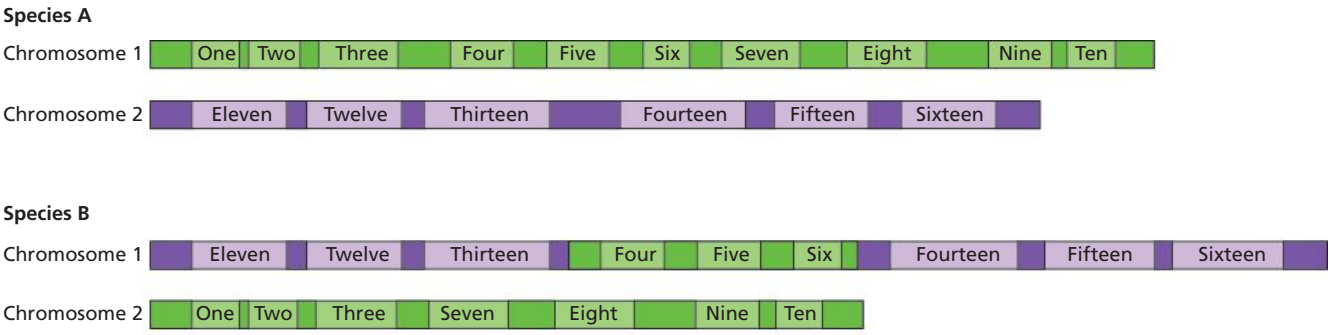
Comparison with a published genome makes it possible to identify unknown genes in a freshly sequenced genome. Identified sets of genes can be diagrammed into metabolic pathways or other operational relationships that describe how the freshly sequenced organism functions. Comparative genomics also exposes how similar genomes are. This provides insights into functionally indispensable regions of both coding and non-coding DNA. Comparing the genomes of different species also reveals mutations that are unique to one lineage or another and contribute to differences in anatomy, biochemistry, physiology or behaviour. In essence, it provides a molecular record of the evolutionary past that is shared by different species or that is unique to distinct species.

Genome composition and organisation reveals shared evolutionary history

Homologous genes, or homologues, in the genome of one species can be identified in the genome of another species. There are two types of homologues. **Orthologues** are homologues that have the same genetic locus in different species. Orthologies therefore evolved by speciation from a common ancestral gene. **Paralogues** are genes that are related by gene duplication and subsequent mutation. During evolution, gene families of paralogues may expand by multiple duplications in some lineages but not in others. Comparative genomics also reveals instances of horizontal gene transfer.

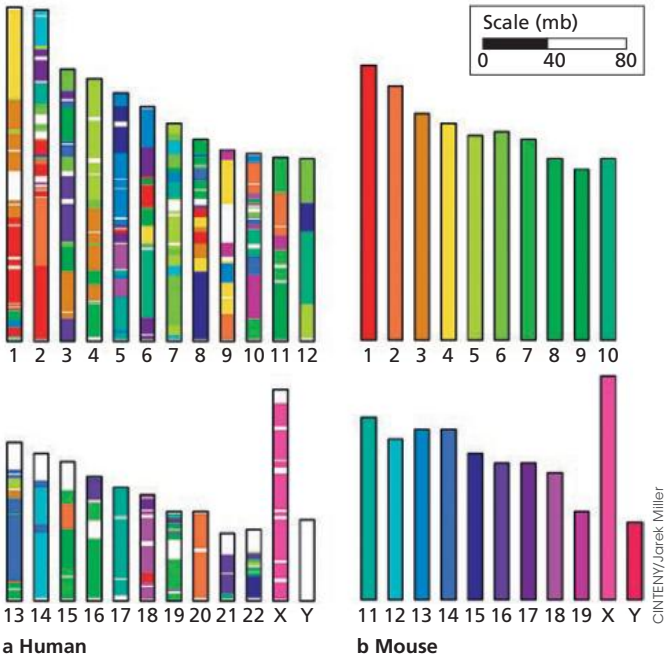
Homologues are characterised by sequence similarity and by their arrangement within the genome. During evolution, chromosomes are broken, rearranged and re-joined (Figure 9.36). When a chromosome fragment is relocated within the genome, the order of the gene loci in the fragment is retained. If the order of gene loci within a section of chromosome is conserved in two different species, the corresponding sections are said to share **synteny** (Figure 9.36). Synteny indicates that the arrangement of the gene loci was inherited from the common ancestor of the two species. Maps of synteny can be drawn to show the relationships in structure between the chromosomes of different organisms (Figure 9.37).

We are in a position to put into perspective the conundrum posed at the beginning of this section. Examination of the human and mouse genomes provides insights into the evolution of these two seemingly disparate species. Significantly, 99% of mouse genes are homologues of human genes, and 99% of human genes



▲ **Figure 9.36** Although the chromosomes of the two species have been rearranged, the order of the gene loci (numbered) is conserved. The green and purple coloured chromosomal blocks represent regions of synteny shared by both species.

are homologues of mouse genes. Of these, 80% are orthologues with a shared evolutionary ancestry. Some differences emerge in certain functional genes. For example, the mouse has more paralogues related to olfaction, indicating it has evolved a more acute sense of smell than humans. More than 90% of the mouse and human genomes can be divided into regions of synteny (Figure 9.37). Despite the overall similarities in their genomes, the two species have evolved immense differences in morphology and behaviour. This is attributed to altered master control genes that have substantially modified the patterns of expression of similar sets of genes during evolution.



◀ **Figure 9.37** Synteny map of human and mouse genomes. The autosomes and sex chromosomes are labelled for each organism. Corresponding colours in both genomes represent regions in which the order of the gene loci are the same in both genomes.

RECALL

- DNA–DNA hybridisation is a method for obtaining a measure of how similar genomes are between species.
- Comparative genomics provides a more detailed account of how similar genomes are between different species by comparing their DNA sequences, gene arrangements and chromosome structures.

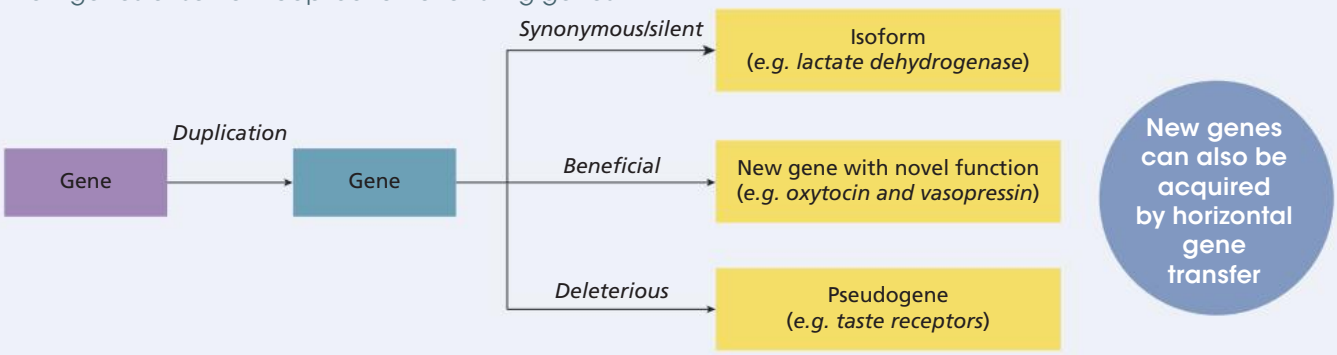
RECAP 9.8

1. Define 'hybridise'.
2. Describe how DNA–DNA hybridisation works and how this can be used to infer evolutionary distance between species.
3. What is the difference between orthologues and paralogues?
4. What does shared synteny mean and what does it reveal about evolution between different species?

CONCEPT SUMMARY

Evolution of genes

New genes arise from duplication of existing genes.



Effects of mutations on genes

- Amino acids critical for a protein's structure and function, such as the amino acids needed by enzymes for binding substrates or carrying out reactions in the active site, are often conserved.
- Surface and core amino acids can often be substituted without altering the protein's shape or function if the substitution is missense.

Molecular homology

Molecular homology analyses the similarity of patterns in the nucleotide sequences of homologous genes or the amino acid sequences of polypeptides from different organisms as evidence for a common evolutionary origin and an indication of evolutionary distance. It is possible because:

- DNA and polypeptides are very long, unbranched polymers
- bioinformatics has enabled large volumes of biological data to be processed (e.g. sequence alignment).

Mitochondrial DNA (mtRNA)

- A double-stranded, circular chromosome (a stable DNA structure) with genes for cellular respiration, rRNA and tRNA
- Convenient for comparison between eukaryotic organisms; DNA 'barcodes' in mitochondrial genes are unique to each species
- The mitochondrial genome is compact and exists in thousands of copies per cell making it easy to extract and manipulate
- Subject to high mutation rates due to oxygen radicals generated by the electron transport chain, creating differences between species
- Maternally inherited and allows tracing of ancestry through the female line

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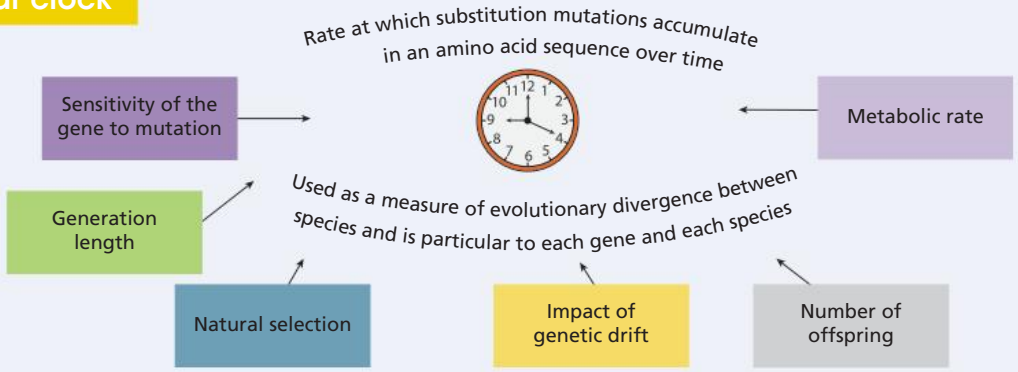
Molecular clock



Phylogeny

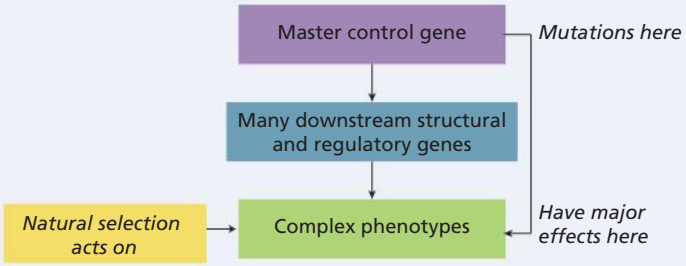
Pairwise comparisons give a measure of molecular homology and are a useful factor in the distance method of determining evolutionary distance between organisms.

Phylogenetic trees

- Unrooted: Evolutionary distance without the direction of evolution
- Rooted: Show passage of time and ancestral history with nodes representing common ancestors
  - Cladogram: Hypothesis-driven, based on clades
    - Systematics categorises organisms according to their phylogeny:
    - involves taxonomy, the system of scientific conventions for naming and classifying organisms
    - aims to define monophyletic (rather than paraphyletic) taxonomic groups
    - modified and updated based on new evidence.
  - Phylogram: Quantitative, with a scale bar

Evolutionary developmental biology

- The study of the developmental processes of different species to determine how the species are related and the genetic basis for how their developmental patterns evolved
- Can involve the study of master control genes because mutations in these genes can affect the expression of many downstream genes and have major effects on phenotypes



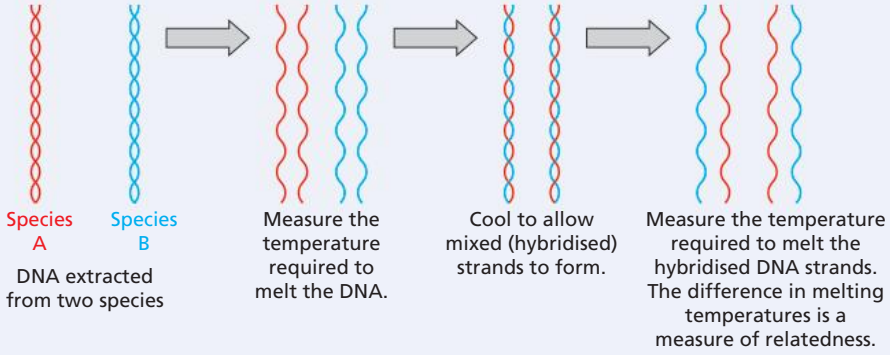
Master control gene *Bmp4*

Bone morphogenetic protein 4

This is a transforming growth factor – a secreted signalling protein that stimulates cell division and differentiation. It stimulates expression of many genes and inhibits expression of many others, so that only the appropriate genes are expressed for the particular developmental program. Differences in the timing, location and abundance of *Bmp4* expression result in species-specific beak morphological differences in Galapagos finches. This is because mutations in *Bmp4* resulted in variation of the phenotypes upon which natural selection could act. Another example of this *Bmp4* effect are the biting mouthparts of cichlid fish.

DNA–DNA hybridisation

The difference in melting temperatures between the original DNA and the hybridised DNA provides a measure of the similarity of the DNA of the two species. The smaller the difference, the more similar is their DNA.



Comparative genomics

Comparative genomics compares DNA sequences, gene arrangements and chromosome structure between different species. Homologues (orthologues and paralogues) in the genome of one species can be identified in the genome of another species and may occur in chromosomal regions of synteny between the species.

CHAPTER GLOSSARY

amino acid sequence see **polypeptide sequence**

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

clade a branch of a cladogram that comprises a common ancestor and all of its descendants

cladogram a rooted phylogenetic tree that depicts a hypothesis about evolution

comparative genomics a field of biology concerned with comparing DNA sequences, gene arrangements and chromosome structure between different species

conjugation the union between two bacterial cells enabling the direct transfer of genetic material from one to the other

conserved refers to amino acids of polypeptide sequences or nucleotides of DNA sequences that remain unchanged during the course of evolution

distance method a strategy for inferring evolutionary relationships based on patterns of evolutionary distance between pairs of species

divergent evolution evolution in which a single ancestral species diverges, or splits, to eventually become two or more descendant species

DNA barcode a nucleotide sequence from a representative gene that uniquely identifies a particular species, subspecies or variety of organism

DNA–DNA hybridisation a technique used to determine how similar the DNA of two species is

DNA sequence the order of the four possible nucleotides in a segment of DNA

evolutionary developmental biology the study of the evolution of species and their developmental processes by comparing the embryonic development of different organisms

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

gene duplication generating an extra copy of a gene within a genome as a result of mutational duplication of a chromosomal segment or because of polyploidy

gene sequence the order of the four possible nucleotides in a gene

homologous in reference to genes or polypeptides, having sequences that are similar and indicate a shared evolutionary ancestry

homologues in reference to genes, genes that have similar nucleotide sequences; usually refers to genes from different species but may also refer to genes from the same species

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

hybridise form double strands with nucleic acids derived from different sources

isoenzymes enzymes that carry out the same biological reaction but are the products of different genes at different loci in the genome

lineage in evolution, the line of descendant species that evolve from an ancestral species

locus the specific location of a gene on a chromosome

master control gene a gene that coordinates expression of many other genes to control body patterning and cell differentiation during embryonic development; also referred to as 'master gene', 'master regulator', 'master switch' or 'toolkit gene'

maternally inherited describes a genotype and phenotype that are transmitted entirely from the female parent to the offspring

melting temperature in DNA–DNA hybridisation, the temperature at which 50% of double-stranded DNA in a sample has separated into single strands

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

nucleotide sequence the order of the four possible nucleotides in a segment of RNA or DNA. See also DNA sequence and gene sequence.

orthologues similar genes in different species that evolved by speciation from a common ancestral gene

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

paralogues similar genes in the same genome that evolved by gene duplication and subsequent mutation

paraphyletic describes a taxonomic group that includes some, but not all, of the species that descended from a common ancestor

phyletic evolution successive evolution of one species into another within a single evolutionary lineage

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny evolutionary relationships that exist between species, often expressed as a tree-like diagram

phylogram a type of rooted tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

polypeptide sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide

pseudogene an obsolete gene for which there are no functional alleles, a consequence of inactivation by mutation during evolution

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

rooted tree a phylogenetic tree that depicts the ancestors and descendants through the course of evolution of a group of organisms

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

speciation the evolution of one or more new species from an ancestral species

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

synteny the conserved order of gene loci in a section of chromosome in two different species

systematics the branch of biology concerned with categorising organisms according to their evolutionary history

taxonomy a system of scientific conventions for naming and classifying organisms

transforming growth factor a secreted signalling protein with a role in stimulating cells to divide and differentiate

unrooted tree a phylogenetic tree that shows only the degree of relatedness between organisms and makes no reference to ancestry

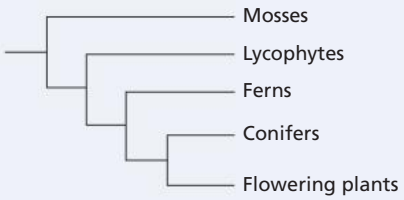
CHAPTER REVIEW QUESTIONS

Remembering

1 A phylogram shows patterns of divergent and phyletic evolution. Explain this statement.
2 What would it imply if the sequences of homologous genes from two organisms were 100% conserved?
3 Describe how DNA–DNA hybridisation data can be used as a distance method for inferring evolution.

Understanding

4 Explain how it is possible that evolution of new species can be accelerated by changes to a single gene.
5 The cladogram in Figure 9.38 represents evolutionary relationships among groups of land plants.
   a Which of the groups diverged the earliest?
   b Which two groups are most closely related?
   c A biologist proposed recognising a taxonomic group of 'non-flowering plants' consisting of mosses, lycophytes, ferns and conifers. What is your recommendation for that proposal? Justify your recommendation.



▲ **Figure 9.38** Cladogram representing evolution of land plants

Applying

6 Amylase is an enzyme that digests starch. Two forms of amylase are found in humans, one that is secreted in saliva, the other secreted from the pancreas. The amylases are coded by two separate but closely located genes on chromosome 1.
   a Explain how this situation has arisen.
   b In populations with a history of farming starchy grains, most individuals have two or more copies of the salivary amylase gene. Every gene is expressed. How has this situation come about?
   c Draw an annotated diagram of the genes on chromosome 1 depicting your answers to parts **a** and **b**.
7 The FTO gene, a gene associated with obesity in humans, is widespread in vertebrate animals (fish, birds, reptiles and mammals), suggesting it emerged early in vertebrate evolution. FTO is absent in insects, worms and fungi but it is found in a few genera of single-celled algae. What might explain the peculiar distribution of the FTO gene across these organisms?

Analysing

8 Consider the data in Figure 9.16 on page 307.

- **a** Why does the rate of the molecular clock vary for different genes?
- **b** If the fibrinopeptide sequences of two species differed by 80 nucleotides out of every hundred, what would be the estimated time since the two species diverged?
- **c** Based on the outcome for part **b**, how many nucleotides out of every hundred would you predict to differ between the haemoglobin sequences of the same two species?
- **d** What other evidence could be used to calibrate these molecular clocks?

9 Molecular biologists have genetically manipulated chicken embryos so that *Bmp4* expression begins earlier and lasts longer during development.

- **a** What do you predict would be the outcome for the bird?
- **b** How else could you genetically manipulate *Bmp4* expression to achieve a similar outcome?
- **c** Do these changes affect the function of the BMP4 protein? Explain.

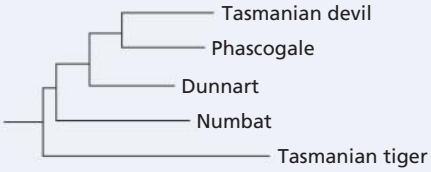
10 Consult the synteny map of the chicken and zebra finch chromosomes in Figure 9.39.

- **a** What do the coloured bars common to the chromosomes of both species represent?
- **b** How is chromosome 1 of the chicken related to the zebra finch chromosomes?
- **c** What might have occurred during evolution to result in this situation?
- **d** How does the map provide evidence in support of your conclusion in part **c**?

Figure 9.39 ▶
Synteny map for chromosome 1 of the chicken genome (top) and two chromosomes of the zebra finch genome (bottom)

11 The last known Tasmanian tiger (thylacine) died in the Hobart Zoo in 1936 and the species is now recognised as extinct. Hypothesising a close evolutionary relationship between the Tasmanian devil and the Tasmanian tiger, scientists sought to explore the phylogeny of Australian carnivorous marsupials using DNA. They sequenced the mitochondrial genome from a pelt of the Tasmanian tiger and from tissues of living marsupials. They compared the mitochondrial genomes and constructed the phylogram shown in Figure 9.40.

- **a** Why did the scientists choose to sequence the mitochondrial genome rather than the nuclear genome?
- **b** What do the branch lengths represent in the phylogram?
- **c** What has happened during evolution to result in the different branch lengths?
- **d** Which is the most closely related species to the Tasmanian devil?
- **e** Was the scientists' hypothesis supported or rejected?
- **f** Decide if the following taxonomic families are monophyletic or not and give reasons to justify your decision:
  - **i** Family Dasyuridae, which includes the Tasmanian devil, phascogale and dunnart
  - **ii** Family Thylacinidae, which includes the Tasmanian tiger and numbat.



▲ Figure 9.40
Phylogram of Australian marsupial carnivores based on mitochondrial genome sequences

12 Consider the sequence alignment for the amino acids in two neighbouring segments of the lysozyme polypeptide (Figure 9.41). The ninth amino acid in the alignment (D, aspartic acid) is involved in the enzyme's catalysis.

- **a** Explain what the alignment shows about the amino acids in the two adjacent regions of the protein. What might be the reason for this observation?
- **b** Imagine the human lysozyme gene was duplicated and the new copy mutated so the codon for S (serine) in the sequence alignment became a STOP codon. What do you predict would happen to the codons for the rest of the copied lysozyme gene over many generations? Explain your reasoning.

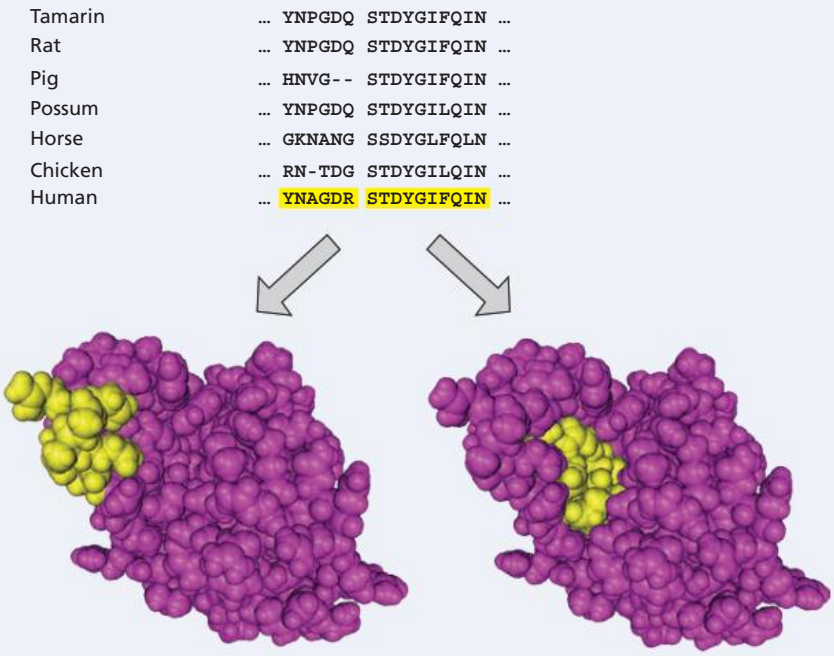
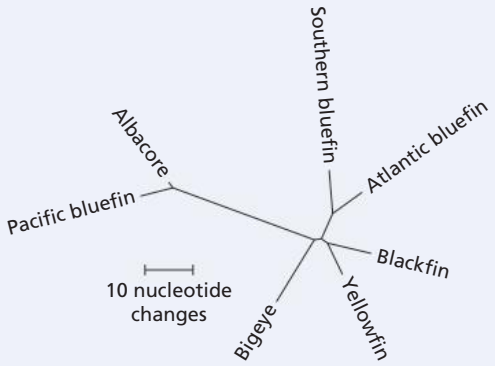


Figure 9.41
An alignment of the amino acids in two neighbouring segments of lysozyme from various organisms. The amino acids are represented by a single letter code (Y = tyrosine, N = asparagine, etc). Two blocks highlighted in the human lysozyme sequence correspond to adjacent regions of the lysozyme protein.

13 Consider the unrooted tree presented in Figure 9.42.

- **a** The *CYTB* gene in tuna species comprises 1141 base pairs. Provide an estimate of the degree of sequence conservation (%) for the *CYTB* gene between the blackfin tuna and
  - **i** yellowfin tuna
  - **ii** bigeye tuna
  - **iii** Pacific bluefin tuna.
- **b** The name 'bluefin' shared by three species suggests they are closely related. What does the *CYTB* gene indicate about the three 'bluefin' species?
- **c** Would you consider the *CYTB* gene suitable for generating DNA barcodes to screen tuna sold at market? Give at least two reasons in support of your answer.

Figure 9.42
An unrooted phylogenetic tree constructed from the *CYTB* gene sequences of seven tuna species



Evaluating

14 You are invited to construct a phylogenetic tree to represent the relationships between *Eucalyptus* species. You have the resources to copy and sequence the genes listed in Table 9.4.

Table 9.4 Genes found in *Eucalyptus* and some of their characteristics

Gene	Genomic location	Approximate size (bp)	Notes
HIS4	Nucleus	500	Codes for a subunit of the proteins around which DNA is wound to form chromatin inside the nucleus
GAPDH	Nucleus	2900	Codes for an enzyme involved in glycolysis
RBCL	Chloroplast	1400	Codes for a subunit of Rubisco, an enzyme involved in the light-independent reactions of photosynthesis
ATP8	Mitochondrion	450	Codes for a subunit of mitochondrial ATP synthase
COX1	Mitochondrion	700	Codes for a protein involved in the mitochondrial electron transport chain
ITS1	Nucleus	200	Transcribed but untranslated region of DNA situated between two rRNA genes; may occur in thousands of copies
HMGB1	Nucleus	4000	Non-functional pseudogene

Consider the merits of using any one of these genes for investigating evolutionary relationships.

- **a** Discuss which features of a gene you would consider important in deciding whether or not to use it for phylogenetic studies.
- **b** Which gene would you choose to explore the phylogenetic relationships of species within the genus *Eucalyptus*? Give reasons to support your choice.
- **c** Which would you choose to explore the phylogenetic relationships between genus *Eucalyptus* and other representatives of kingdom Plantae? Give reasons to support your choice.
- **d** In each of **b** and **c**, would you choose to represent the evolutionary relationships as a rooted or an unrooted phylogenetic tree, and why?

15 Defend or refute the statement that 'Gene phylogeny is the same as species phylogeny', and outline the arguments in support of your position.

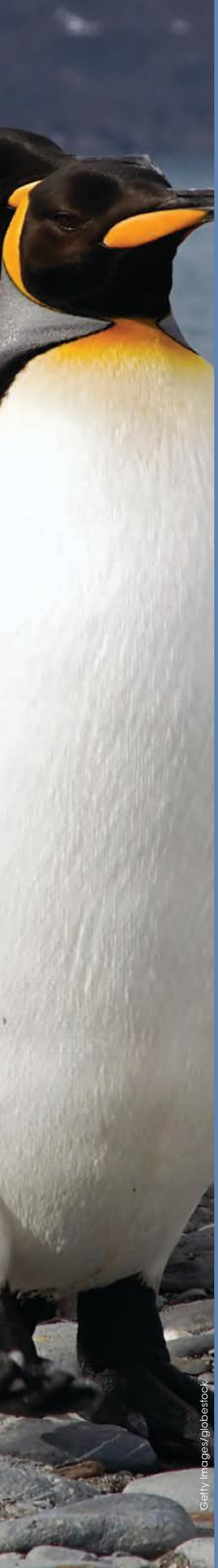
Creating

16 Consider the gene sequences shown in Table 9.5.

Table 9.5 A 100-nucleotide segment of the *COX1* gene from four bird species

Species name	Common name	Locality	DNA sequence
Eclectus roratus	Eclectus parrot	Northern Australia, New Guinea	CTTCGGCGCATGAGCTGGCATAATC GGTACCGCCCTAAGCCTACTTATCCG CGCAGAACTAGGCCAACCTGGAAC CCTACTAGGAGACGACCAAATCTAC
Ciconia boyciana	Oriental stork	China, Russia	CTTCGGCGCATGAGCTGGCATAGTTG GAACCGCCCTTAGCCTTCTTATTCG CGCAGAACTTGGTCAACCAGGAAC CCTCCTAGGAGACGACCAAATCTAC
Agapornis roseicollis	Rosy-faced lovebird	South-western Africa	CTTCGGCGCATGAGCTGGCATGATTG GTACATCCCTAAGCCTCCTCATCCGCG CAGAACTAGGCCAGCCAGGAACCCT GCTAGGAGACGACCAAATCTAC
Rhynchopsitta terrisi	Maroon-fronted parrot	Mexico	CTTCGGCGCATGAGCAGGCATGGTCG GTACCGCCCTAAGCTTGCTTATTCGTGCA GAGCTCGGTCAACCAGGGACCCTCCTAG GAGACGACCAGATCTAC

- **a** Propose a series of operations for using the sequences to infer the evolutionary relationships between the four organisms. Depict your proposal as a flow chart.
- **b** Construct an unrooted phylogenetic tree from the sequences and outline your rationale for the length and arrangement of the branches.



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CHAPTER 10
THE HUMAN ANIMAL

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

KEY KNOWLEDGE

Human change over time

- shared characteristics that define primates, hominoids and hominins
- major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo* including structural, functional and cognitive changes and the consequences for cultural evolution
- the human fossil record as an example of a classification scheme that is open to interpretations that are contested, refined or replaced when new evidence challenges them or when a new model has greater explanatory power, including whether *Homo sapiens* and *Homo neanderthalensis* interbred and the placement of *Homo denisovans* into the *Homo* evolutionary tree.

KEY SCIENCE SKILLS

Draw evidence-based conclusions

- Draw conclusions consistent with evidence and relevant to the question under investigation

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Figure 10.1 ▲
What has occurred during evolution to enable *Homo sapiens* to rise to prominence?

Imagine you could weigh all the living land vertebrates – that is, mammals, marsupials, birds, reptiles and amphibians. Then you could divide them into three groups: all the wild animals, all the humans and all the domesticated animals that humans depend upon. What proportion of the total weight would you allocate to each? The ratio estimated for wild animals : humans : domesticated animals is 3 : 30 : 67. Notwithstanding the limitations of making such a calculation, the result underscores what is intuitive to most of us. *Homo sapiens* is the dominant vertebrate animal on the planet, and humanity's activities have a profound impact on other species.

As biologists we cannot assume humans are special, separate from nature, on Earth because of some divine plan. Rather, *Homo sapiens* is one species that has evolved under the same constraints and biological principles that apply to all species. In this chapter we will explore the various lines of evidence that argue for the evolutionary history of *Homo sapiens* and the features that have emerged to make the species in some ways unique.

Our understanding of human evolution is incomplete, sometimes contentious, and continually reshaped as new evidence arises. The evidence may come from new discoveries that are made by utilising existing investigative methods or from the development and application of novel methods. Consequently, the study of human evolution serves as an illustrative model for how biologists infer evolutionary relationships between organisms and assess how closely related one group of organisms is to another. This chapter therefore discusses what has been elucidated about human evolution at this point in time and addresses some of the gaps in our understanding.

Throughout the chapter, our overarching question is: what course has evolution taken to enable this one species, *H. sapiens*, to rise to such prominence (Figure 10.1)?

The taxonomy of modern humans

See Chapter 9 for more on taxonomy. See Chapter 5, Nelson Biology VCE Units 1 & 2 *to revise the features that identify chordates and mammals.*

Humans are animals and, like all other animals, they can be classified within taxonomic schemes. As a tool of systematics, the taxonomy reflects a hypothesis for the evolutionary relationships between humans and other organisms. Within formal taxonomic schemes, modern humans are members of the phylum Chordata and the class Mammalia. Among mammals, they are classified within the **infraorder** Eutheria, or placental mammals. Our review focuses on classification at the lower taxonomic ranks. What does the classification of modern humans indicate about our evolutionary descent? Which animals are our most closely related living species? And how has that perspective changed over time?

Humans are primates

When we examine and compare anatomical features of humans with those of other living mammals, it is apparent that there are certain animals with which humans share an affinity. Intuitively, humans bear similarities to apes, as well as to monkeys and an even broader group: the order Primata, or 'primates'. It was these similarities that inspired Charles Darwin to study primates in the later part of his career, drawing conclusions about human evolution that were publicly derided in his day but are supported today by the evidence collected by modern science.

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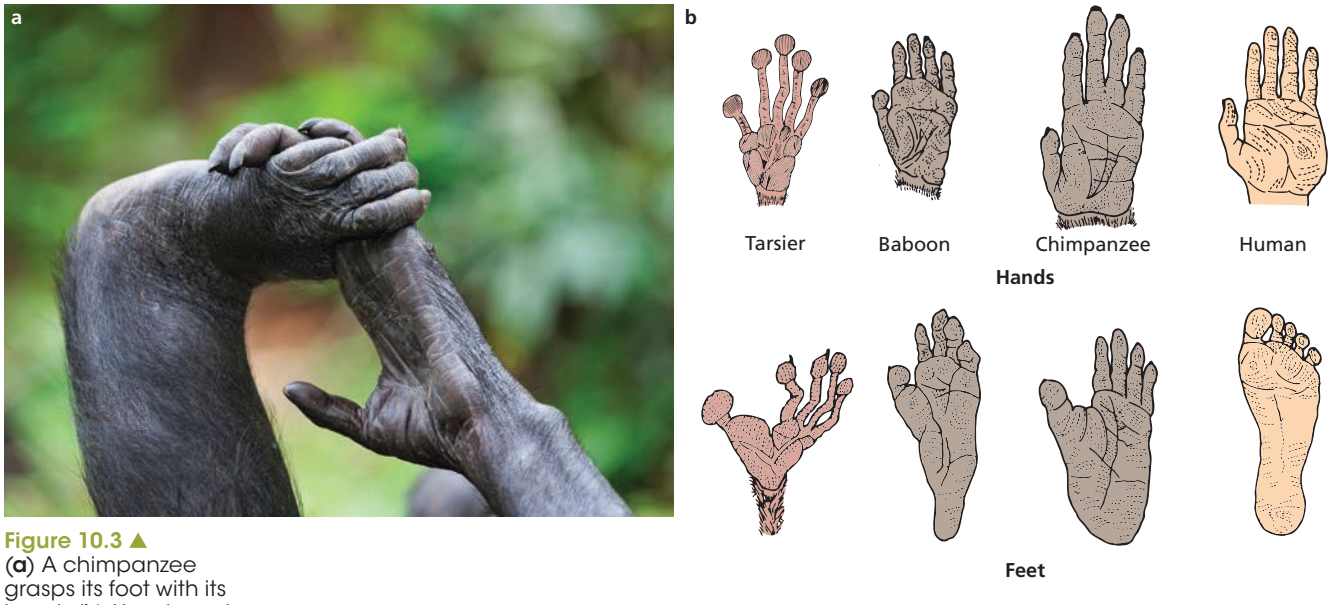
◀ Figure 10.2
Representative primates: (**a**) crowned lemur; (**b**) pygmy loris; (**c**) Siau Island tarsier; (**d**) a New World monkey, the Colombian night monkey; (**e**) an Old World monkey, the mandrill; and (**f**) an ape, the western gorilla

The primates comprise animals referred to as lemurs, lorises, tarsiers, New World monkeys, Old World monkeys and apes, as well as modern humans (Figure 10.2). What features unite this group?

Features that characterise primates

The primates share a suite of features that distinguish them from other mammals. First and foremost, primates have hands and feet that bear five digits that include an innermost, opposable digit (Figures 10.2 and 10.3). These are the thumb of the hand and the **hallux** (enlarged toe) of the foot. The hands and feet are **prehensile** in that they curl. The tips of the digits are also endowed with sensitive touch receptors. In contrast to non-primates, the digits are typically furnished with flattened nails instead of claws. These adaptations enable primates to grasp, climb, or manipulate objects.

Primates depend heavily on their sense of vision and this is reinforced by distinctive features (Figure 10.2). They have forward-facing eyes enabling **stereoscopic** (3D) vision. This is presumably an adaptation for an **arboreal** lifestyle as primates tend to live mostly in trees. If an animal is to leap from tree to tree, judging distance is critically important. Most primates also have retinas that are richly endowed with cone cells (colour photoreceptors), enabling them to see in colour. Primate snouts tend to be reduced as they are less reliant on a sense of smell than many other mammals. To accommodate the reduced snout, primates have a decreased number of teeth compared with other mammals. However, the precise number, structure and arrangement of teeth varies among different primates.



Alamy/Martin Harvey

Figure 10.3 ▲
(**a**) A chimpanzee grasps its foot with its hand. (**b**) Hands and feet of four primates. Note that only the human lacks an opposable hallux.

Compared with other mammals, primates have a relatively enlarged **cranium** for their body weight. This is due to the expansion of the **brain case**. It is the outer region of the brain, called the **cerebral cortex**, that has expanded the most during primate evolution. The cerebral cortex is responsible for the so-called higher functions of the brain. These include processing visual and tactile sensory information, as well as memory and reasoning. The increase is proposed to be an adaptation to life in the treetops, which depends heavily on processing visual and tactile input and coordinating it with responses from the body's muscles.

Primates have flexible spines and possess considerable rotation about the hips and shoulders, enabling them to move in various ways. Their modes of locomotion include climbing, **brachiation** (swinging through the air between branches), **suspensory locomotion** (using all fours to amble while hanging from a limb), **quadrupedalism** (moving on all four limbs) and **bipedalism** (moving on the hind limbs). Primates tend to shift their body weight onto their hind limbs, in contrast to most other terrestrial mammals, which shift their body weight onto the forelimbs (e.g. dogs, horses).

Primates also have intangible characteristics that are not necessarily useful for classification but are shared by most members of the group to a greater or lesser extent. For example, many primates are social animals, often living in extended family and social groups. Most give birth to a single offspring at a time, and the juveniles demand relatively intense care for an extended period of time.

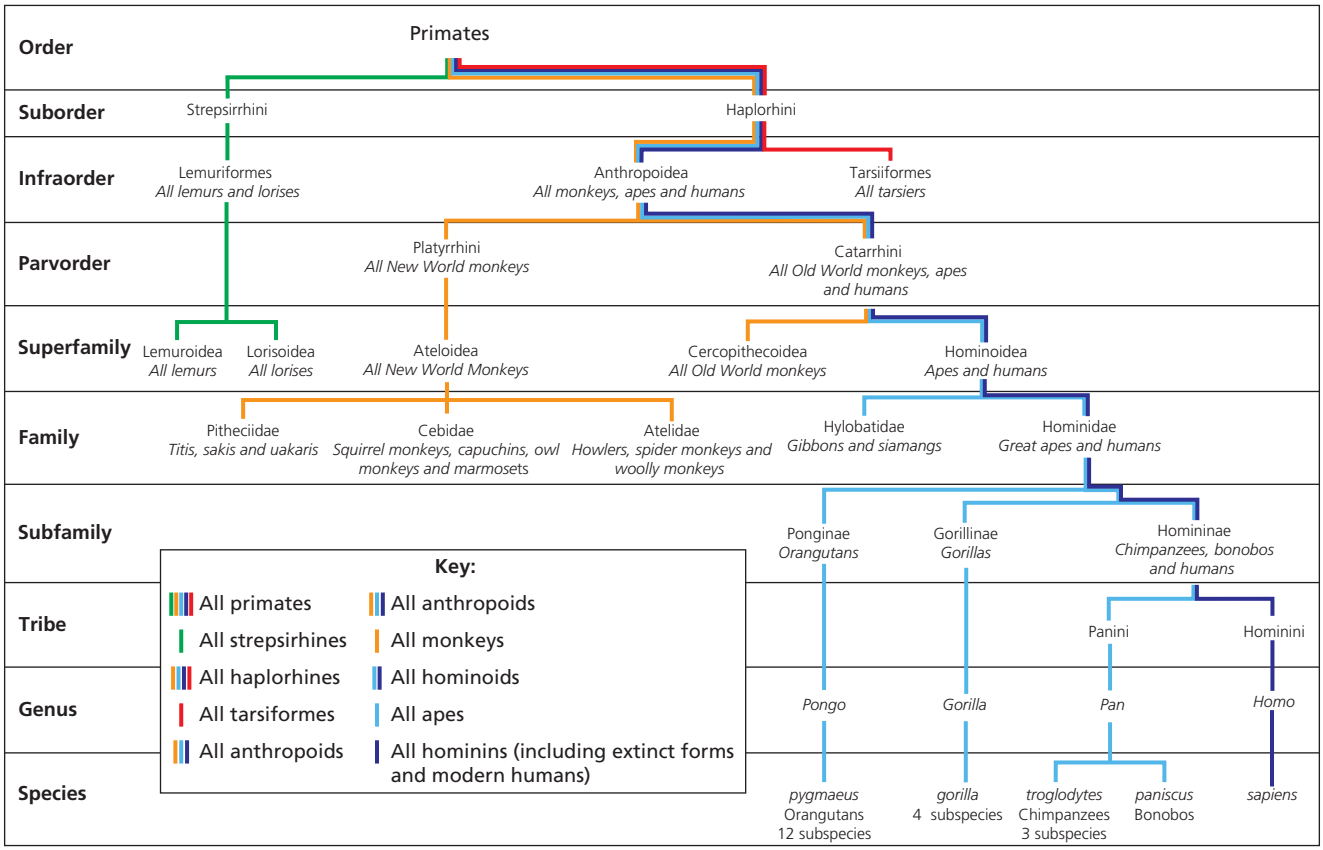
It is within the context of these features that modern humans evolved.

Classifying the primates

The Primata is divided into two suborders: the Strepsirrhini or 'wet-nosed primates' and the Haplorrhini or 'dry-nosed primates' (Figure 10.4). The Strepsirrhini comprises the lemurs and lorises, whereas the other primates are grouped under the Haplorrhini. This division has not always been clear-cut and debate continues on the position of the tarsiers. Traditionally tarsiers were considered more closely related to the lemurs and lorises based on presumably shared morphological features. The modern classification reflects DNA sequence data, which has tended to support tarsiers representing the earliest split within the Haplorrhini.

See Chapter 9 for more on taxonomy and systematics.

Humans are classified within the suborder Haplorrhini. While the tarsiers are regarded as a distant relation within the suborder, the remainder of the Haplorrhini comprises the monkeys, apes and humans.



▲ **Figure 10.4** An abbreviated taxonomy for the primates superimposed on a cladogram showing the position of modern humans. Additional levels of classification are introduced (e.g. superfamily, tribe) to represent the systematics of the order better.

Classifying the monkeys

Monkeys are divided into Old World monkeys and New World monkeys. These common names refer to the continents the monkeys inhabit. Historically, the 'Old World' refers to Europe, Africa and Asia, whereas the 'New World', discovered and explored by European mariners during the 15th and 16th centuries, refers to the Americas. The geographical isolation imposed by continental separation shaped the evolution of the monkeys on each continent and they are now discernibly different in their anatomy, physiology and behaviour. The fundamental feature that distinguishes them is that New World monkeys have compact noses with nostrils that angle sideways ('platyrrhine', Figure 10.5a), whereas Old World monkeys, as well as apes, have expanded noses with downward facing nostrils ('catarrhine', Figure 10.5b). New World monkeys are thus classified in the taxonomic group of Platyrrhini, and Old World monkeys are classified in the Catarrhini (Figure 10.4). Additional differences relate to the number and pattern of premolar teeth, the form of the bony ring in the ear drum and the types of tails in each group.

The New World monkeys are herbivores and inhabit the jungles of Central and South America. One family of New World monkeys (Cebidae) includes the capuchin, squirrel, howler and spider monkeys, and is characterised by possession of a sturdy, prehensile tail that curls tightly and assists with climbing (Figure 10.5c). Other monkeys have tails but they are not prehensile. The other family (Callitricidae) includes tamarins and marmosets. Marmosets are regarded as the most primitive monkeys. For example, marmosets have flattened nails only on their big toes but claws on their other digits (Figure 10.5d).

There are two subfamilies of Old World monkeys (family Cercopithecidae). The members of one subfamily (Colobinae) are herbivorous with multi-chambered stomachs

Figure 10.5 New World and Old World monkeys. (**a**) Common woolly monkey has the platyrrhine nose of New World monkeys. (**b**) Yellow baboon has the catarrhine nose of Old World monkeys. (**c**) Poeppig's woolly monkey hanging by its prehensile tail (**d**) Common marmoset, showing claws (**e**) Proboscis monkey (**f**) Drill displaying its rump

analogous to those of cows. This aids digestion of hardy vegetable matter, making them uniquely ruminant primates. These are predominantly Asian, such as proboscis monkeys (Figure 10.5e), but also include the Colobine monkeys of Africa. The monkeys of the other subfamily (Cercopithecinae) include various baboons, mandrills, macaques and vervets. They are recognisable for the often coloured, calloused bare patches on their rumps (Figure 10.5f). They are mostly African (with some macaques found in Asia), are relatively large, omnivorous, and have cheek pouches to store and pre-digest their food.

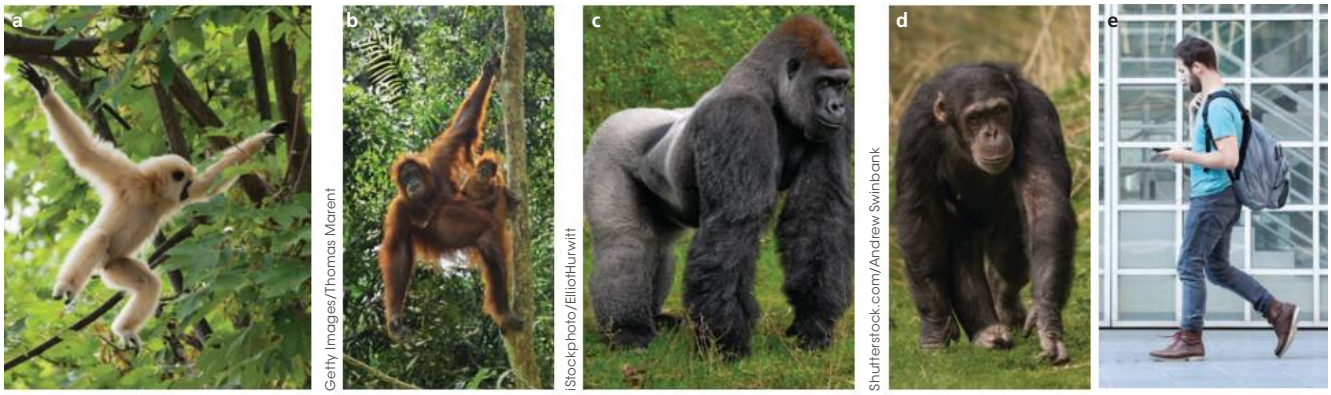
On the basis of anatomy, morphology, physiology, biogeography and DNA sequence data, the Old World monkeys are considered most closely related to the apes. The apes are therefore classified with Old World monkeys in the Catarrhini (Figure 10.4).

RECALL

- Modern humans are classified in the order Primata (primates), within a group called the Catarrhini.
- Key characteristics of primates include opposable digits, stereoscopic colour vision and a relatively enlarged brain.

RECAP 10.1

1. What does 'prehensile' mean?
2. What are four different modes of locomotion among primates?
3. How are the key characteristics of primates relevant to organisms that live high in the treetops?
4. What is the fundamental anatomical difference between Old World monkeys and New World monkeys?



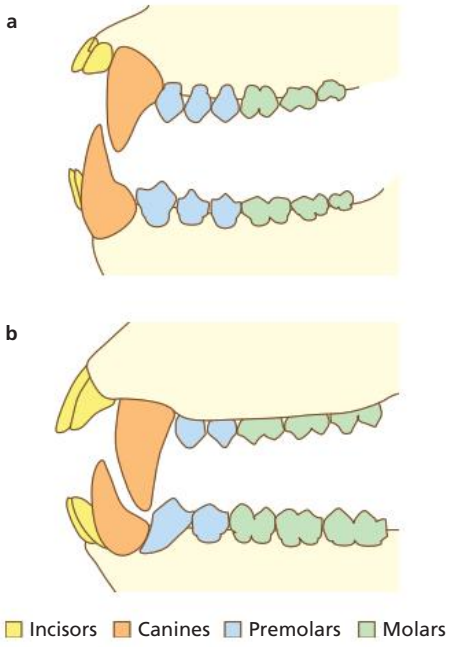
▲ **Figure 10.6** Representative hominoids displaying their chief mode of locomotion. (**a**) Lar gibbon brachiating (**b**) Bornean orangutan moving by suspensory locomotion (**c**) western gorilla knuckle-walking (quadrupedal locomotion) (**d**) chimpanzee knuckle-walking (**e**) human walking upright (bipedal locomotion).

Humans are hominoids

An animal that is a **hominoid** is by definition classified within the formal taxonomic grouping of the **superfamily** Hominoidea (Figure 10.4). The superfamily is a taxonomic rank immediately superior to the traditional rank of family. The Hominoids comprise the gibbons, orangutans, gorillas, chimpanzees and humans (Figure 10.6). Hominoids are fundamentally apes. They are distinguished from monkeys by lacking a tail.

Hominoid dentition

The dentition of hominoids has features in common with Old World monkeys but contrasts with that of other primates. For example, adult hominoids and Old World monkeys have eight premolars, whereas New World monkeys have 12 (Figure 10.7). The molars of hominoids and Old World monkeys have five cusps on their molars with a Y-shaped upper valley. By contrast, New World monkeys have four cusps with a cross-shaped valley (Figure 10.8). These observations support a closer evolutionary relationship between Old World monkeys and hominoids.

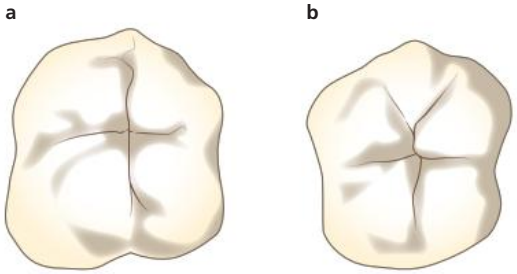


◀ **Figure 10.7** The dentition of (**a**) New World monkeys and (**b**) hominoids and Old World monkeys

What makes apes sit upright?

The rib cage in hominoids is flattened compared to that in monkeys, with the effect that hominoids have a broader chest. In addition, for their body length, hominoids have a comparatively shorter spine between the rib cage and the pelvis. It is proposed that these features enable hominoids to sit comfortably with an upright posture.

The relative length of the forelimbs and hind limbs varies among primates according to the animal's lifestyle and preferred mode of locomotion. Most hominoids, however, have forelimbs (arms) that are longer than their hind limbs (legs), with the exception of humans, and flexible shoulder and elbow joints facilitating a range of locomotion styles (Figure 10.6). The shoulder blades of hominoids sit further back than in other primates. This allows hominoids to move



◀ **Figure 10.8** (**a**) Molars of New World monkeys have four cusps. (**b**) Molars of hominoids and Old World monkeys have five cusps.

their arms freely around the shoulders. To maintain their balance when moving, hominoids display an **orthograde** form of locomotion. The limbs on the same side of the body move in opposition to each other so that the forelimb swings back as the hind limb swings forward.

By contrast, monkeys have relatively narrow rib cages, comparatively longer spines for their body length, and most monkeys have fore- and hind limbs of approximately equal length, adapting them for quadrupedalism on tree limbs or on the ground. Monkeys are highly dependent on their tails for controlling their balance. Consequently, when monkeys 'walk' on their hind legs, they tend to dangle their forelimbs. Some monkeys, such as New World monkeys of the Cebidae, use their tail essentially as a fifth limb for gripping, swinging and climbing (Figure 10.5c).

Humans are great apes

Hominoids are divided into the lesser apes and the great apes (Figure 10.4). The lesser apes (family Hylobatidae) comprise the gibbons, whereas great apes (family Hominidae) comprise the orangutans, gorillas, chimpanzees and humans.

The division between the lesser apes and the great apes reduces to a key difference in the anatomy of the wrist bones. In lesser apes (gibbons), the wrist is characterised by a ball-and-socket joint, which permits the animal's wrist to swivel and facilitates highly agile brachiation. Gibbons move by swinging briskly between tree branches (Figure 10.6), and a gibbon can freely reverse its direction by pivoting on one wrist while in motion.

By contrast, great apes, including humans, have a gliding wrist joint. The joint moves up and down in a vertical manner with limited twisting movement. Great apes do not practise brachiation in the same sense as gibbons do. Suspensory climbing and knuckle walking are the common locomotion styles of the great apes. Great apes can also stand and walk on their hind legs to a limited extent. Humans are exceptional among the great apes in that they are exclusively bipedal.

Humans are hominins

A **hominin** is any organism classified in the taxonomic tribe Hominini (Figure 10.4). The **tribe** as a taxonomic rank occupies a position between family and genus. The defining feature of hominins is that they are principally bipedal and walk on their hind limbs. Humans are uniquely classified as hominins for this reason; however, they also have a constellation of features that distinguish them from other apes. This will be the subject of the next section. For the remainder of this section we will explore how the systematics of humans was established.

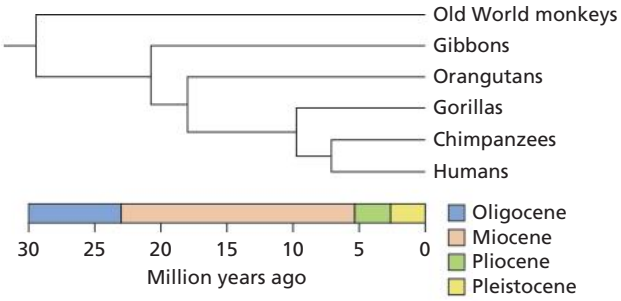
Table 10.1 Summary of defining features for taxonomic groups within which *Homo sapiens* is classified

Group	Defining features
Primates (Primata)	Hands and feet with five digits that include an innermost, opposable digit Forward-facing eyes enabling stereoscopic colour vision Relatively enlarged cranium relative to body weight Flexible spine with considerable rotation about the hips and shoulders
Hominoids (apes – Hominoidea)	Distinguished from monkeys by lacking a tail Dentition includes 8 premolar teeth Molars with 5 cusps and grooves in a Y-shaped pattern Broad, flattened rib cage Arms generally longer than the legs (except for humans)
Great apes (Hominidae)	Gliding wrist joint with limited twisting movement
Hominins (Hominini)	Bipedal mode of locomotion

Delineating the human lineage

Within the Hominini, humans are classified in the genus *Homo* as the species *sapiens* (Figure 10.4). The *Homo sapiens* identifier derived from Latin translates as 'intelligent human being'.

The scientific name applied by Linnaeus to humans in 1758 highlights a bias of reasoning that confounded taxonomy for centuries. Humans seemed so different from other great apes that they were placed in a separate taxonomic family. During the second half of the 20th century, the taxonomy was progressively amended. Humans were grouped together with the other great apes in a single family. By the mid-1980s, orangutans were regarded as the earliest divergence of the great ape lineage, based on morphology and molecular evidence. Which two were more closely related among gorillas, chimpanzees and humans was, however, a matter of substantial debate. As a compromise, the three were for a time regarded as equally related.



▲ **Figure 10.9** Phylogenetic tree depicting molecular clock estimates for divergences between major hominoid lineages

In the late 1980s, a clearer picture of the phylogeny of humans emerged with DNA–DNA hybridisation data. These data showed that the gorilla lineage split first and that the chimpanzee and human lineages diverged later (Figures 10.4 and 10.9). The data implicate humans and the chimpanzees as the most closely related great apes. This phylogeny is now strongly supported by molecular homology of many gene sequences, as well as comparative genomics. Molecular clock estimates tend to be controversial because they vary with the molecular data, statistical methods and calibration systems used. However, recent molecular clock estimates based on whole-genome analyses (Figure 10.9) indicate the human and chimpanzee lineages diverged ~7.5 mya. The gorilla lineage is estimated to have diverged ~10 mya. These estimates may be refined in the future.

See Chapter 9 for more on DNA–DNA hybridisation, molecular homology and molecular clock estimates.

RECALL

- In contrast to other primates, hominoids lack tails, have eight premolars rather than twelve, and molars with five cusps rather than four.
- Hominins are uniquely bipedal.
- Chimpanzees are the living species that are most closely related to humans.

RECAP 10.2

1. Explain the difference between a hominoid and a hominin.
2. How many living hominin species are there?
3. Outline the evidence that suggests hominoids are more closely related to Old World monkeys than to New World monkeys.
4. Outline the evidence that suggests chimpanzees and humans are the most closely related great apes.

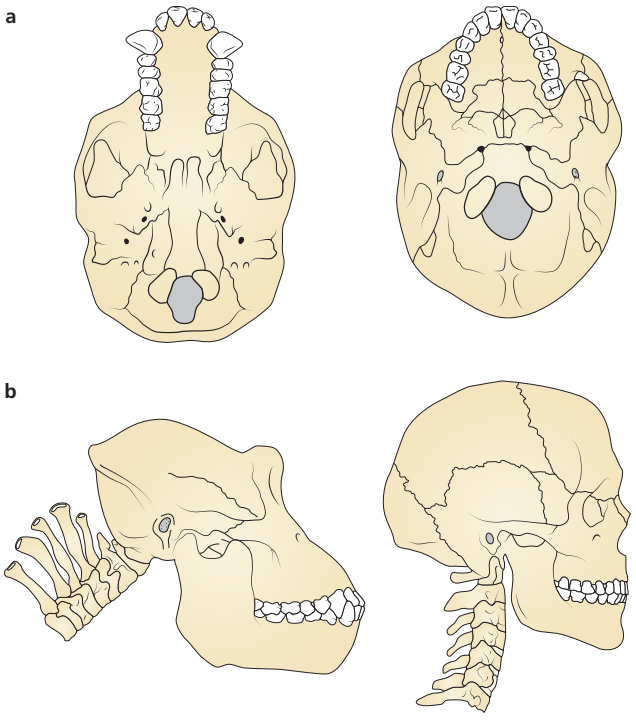


Figure 10.10 ▲
(**a**) Position of the foramen magnum (shaded) in the gorilla and the human (**b**) The corresponding position of the spine and skull in each

Adaptations that define humans

Humans have the same basic characteristics as apes, which places them in the hominoid superfamily. Humans also have features that distinguish them from other hominoids. Key characteristics include the anatomical features that allow humans to stand upright and walk with a fully striding gait. These fundamentally define humans as hominins. Humans also are relatively hairless, have a greatly expanded brain, and have modified teeth and jaws. Furthermore, humans behave very differently from other apes. They communicate with speech, display advanced intellectual abilities, apply sophisticated technology, and practise elaborate symbolism. In this section, we review some of the adaptations that set humans apart from the other great apes.

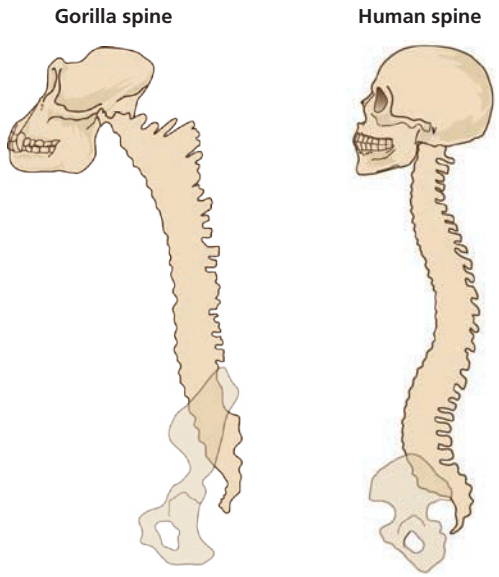
Adaptations for bipedalism

Most hominoids are fundamentally quadrupedal in that they use all four limbs for locomotion. Our closest living relatives, the gorillas and chimpanzees, mainly amble by knuckle-walking (Figure 10.6c and d). Humans are distinctly different, as they stand upright and walk on their hind limbs. They are bipedal (Figure 10.6e). The shift to bipedalism has been accommodated by changes throughout the skeleton. No other animal is as adept as the human at full striding bipedal locomotion. From head to toes, the human body is configured for bipedalism.

Position of the foramen magnum

In vertebrate animals, the spinal cord feeds through a hole in the skull called the **foramen magnum** to connect with the brain. The position of the foramen magnum in the base of the skull varies according to how the animal moves. In quadrupedal animals, such as the gorilla or the chimpanzee, the foramen magnum is positioned towards the back of the cranium (Figure 10.10). This is because the spine is almost horizontal where the spinal cord enters the skull. In the fully bipedal human, the foramen magnum is positioned more centrally at the base of the skull (Figure 10.10). This permits the head to face forward comfortably while resting almost vertically over the spinal cord when upright.

Figure 10.11 ▼
A comparison of the spinal column of the gorilla and the human



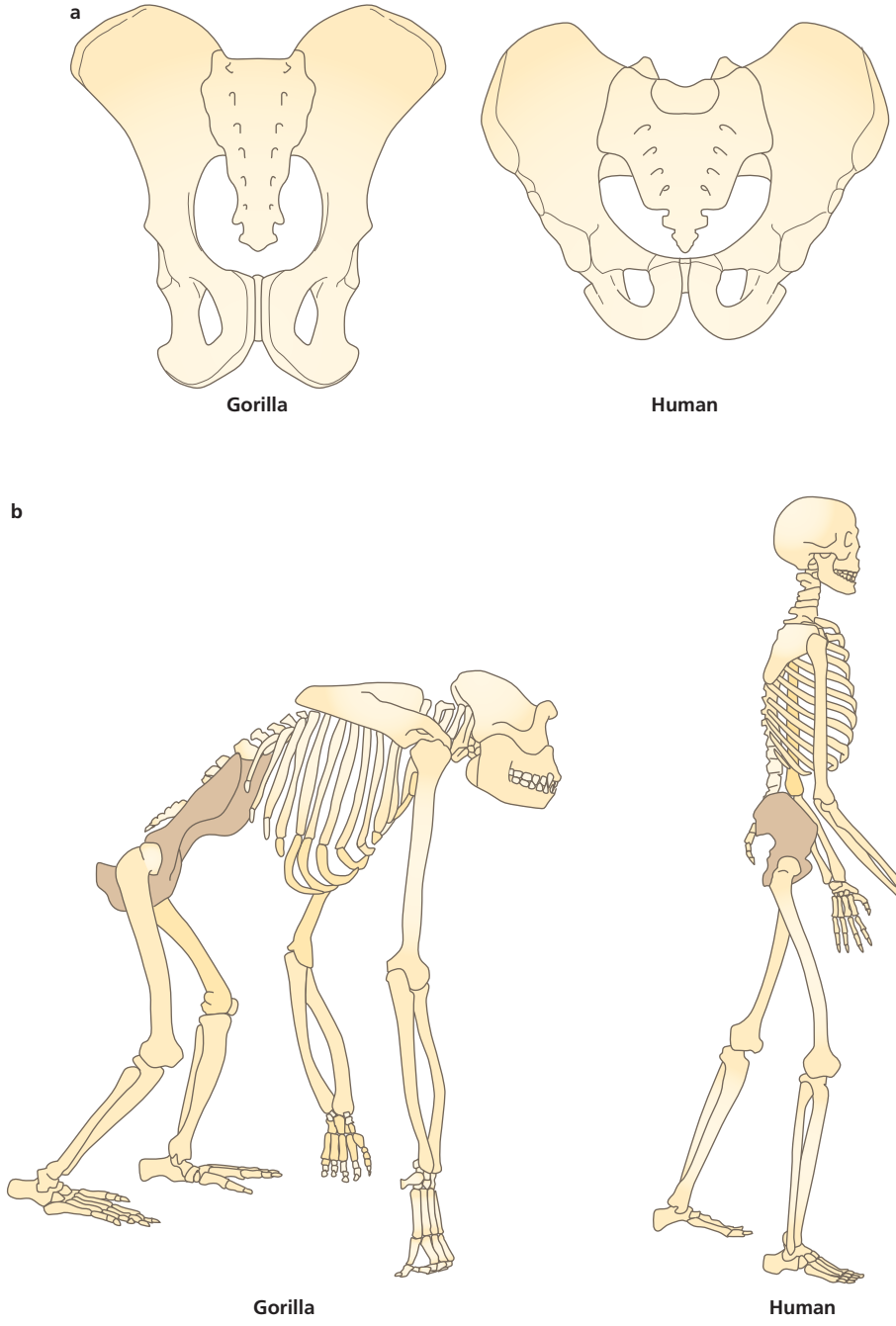
Curvature of the spinal column

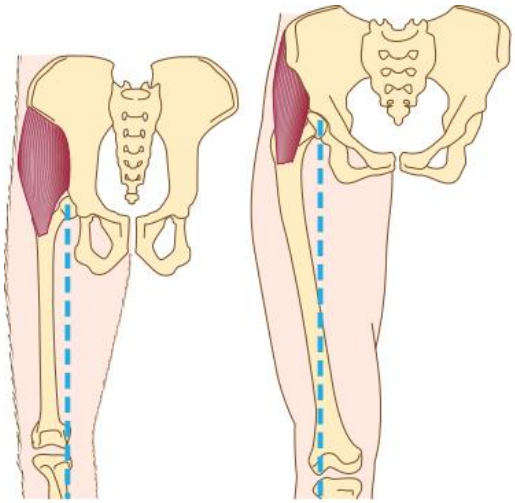
The posture of modern humans contrasts with that of other apes. In apes such as gorillas and chimpanzees, the spine curves forward (Figure 10.11). Their body weight is evenly distributed by support from the forelimbs during quadrupedal locomotion (Figure 10.6c and d). The curve of the human spine, however, follows an S-bend to support the weight vertically (Figure 10.11). The vertebrae in the lumbar (lower back) region of humans are wedge-shaped and pack together with the thin edges pointing backwards. This causes the lower spinal column to adopt a convex (forward) curve.

Pelvis

The lower spinal column connects with the pelvis. In most apes, the pelvis is relatively long and narrow (Figure 10.12). In humans, however, the pelvis is comparatively shallow and bowl-shaped (Figure 10.12). This shape creates a basin that sustains the weight of the abdomen and provides support for the upper body. The broader hip bones also provide expanded attachment sites for the buttock muscles. The relatively enlarged buttock muscles of humans extend the leg and help steady the pelvis and upper body during walking.

▼ **Figure 10.12**
(**a**) Comparison of the pelvises of the gorilla and human
(**b**) Illustration of the position and orientation of the pelvis during locomotion in the gorilla and human





Chimpanzee Human

Figure 10.13 ▲
Comparison of the pelvis and femur of the chimpanzee and human, showing how the femur of the human is angled inward towards the knee. The dotted line shows the direction weight is transmitted.

The carrying angle

In humans, the flaring of the pelvis aligns the hip joints directly beneath the head and torso. The weight of the upper body is therefore transferred via the pelvis to the legs. However, the broad pelvis spreads the top of the femurs (thigh bones) away from the midline of the body. This is potentially destabilising. The body would have to sway from side to side during walking so one leg could support the body's weight. The femurs are therefore angled in towards the knees. This **carrying angle** relative to the vertical is evident when viewed front-on (Figure 10.13). The angle ensures one knee and foot are directly beneath the body while the other knee and foot are lifted during walking. It allows the body to rotate about the lower leg and foot, and one foot to be set ahead of the other when striding. Orthograde movement of the arms compensates for body rotation. The knees are strengthened to support the weight at the lower end of the femur. This arrangement allows humans to extend the leg fully during walking. The longer legs of the human relative to other great apes increase the length of the stride overall.

The foot

Unlike other apes, humans use their feet primarily for propulsion rather than for grasping or climbing. As a result, the human foot has lost its prehensile capacity and the hallux aligns alongside the other toes. In addition, the human foot has a comparatively wide heel (Figure 10.14) that serves as a shock absorber upon heel strike. Apes' feet have a single longitudinal arch that runs from the back to the front of the foot. By contrast, human feet have two arches, one longitudinal and one transverse, which cross over the foot (Figure 10.14). When a human is standing erect, the foot acts as a pedestal to support the body's weight (Figure 10.15). When the human foot presses to the ground during walking, weight is transferred progressively forward and across the foot via the arches (Figure 10.15). With the weight transmitted to the toes, the big toe pushes off to launch the next step.

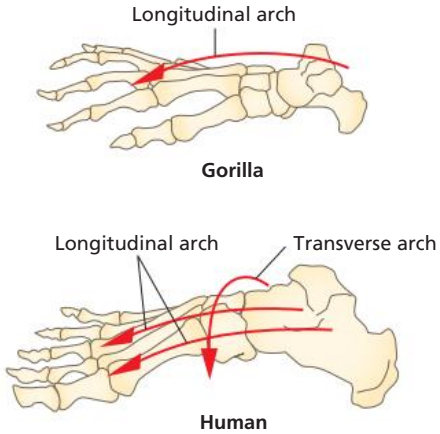


Figure 10.14 ▲
Comparison of the arches of a gorilla foot and a human foot

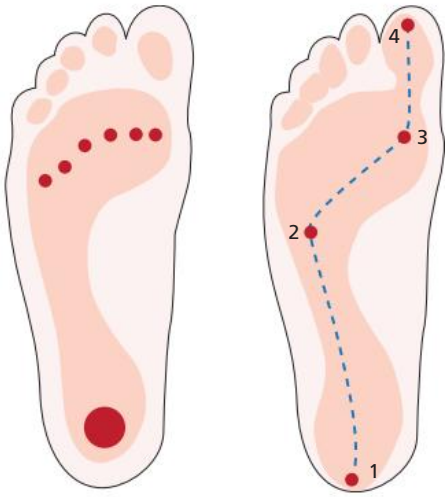


Figure 10.15 ▲
How body weight is distributed across the human foot when standing (left) and walking (right). When walking, the body weight is shifted in sequence from position 1 (heel strike) to positon 4 (the big toe thrusts off).

RECALL

- Many features of the human skeleton, including the skull, spine, pelvis, femurs and feet, display a suite of adaptations for bipedalism.

RECAP 10.3

1. What is the foramen magnum, what is its function, and what does its position indicate about a hominoid's locomotion?
2. How does the shape of the human spine differ from that of other apes, and why is this the case?
3. What benefit is there in the shape of the human pelvis?
4. What is meant by 'carrying angle' and why is it different in humans compared with other apes?
5. What features does the human foot have that adapt it to walking rather than climbing?

The human hand

Bipedalism makes it unnecessary for the human forelimbs to carry the body's weight. The hands have instead become adapted for manipulation. Compared with other primates, the human hand is short and broad with relatively short, straight fingers and a long, strong thumb. These modifications give the human thumb a substantial amount of freedom and the ability to extend to the fingertips. The degree of contact between the thumb and forefinger allows humans to grip and manoeuvre small or delicate objects, such as a needle for sewing or a pencil for writing (Figure 10.16). It is the basis of the **precision grip**, which enables humans to grasp and manipulate objects with exquisite dexterity.

▲ Figure 10.16
The precision grip

Expansion of the cranium

Humans clearly behave in unique ways compared with other apes. Humans are profoundly inventive and technologically sophisticated. Humans have an unparalleled aptitude for manipulating and interpreting symbols. They are capable of complex language. They indulge in personal ornamentation, art and music. They establish extensive communities and observe societal conventions. To practise these faculties is to celebrate what it means to be human.

Compared with other apes, humans have an advanced cognitive capacity. **Cognitive capacity** describes an organism's innate intelligence, ability to learn, plan, evaluate, make decisions, and apply new knowledge and skills. To a great extent, the cognitive capacity of the human is credited to its relatively enlarged brain (Figure 10.17). The brain volumes of most apes vary between about 350 and 500 cm^3. The volume of human brains varies between 900 and 2200 cm^3 but the average is ~1350 cm^3. Most of the enlargement is associated with expansion of the cerebral cortex, the outermost region of the brain. The surface area of the primate cerebral cortex is further increased by extensive folding, called **convolutions**. Convolutions are not random. Rather, they form specific patterns in the cerebral cortex of different species. Relative to brain size,

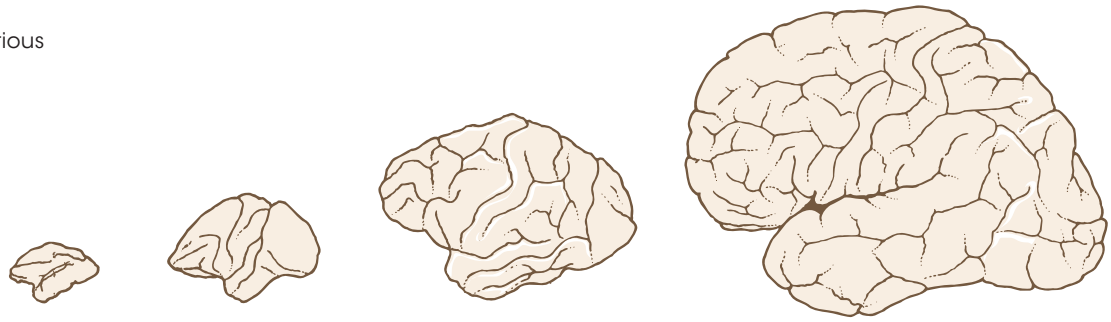


Figure 10.17 ▶
The brain sizes of various primates (drawn to scale)

the cerebral cortex is estimated to have ~40% greater surface area in the human brain than in the chimpanzee brain.

Differences arise not just in the size of the brain but also in how the brain operates. The cerebral cortex is associated with the so-called executive functions of the brain, including reasoning, planning and judgement. The **prefrontal cortex**, which covers the front part of the brain, is the portion of cerebral cortex that has undergone the greatest expansion. The relative size of the human prefrontal cortex is about six times that of other apes. The prefrontal cortex governs a variety of functions, including abstract thinking, analysis of conflicting outcomes, and planning and strategising. Not least, it is associated with complex social behaviours, such as those demanding impulse control and ethical choices.

See Chapter 9 for more on master control genes.

The human cerebral cortex can be divided into more than 50 distinct regions based on cellular staining patterns that were described over a century ago. Master control genes regulate the position and size of the cerebral cortical regions, as well as the formation of convolutions. The difference between the human and chimpanzee genomes seems small, approximately 1% of total gene sequences. However, it is likely that changes to the genome have affected master genes, resulting in substantial differences in brain development, anatomy and function.

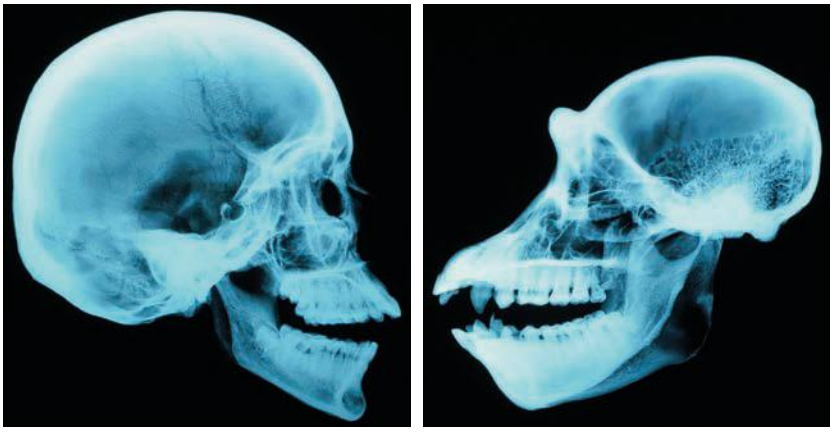


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Figure 10.18 ▼
Profile images of human and chimpanzee skulls showing differences in cranial capacity, brow ridges, jaws and teeth

Modification of the skull

To adapt to the extraordinary change in brain size, the human cranium has undergone substantial modification during evolution (Figure 10.18). The human has an enlarged brain case to accommodate the brain. **Cranial capacity** is a measure of the volume of the brain case. The shape of the cranium in humans is also altered. In the skulls of most apes, the forehead is sloped back. The **brow ridges**, the bony ridges above the eye sockets, are typically prominent. In humans, however, the front of the cranium is higher and more rounded, and the brow ridges are significantly reduced. These changes accommodate the enlarged prefrontal cortex. The effect is the distinctively raised forehead of humans.

Chimpanzees and other apes have protruding jaws (Figure 10.18), a condition described as **prognathism**. In humans, the jaw does not protrude so far. Corresponding to the difference is the shape of the lower jaw, or **mandible**. The chimpanzee mandible has an extended rectangular shape, whereas the human has a

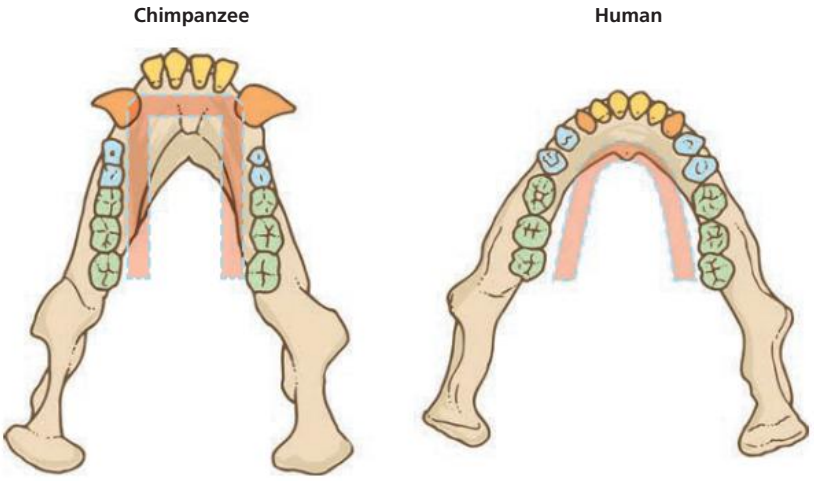

Figure 10.19 The mandible of a chimpanzee and a human. The pink shapes highlight the rectangular shape of the chimpanzee mandible and the parabolic shape of the human mandible.

shallower, parabolic mandible (Figure 10.19). Most apes have prominent, interlocking canine teeth, whereas human canines are reduced so that they appear similar to the incisors (Figure 10.19). The difference in canines is hypothesised to relate to differences in behaviour rather than diet. The canine teeth are most conspicuous in male apes. Male apes exhibit their canines in competitive displays so as to avoid violent aggression.

Overall, the changes to the cranium and jaws have flattened the human face. The chin and prominent nose are distinctly human characteristics.

Table 10.2 Summary of anatomical features that distinguish *Homo sapiens* from other hominoids (apes)

Feature	*Homo sapiens*	Other hominoids
Body covering	Relatively hairless	Relatively hairy
Mode of locomotion	Bipedal	Quadrupedal
Position of foramen magnum	Closer to the centre of the base of the skull	Closer to the rear of the skull
Spinal curvature	S-bend with convex curve near the base of the spine	C-shape forward curvature
Pelvis	Shallow and bowl-shaped	Long and narrow
Carrying angle of femur	Relatively high	Relatively low
Feet arches	One longitudinal and one transverse	One longitudinal only
Hallux	Not prehensile	Prehensile
Hand	Thumb long compared to fingers for precision grip	Thumb short compared to fingers
Cranial capacity	Relatively large	Relatively small
Brain	Expanded prefrontal cortex, many more convolutions	Smaller prefrontal cortex, fewer convolutions
Brow ridges	Subtle or absent	Prominent
Prognathism (jaw protrusion)	Subtle	Substantial
Mandible shape	Parabolic	Rectangular
Canines	Reduced	Enlarged

RECALL

- Humans have a precision grip that enables manual dexterity.
- The anatomy of the brain, including the enlarged prefrontal cortex and increased convolutions, enhances the cognitive capacity of humans.

RECAP 10.4

1 Describe how the anatomy of the human hand differs from that of other apes, and how it adapts the human hand for a precision grip.
2 What is meant by 'cognitive capacity'?
3 Describe three ways the human brain evolved to become different from those of other apes.
4 Describe how the shape of the modern human skull differs from that of other apes, and how these changes accommodate the human brain.

Communication, technology and culture

The most conspicuous physical legacies of human evolution are bipedalism and the expansion and development of the brain. As a consequence of bipedalism, the hands are also freed for fine manipulation. These result is an organism that combines an enhanced capacity to imagine and plan with the manual acuity to modify items and reshape its environment. These are the basic biological ingredients for developing tools and utilising technology.

Humans are social creatures, like many other primates. However, humans have a unique ability to communicate abstract ideas in detail. This has ensured that knowledge and ideas are transmitted freely between individuals. It is also the foundation for extensive cooperation, which accelerates the pace and scale of innovation. In this section we explore some of the behavioural adaptations that distinguish humans from other primates.

Language: a mechanism for innovation

Humans are not the only animals to use tools. Among apes, for example, chimpanzees employ a kind of 'toolkit' for capturing termites (Figure 10.20). The chimpanzee uses one stick with brush-like leaves to clear the entrance leading into the termite mound. They use a second stick deliberately stripped of leaves for 'fishing' the termites by inserting it into the hole. When the stick is withdrawn, it is covered in termites, which the chimpanzee licks off with its tongue. Juvenile chimpanzees watch and learn these techniques from the adults. A feature of the primate brain that assists this kind of learning is the mirror neurons. **Mirror neurons** are neurons that fire when the animal either performs an action or observes another animal performing the same action. Mirror neurons allow the observer to imagine themselves in the position of the individual they are watching and to imitate their behaviour.

Figure 10.20 ▼
Common chimpanzees (*Pan troglodytes*) using sticks to 'fish' for termites

Alamy/Steve Bloom Images

Humans also have mirror neurons; however, humans have a unique capacity to communicate far more complex and abstract ideas. Humans have the astounding ability to vocalise thousands of sounds, to attach meaning to each, and to reorganise them in expressive new sequences. Humans have the anatomy and neural wiring to coordinate the lungs, mouth, throat and nasal organs to make talking possible. **Language** provides a functional framework for human speech. As the utterings of infants demonstrate, humans are genetically predisposed to learn vocabulary and to order words according to strict grammatical rules. The actual language learned by any individual human is, however, influenced by their environment. If Chinese parents in China adopt a native-born German baby, the infant learns Chinese as her principal language.

Recording symbols to represent spoken language is the foundation of written language. Writing allows humans to formalise, store and reference abstract ideas. The ability to manipulate and interpret symbols also allows humans to express meaning in fields such as music or mathematics (Figure 10.21). Spoken language is immediate. It transmits knowledge directly among people at a particular time. Written language preserves ideas for transmission to wider audiences, between generations, and over extended periods of time. For example, Charles Darwin's 19th century publications can still inspire biologists more than a century after his death. The result is a collective knowledge that is accumulated over time. New ideas and technology can be developed by people today that are based on those of people who lived in another time and place.

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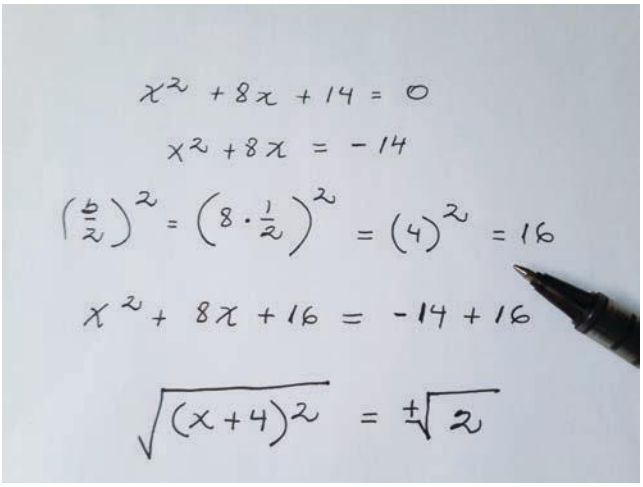

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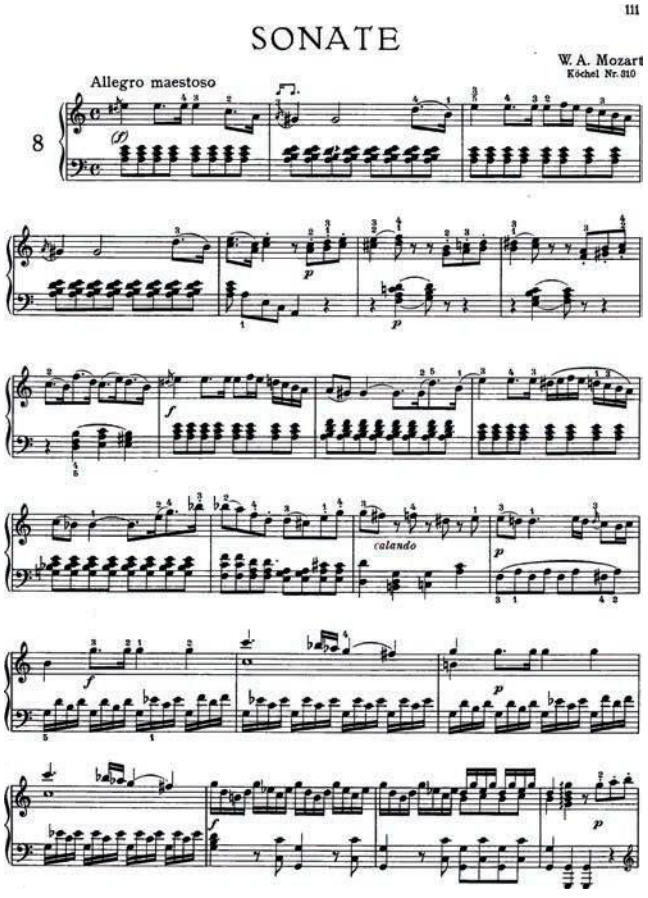


◀ **Figure 10.21**
Examples of written communication

Other animals, including chimpanzees, can recognise and communicate using sounds or abstract symbols. A famous case is that of Washoe, a common chimpanzee, who learnt to communicate with up to 200 symbols of American Sign Language. Washoe could string up to three signs into short expressions, and she set about teaching her adopted infant chimp the language. Chimpanzees in the wild also produce scores of vocalised sounds to communicate with one another.

The difference between humans and other animals is a matter of scale and sophistication. Vocabulary size varies from one person to the next, but it has been estimated that, by age 4, most humans know ~4000 words. For adults, it is suggested to be between 15 000 and 25 000 words. A standard English dictionary contains over 170 000 definitions. The size and versatility of human language gives those using it an exceptional ability to convey abstract ideas. It enables models to be described for concepts that are beyond the visceral experience of most people, or indeed any animal. The structure of the atom, principles in electromagnetic theory, supermassive black holes and evolution on geological time scales are all examples.

Cultural evolution

Humans' aptitude for communication has enabled knowledge to spread rapidly between individuals, throughout populations and between different populations. It also enabled individuals to organise themselves and work cooperatively in ever larger groups. The humans' suite of physical, cognitive and communicative characteristics underpin the evolution of human culture. **Cultural evolution** describes the way human beliefs, social practices, skills and technology change over time.

Cultural evolution contrasts with biological evolution in the speed and means by which it is transmitted. The significant differences between cultural and biological evolution are summarised in Table 10.3. Biological characteristics are exclusively transmitted from parent to offspring. It normally takes many generations to observe biological changes in a population over time. Culture, however, can be transmitted rapidly between unrelated individuals of the same generation or of different generations. Cultural characteristics can be communicated informally (e.g. spoken word), formally (e.g. education), and even over long distances without the individuals concerned ever meeting each other (e.g. via books or the Internet). Consequently, cultural evolution occurs rapidly within the lifetime of the individual.

Table 10.3 Summary of differences between features of biological and cultural evolution

Feature	Biological	Cultural
Data coding	Genetic	In written, spoken or symbolic language
Transmission of traits	Inherited from parents. No choice in traits acquired.	Communicated from unrelated individuals. Taught and learnt. Choice in traits acquired.
Generation	From one generation to the next	Within or between generations
Speed of dispersal	Slow. Many generations required to spread trait in population.	Fast. Can spread rapidly in population by immediate learning.
Intent	None. Unplanned, resulting from random processes.	Deliberate, result of conscious action.

RECALL

- Written and spoken language enables humans to convey abstract concepts.
- Cultural evolution is distinguished from biological evolution by the speed, the means and the choice exercised in its dispersal.

RECAP 10.5

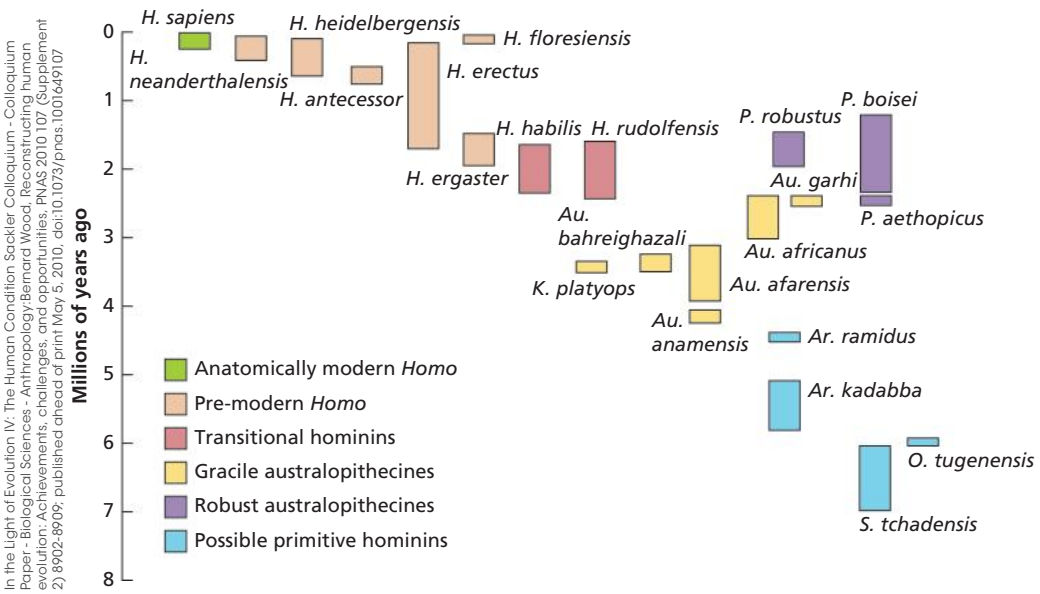
1 What is 'cultural evolution'?
2 List five ways cultural evolution contrasts with biological evolution.
3 For the following examples, explain whether they represent cultural or biological evolution:
   a invention and subsequeant miniaturisation of mobile phones
   b changes in hairstyles in a population
   c increasing uptake of the 'paleo' diet in a population
   d increasing resistance to malaria over many generations.
4 How does written language contribute to cultural evolution?

Meet the ancestors

We now turn our attention to the natural history that led to the evolution of modern humans.

When examining fossils, scientists are often trying to deduce the morphology, lifestyle and behaviour of an extinct organism from a few fragments. Interpreting the anatomy of extinct organisms relies on comparing fossils with the skeletal structures of living organisms. Knowing the living organism's appearance and lifestyle allows scientists to infer some things about the appearance and behaviour of the extinct organism.

Hominin classification includes species of fossil great apes that share similarities with modern humans. As a taxonomic group, hominins therefore are modern humans and their extinct bipedal ancestors. The hominin fossil record (Figure 10.22) demonstrates that human evolution was not a simple, linear progression from one species to the next towards modern humans. Instead, multiple human species coexisted at one time.



In the Light of Evolution IV: The Human Condition Sackler Colloquium - Colloquium Paper - Biological Sciences - Anthropology:Bernard Wood. Reconstructing human evolution: Achievements, challenges, and opportunities, PNAS 2010 107 (Supplement 2) 8902-8909; published ahead of print May 5, 2010, doi:10.1073/pnas.1001649107

Figure 10.22 Species recognised in the hominin fossil record under the genera *Homo* (*H.*), *Ardipithecus* (*Ar.*), *Australopithecus* (*Au.*), *Kenyanthropus* (*K.*), *Orrorin* (*O.*), *Paranthropus* (*P.*) and *Sahelanthropus* (*S.*). The rectangles represent an estimate of the periods in the geological record during which the species are presumed to have lived, based on fossil evidence.

Some may have been competitors, others may have occupied different niches. Some species persisted a long time, others less so. Palaeoanthropologists are compelled to draw lines that connect these species into some sort of evolutionary tree. What that tree looks like is inevitably contentious because interpretations vary among scientists. New fossil discoveries occasionally challenge existing hypotheses. It is evident, however, that the hominin evolutionary tree has many branches, and all but one of those branches terminates in extinction. Modern humans are the last living legacy of this rich evolutionary history.

In this section we review specific hominins and what they reveal about the course of human evolution. In order to tease out the trends, we consider fossil hominins mostly in the chronological order of their appearance in the geological record. The oldest of these were recovered in Africa but many more recent ones have been found across Europe and Asia (Figure 10.23). This observation reveals an African origin for the hominins followed by subsequent migration.

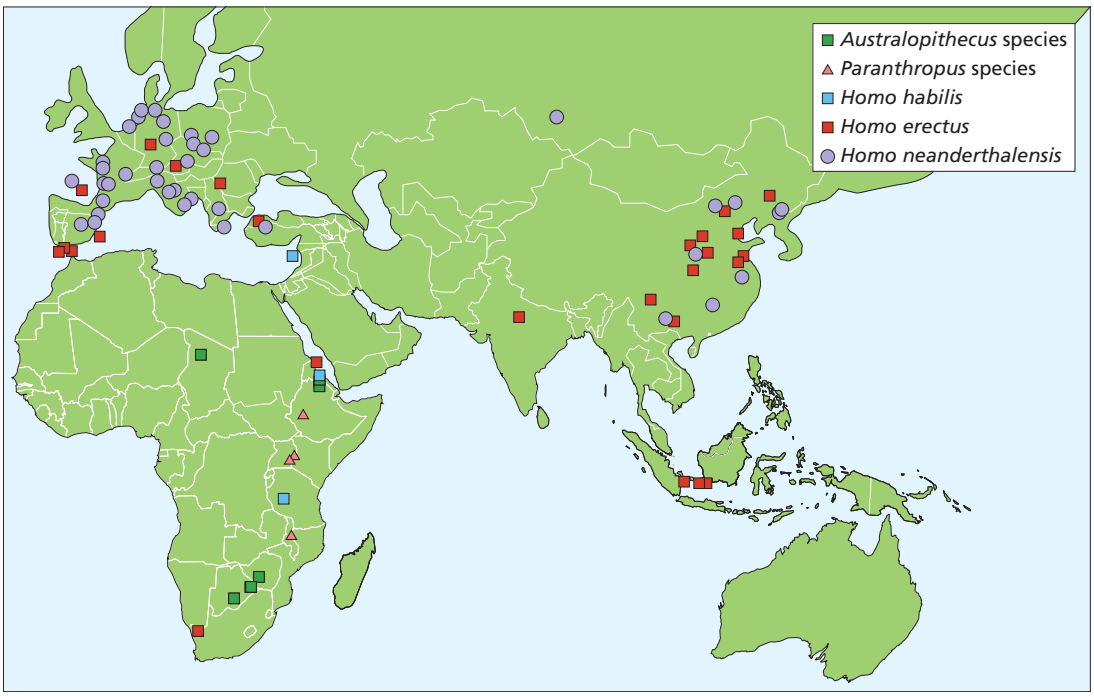


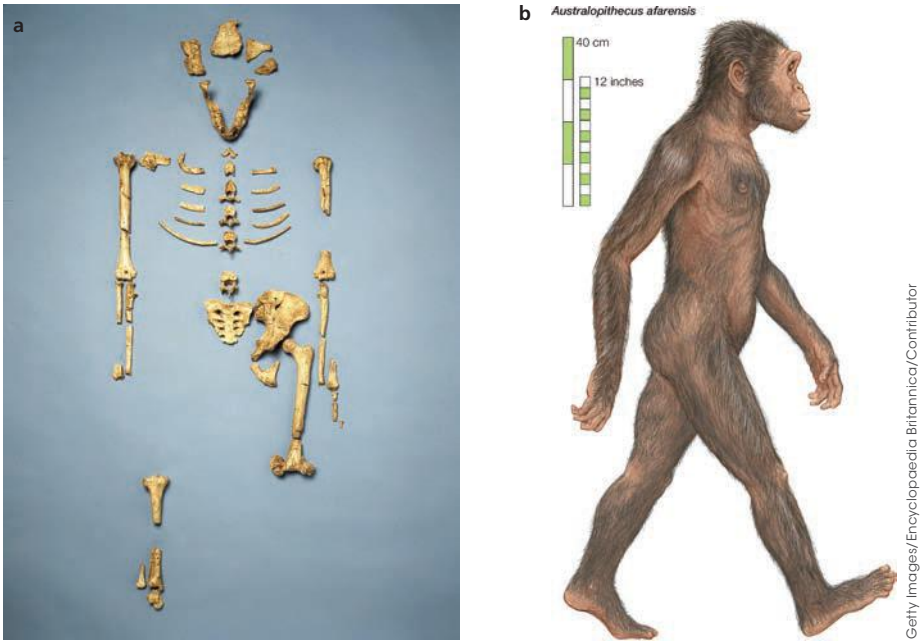
Figure 10.23 ▶ Location of major hominin fossil finds

The australopithecines

The fossil record for hominins older than ~4.2 million years is limited. There are fossils of possible hominins that date to ~7 million years old (Figure 10.22) but their identity and significance as hominins are frequently debated. The earliest universally accepted hominin fossils, those from which modern humans evolved, are the **australopithecines**. These are a varied group of small, bipedal apes that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya. They were evolving during a time of climatic change accompanied by a shift from forests to wooded grasslands. During that period, the australopithecines flourished and diversified into a number of species. The species are distinguished by their morphology as slender **gracile** forms or stocky **robust** forms. The gracile australopithecines, of which there are five generally recognised species, are classified in the genus *Australopithecus*. The robust forms are placed in the genus *Paranthropus*, which contains three accepted species (Figure 10.22). Some australopithecines, such as *Australopithecus afarensis*, are widely regarded as direct ancestors of modern humans. A number of others are considered evolutionary dead-ends.

The archetype for genus *Australopithecus*: *Au. afarensis*

Australopithecus afarensis was discovered by chance in 1974 when paleoanthropologists were surveying potential excavation sites in the Afar region in Ethiopia. The specimen eventually unearthed and reconstructed to 40% completion was nicknamed 'Lucy' (Figure 10.24). Now known from hundreds of fossils collected in eastern Africa, *Australopithecus afarensis* is considered a key australopithecine. The species persisted between 3.8 and 2.9 mya and is believed to be a direct ancestor of the modern human. Like other australopithecines, the species displayed **sexual dimorphism**, with adult males significantly bigger than females. Males bore a **sagittal crest** at the top of the skull. Male gorillas also have a sagittal crest, which provides expanded surface area for the uppermost attachment of their powerful jaw muscles. By implication, the male *Au. afarensis* had a strong bite.



Alamy/The Natural History Museum

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◀ Figure 10.24 *Australopithecus afarensis* (**a**) The specimen dubbed 'Lucy' (**b**) An artist's impression of the organism

As a model for the genus, *Australopithecus afarensis* clearly showed bipedal features (Figure 10.25). It had a relatively wide and shallow pelvis, femurs angled in towards the knees, strengthened weight-bearing knees, arched feet, wide heels, and its hallux aligned with the other toes. The interpretation was substantiated by a discovery in 1978 near Laetoli in Tanzania. This was a set of fossilised footprint impressions in a volcanic ash bed laid down some 3.6 mya (Figure 10.26). Attributed to *Au. afarensis*, they show the tracks left by two adults walking one in front of the other and a juvenile walking beside them. These provided direct evidence that *Au. afarensis* was capable of bipedal locomotion. Yet *Au. afarensis* also had relatively long forearms, long curling fingers and toes, and shoulder blades akin to those of other great apes rather than to modern humans. These adaptations indicate *Au. afarensis* was a proficient tree climber. The mosaic of features suggests this species lived in mixed habitats that included forest and grassland, and it utilised both climbing and walking. Microanalysis of fossil teeth indicates its diet consisted mainly of leaves and fruits.

For its body size, *Au. afarensis* had a relatively small cranial capacity (~430 cm^3). The cranium of its successor, *Au. africanus*, was only marginally larger (~480 cm^3). In fact, relative to their estimated body masses, the brains of australopithecines were comparable in size to those of modern chimpanzees.

The key lesson to be learnt from the australopithecines is that bipedalism preceded expansion of the cranium during hominin evolution.

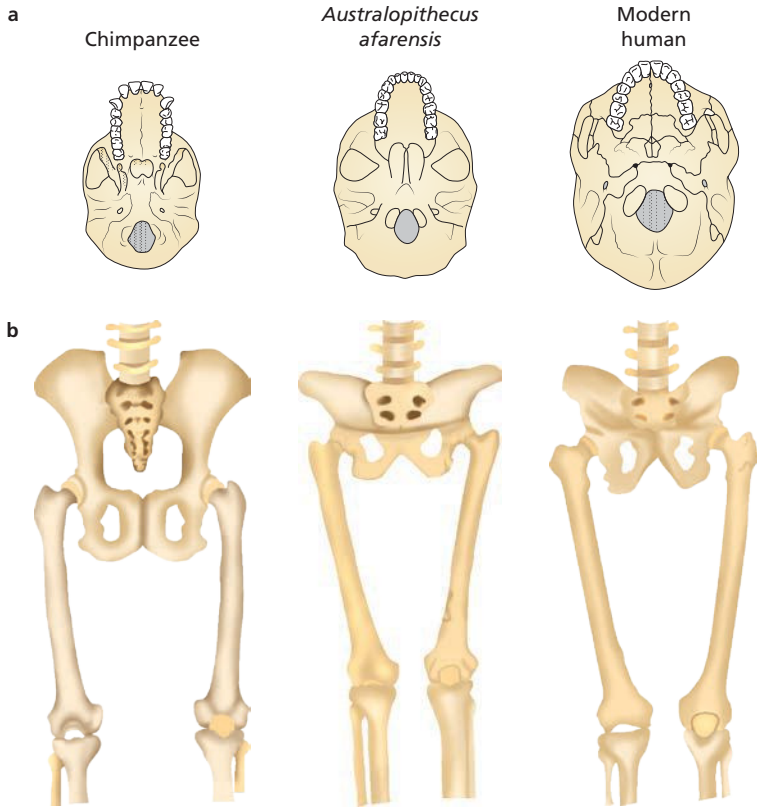

Figure 10.25 ▲
Australopithecus afarensis showed bipedal features. (**a**) The position of the foramen magnum was intermediate between that of the chimpanzee and modern human. (**b**) It had a bowl-shaped pelvis and carrying angle more like those of the modern human.

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Figure 10.26 ▲
The Laetoli footprints in Tanzania provide evidence that australopithecines were bipedal.

Genus *Paranthropus*

The name *Paranthropus* is derived from two Greek words that translate into 'beside human'. The 'robust' australopithecines are classified in *Paranthropus*. 'Robust' in this case refers not only to their burlier build but also to their extremely large jaws, premolars and molar teeth (Figure 10.27). One species, *P. boisei*, discovered in Ethiopia in 1959, was nicknamed 'nutcracker man' because it was assumed its strong jaws and large molars were used for crushing and grinding hard, fibrous foods, such as nuts. More recent microwear evidence of fossil teeth suggests the diet of *P. boisei* was much more varied, and nuts were not a staple food item. Despite the striking fossil evidence for *Paranthropus* skulls, there is limited evidence for the remainder of the skeleton. The little that has been recovered for *P. boisei*, for example, shows the pelvis and hip joint are similar to members of genus *Australopithecus*. This suggests it was bipedal but does not prove that walking was its main mode of locomotion.

Paranthropus species persist in the fossil record from around 2.5 to 1 mya. Based on their age and on anatomical features, scientists variously consider *Paranthropus* species to be descendants of *Australopithecus* or as yet unidentified ancestral *Paranthropus* species. Whatever the origin, scientists agree that *Paranthropus* is an extinct side branch to the direct line from which modern humans evolved.

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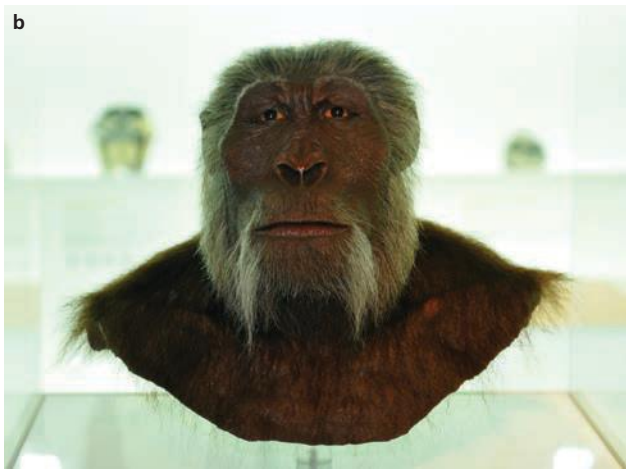

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◀ **Figure 10.27** *Paranthropus boisei*: (**a**) fossil skull showing the prominent mandible and the saggital crest along the midline of the cranium (**b**) artist's reconstruction

Oldowan technology

Australopithecines apparently occupied home bases from which they ventured to forage for food. There is no evidence to date for fire use among australopithecines. Stone tools, such as choppers, scrapers and chisels, have been recovered in areas where australopithecine fossils are found. These relatively simple tools are described as **Oldowan** technology, named for the site in Tanzania where they were first discovered. Most implements are about the size of a tennis ball or smaller (Figure 10.28). Oldowan tools were sculpted to shape by striking the tool stone with a **hammerstone** to chip off flakes. Although relatively simple, Oldowan tools demonstrate their makers were fashioning materials towards some imagined end product. They were also using precision grip to carry out the work. Oldowan tools represent the first stage of technological evolution in hominins, presumably enabling australopithecines to exploit their environment more effectively.

Getty Images/Javier Trueba/MSF/SPL

▲ **Figure 10.28** Samples of Oldowan tools

Oldowan tools date from around 2.5 to 1.2 mya. They are found dispersed along the east coast of the African continent and throughout the Old World. These observations suggest Oldowan technology was migrating with early hominins. They also show that cultural evolution was underway. The technology was transmitted across massive geographical areas over many generations.

RECALL

- Hominin evolution is represented by a 'bushy' evolutionary tree. Interpretations about the fossil record are a matter of ongoing debate and refinement as new discoveries are made.
- Australopithecines were relatively small, bipedal apes.
- Australopithecine species are grouped into the genera *Australopithecus* and *Paranthropus*, distinguished as gracile and robust forms, respectively.

RECAP 10.6

1 What does it mean if a species is sexually dimorphic?
2 What was the evolutionary fate of genus *Paranthropus*?
3 *Australopithecus afarensis* shows a mosaic of features that suggests it was both arboreal and bipedal. Defend this statement with evidence.
4 What does the craftsmanship and distribution of Oldowan stone tools indicate about hominin evolution?

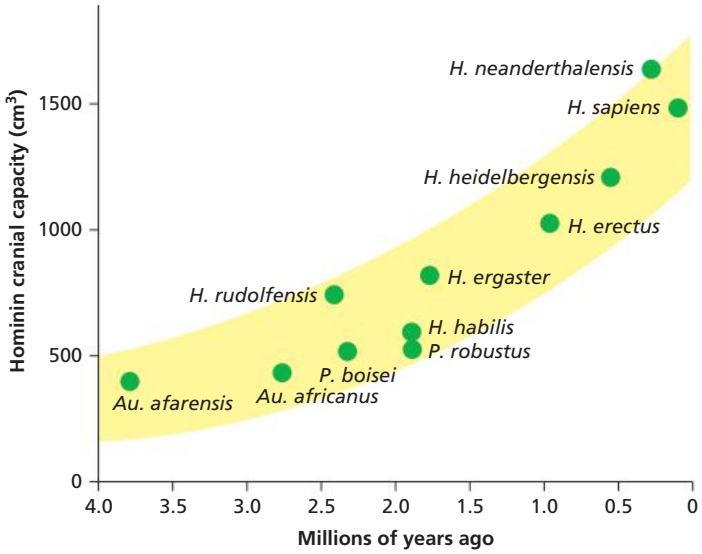

Figure 10.29 ▲
Graph showing the gradual increase in cranial capacity of hominins over time. The average cranial capacity for each species is presented.

Evolution of genus *Homo*

The evolution of genus *Homo* is associated not only with refinements to bipedalism but also expansion of the cranium (Figure 10.29). There are ten species classified in genus *Homo*, including *H. sapiens*. The composition of genus *Homo* is continually being revised. For example, a new species, *H. naledi*, was added in 2015 based on the discovery of ancient skeletal remains in a South African cave system. These remains are yet to be dated. In addition, the validity of a few revisions is often questioned. Some argue that one or another of the existing species should be split into more. In this section we review three key fossil species that highlight what is understood and what is debatable about the course of hominin evolution.

A transitional fossil: *Homo habilis*

Homo habilis is described mainly from fragments of skull (Figure 10.30), hand and arm bones discovered in Tanzania in 1960. Dating to 1.8 mya, *H. habilis* is one of the earliest fossil hominins verified to show an increased cranial capacity (Figure 10.29), although its total volume was less than half that of modern humans. It was also the earliest hominin

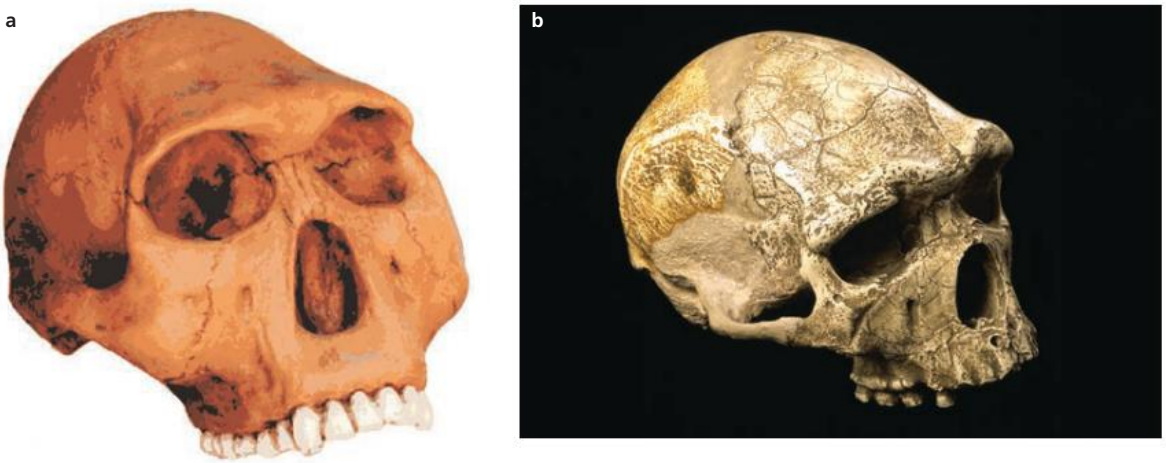

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Figure 10.30 ▲
Detail of skulls from (**a**) *Homo habilis* and (**b**) *Homo erectus*. Note the thickened midline on the *Homo erectus* skull, referred to as a **sagittal keel**.

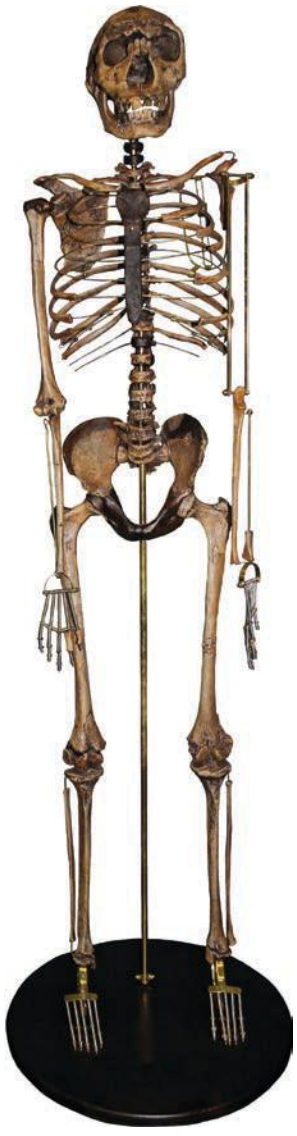
to be found unequivocally associated with Oldowan stone tools, providing direct evidence for the use of technology. These features were used to justify its placement in genus *Homo* and it was accordingly named *Homo habilis*, or 'handy man'. The assertion has been contested by some scientists, however, because selected fossils assigned as *H. habilis* have arm and leg dimensions resembling those of australopithecines.

H. habilis is currently interpreted as a transitional fossil showing features of both the australopithecines and the genus *Homo*. Use of stone tools by *H. habilis* indicates a significant advance had occurred in the cognitive abilities of hominins.

Homo erectus

With the evolution of *Homo erectus* (Figure 10.30b), humans figuratively and literally hit their stride. The **postcranial** anatomy of this species (Figure 10.31) resembles that of modern humans, indicating they were dedicated bipeds capable of walking long distances and running, if necessary. Its fossils are found throughout Africa, Europe and predominantly in Indonesia and China. This indicates *H. erectus* is the earliest known hominin to migrate out of Africa, dispersing across the Old World. In China, repeated cooling and drying through three glacial periods encouraged the establishment of grasslands, attracting large grazing animals that *H. erectus* might have hunted.

The cranial capacity of *H. erectus* was greater than that of the australopithecines and *H. habilis* (Figure 10.29), suggesting further evolution of cognitive abilities. Hands were no longer used for climbing but had become more refined for manipulating objects. Stone tools have been found with *H. erectus* fossils in western Asia, Europe and Africa (see below). Their smaller teeth indicate the diet of these hominins had changed in some way compared with australopithecines. *Homo* species were possibly eating different foods or possibly eating the same foods but preparing them differently, for example by cooking them before eating them. Deer, antelope, boar and fish bones found at various sites indicate some of the prey items of the *H. erectus* diet. Burnt stone and animal bones, charcoal and ash deposits dating to ~0.5 mya suggest *H. erectus* used fire (Figure 10.32), but it is difficult to prove that fire was controlled.



Alamy/Sabena Jane Blackbird

▲ **Figure 10.31**
A relatively complete skeleton of *Homo erectus*

Getty Images/Christian Jegou

◀ **Figure 10.32**
An artist's impression of a *Homo erectus* camp site. Males in the foreground use fire to fashion spears, while a couple in the background skin an animal.

Getty Images/Prisma/UIG

Figure 10.33 ▲
Acheulean hand axes

H. erectus is the earliest hominin that combines modern human dentition, fully upright posture, obligatory long-range bipedalism, and at least a middle-sized brain.

Acheulean technology

The stone tools found with *H. erectus* fossils are characterised mainly by tear-drop or pear-shaped hand axes. The tools are described as **Acheulean** technology, named for the site in France (St Acheul) where they were first discovered. The axes are 12–20 cm long and are crafted by chipping on both faces of the stone (Figure 10.33). These tools appear in the fossil record from around 1.6 million to 200 000 years ago.

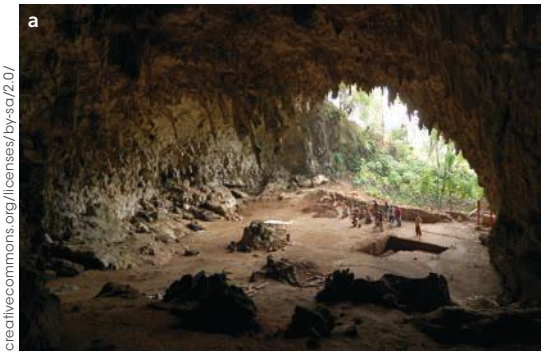
Homo floresiensis

Widely regarded as the most surprising fossil hominin find in decades, *Homo floresiensis* was discovered in 2003 by a joint Australian–Indonesian team. Skeletal remains unearthed in Liang Bua cave on the island of Flores in Indonesia (Figure 10.34a) are dated to between 100 000 and 60 000 years old. The most important specimen, dubbed 'the Hobbit', is described from unfossilised skeletal remains of an adult female (Figure 10.34b, c). Standing at just 1.1 m tall, the Hobbit was a diminutive hominin. Associated with ample stone tool artefacts, *H. floresiensis* evidently hunted and processed island fauna for meat. Charred bones demonstrate that *H. floresiensis* exploited fire for cooking. How *H. floresiensis* arrived on Flores is unknown. Even with the sea level changes occurring in the last million years, Flores was never connected to mainland Asia and is separated by tens of kilometres of sea. Chance colonisation by drift rafting is a possibility. Archaeological evidence indicates *H. floresiensis* occupied Liang Bua cave from at least 190 000 years ago until 50 000 years ago. Stone tools older than 800 000 years also have been found on Flores and may belong to *H. floresiensis* or to an earlier hominin. The disappearance of *H. floresiensis* broadly correlates with the timing of modern humans' arrival on Flores. There is, however, no direct evidence that the two species interacted.

The discovery of *H. floresiensis* rattled the field of **palaeoanthropology** for a couple of reasons. First, the remains were originally dated to between 38 000 and 18 000 years old, suggesting that *H. floresiensis* coexisted with modern humans until relatively recently. That suggestion was rejected in 2016 after the dating evidence for the remains and surrounding deposits was re-examined. The remains are now considered to be older, from 100 000 to 60 000 years old.

Second, and more compelling, the origin of *H. floresiensis* is mysterious. One possibility is that *H. floresiensis* evolved from *H. erectus* that had migrated into Asia. This interpretation recognises similarities in the shapes of their skulls, particularly the

Figure 10.34 ▼
(**a**) Liang Bua cave on the island of Flores, the excavation site where *Homo floresiensis* was unearthed (**b**) The skeleton and (**c**) detail of the skull of the *H. floresiensis* specimen dubbed 'the Hobbit'.



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Chip Clark, Smithsonian, Human Origins Program

Alamy/Sabena Jane Blackbird

brow ridges and sagittal keel. If true, *H. floresiensis* must have evolved to become smaller after its ancestors settled on Flores. Examples of **insular dwarfism** have occurred on other islands around the world. Dwarfism presumably evolves in colonising species that experience long-term isolation with a restricted food supply and limited predators. The smaller cranium of *H. floresiensis* (~400 cm^3 compared with other ancient hominins) (Figure 10.34c) may be such an evolutionary adaptation to reduce the brain's energy demand. This proposal is supported by fossils of other extinct miniature species on Flores, such as those of *Stegodon*, an unusual form of pygmy elephant.

An alternative proposition for the origin of *H. floresiensis* is drawn from the primitive features of its body. *H. floresiensis* had relatively long arms and short legs with long feet, which are more like those of australopithecines. This interpretation is supported by australopithecine features of the wrist, hip and collar bones. It may be that *H. floresiensis* descended from an australopithecine or a *Homo habilis*-like ancestor and always was of a comparable size to them. If this were the case, *H. floresiensis* could have initiated the earliest independent migration of hominins out of Africa. Whatever its origin, *H. floresiensis* ultimately represents an extinct side branch to the direct line of human evolution.

RECALL

- Evolution of genus *Homo* is associated with expansion of the cranium, as well as enhancements in bipedal evolution.
- The discovery of *Homo floresiensis* challenged existing assumptions about hominin evolution.

RECAP 10.7

1 What evidence demonstrates that the cognitive abilities of hominins had advanced with the evolution of *Homo habilis*?

2 What do the postcranial anatomy and the global distribution of fossils indicate about bipedalism in *Homo erectus*?

3 What does its dentition suggest about the *Homo erectus* diet? What other evidence may support or refute that assertion?

4 Outline two aspects of the *Homo floresiensis* anatomy that argue for contradictory origins.

EXPERIMENT 10.1

IS 'ARDI' A HOMININ?

There are very few fossils of possible hominins dating to before ~4.2 mya and their identity as hominins is frequently debated. The most informative fossil from that time period is a nearly half-complete skeleton of a species described as *Ardipithecus ramidus* (nicknamed 'Ardi'). Discovered in Ethiopia in 1994, the delicate work of excavating and reconstructing Ardi took 15 years.

Scientists debate whether Ardi is a direct ancestor of modern humans, an early side branch of hominin evolution, or a representative of an altogether separate group from the humans and the chimps.

Aim

To examine the characteristics of *Ardipithecus ramidus* and evaluate its classification as a hominin

Observations

Examine the data for *Ardipithecus ramidus* outlined in Table 10.4 and Figures 10.35 and 10.36. Record your observations for the following features:

- cranial capacity
- relative size of the brow ridges

- relative size of the canines
- amount of prognathism
- length of limbs relative to the rest of the body
- length of the thumb relative to other digits of the hand
- shape of the spine
- shape of the pelvis
- carrying angle
- length of hallux relative to other toes of the foot.

Table 10.4 Characteristics of *Ardipithecus ramidus*

Characteristic	Value
Age	~4.4 million years
Sex	Female
Height	1.2 m
Weight	50 kg
Cranial capacity	350 cm^3

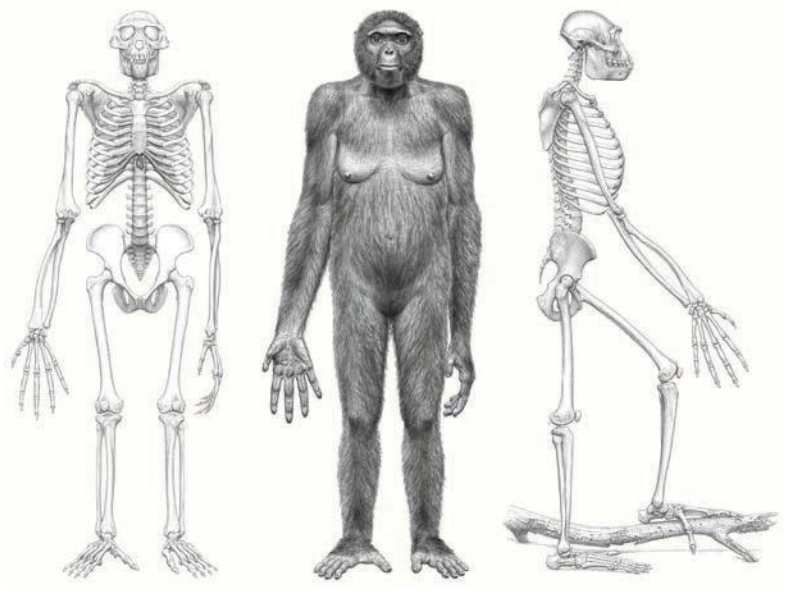


Figure 10.35 ▲
Sketches of the front view (left) and profile view (right) of the skeleton of *Ardipithecus ramidus*, together with an artist's reconstruction (centre)

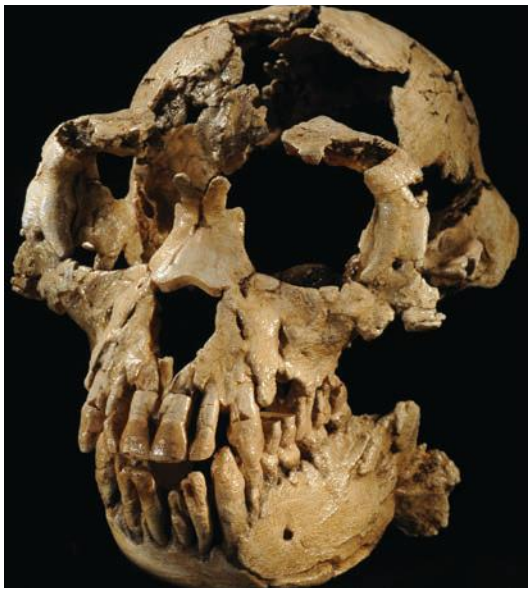


Figure 10.36 ▲
A 4.4 million-year-old reconstructed fossil skull of *Ardipithecus ramidus, found in the Aramis site, Middle Awash Valley, Afar depression, in northeast Ethiopia, housed in the National Museum of Ethiopia*

Discussion

1. What attribute(s) would you consider important in determining whether a hominoid was a hominin? Explain what features you might expect to see in the skeleton of the hominoid if it possessed those attribute(s).
2. What can you deduce about Ardi's mode of locomotion? Would you say she was quadrupedal, bipedal or both? Did she climb trees? What evidence do you have to support your interpretations?
3. Do the cranial features of Ardi (brow ridges, canines, prognathism) show more similarities with hominins or with other hominoids?
4. What is cranial capacity supposed to indicate about a hominoid? What does Ardi's cranial capacity?
5. Use the evidence and your interpretation to argue the case for including or excluding *Ardipithecus ramidus* in the hominins. Is there anything more you would need to know about the fossil that would help you make your decision?

Conclusion

Write a brief conclusion stating your decision to include or exclude Ardi as a hominin and summarise your reasons.

Modern humans and Neandertals: a case study in current palaeoanthropology

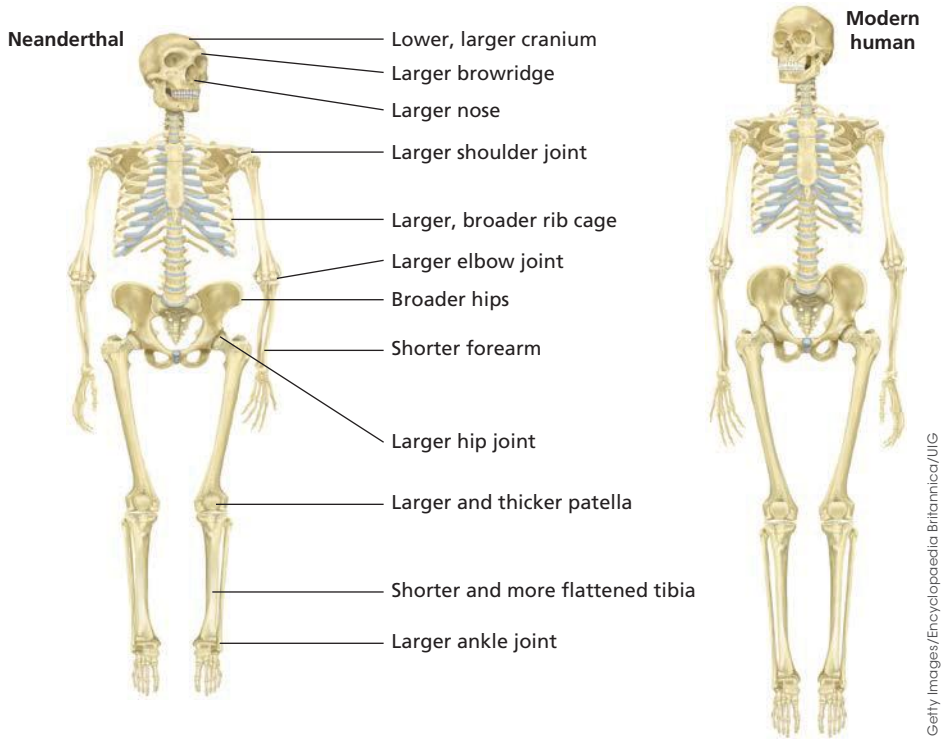
The first formal record of fossil hominins to be excavated was in 1856 in north-western Germany. Initially mistaken for the remains of a bear, the fossils would famously come to be known by the name of the valley in which they were exhumed: Neandertal (traditionally 'Neanderthal'). The discovery launched the field of palaeoanthropology and ignited public imagination. An icon of pop culture, the Neandertal was rendered as a 'dull-witted prehistoric brute' (to paraphrase the early 20th century palaeontologist Marcellin Boule). No other ancient hominin has enjoyed as much attention or fascination.

The relationship between Neandertals and modern humans spawns many contentious questions that engage scientists and the public alike. Why did the Neandertal disappear while modern humans survived? Did Neandertals and modern humans coexist peacefully or were they competitors, or adversaries? Did they interbreed? Are modern humans today descended from Neandertals? In this section, we investigate these questions. Pursuing the answers is as much a study of the advances in biology as it is about understanding human evolution. We draw on a range of evidence, from over a century and a half of palaeontology to the most recent developments in biotechnology.

Fossil evidence for *Homo neanderthalensis*

Today, thousands of Neandertal fossils have been recovered, portraying the image of a hardy, resourceful people that is at odds with the pop culture stereotype.

The distinctive facial appearance of Neandertals arises from the enlarged brow ridge, sloping forehead and expanded nose. They also had a larger average cranial capacity than modern humans. Stockier than modern humans, Neandertals had a flared rib cage, accommodating an expanded abdomen, and relatively shorter limbs (Figure 10.37). Well-formed muscle attachments indicate strong muscular bodies and a strenuous



◀ **Figure 10.37**
Skeletal reconstruction of *Homo neanderthalensis* (left) and modern *Homo sapiens* (right).

lifestyle. Given that Neandertals had to survive episodes of glaciation, these features have been traditionally interpreted as adaptations for conserving heat in a cold climate. This view is contentious. For example, it was accepted for a long time that the broader nose was associated with a larger sinus cavity, enabling a greater volume of air to be warmed during inhalation. This view was contradicted by 2D X-ray analysis of skulls that revealed Neandertal sinus cavities are similar in size to those of modern humans. An alternative hypothesis is that the unique Neandertal morphology arose by genetic drift in a relatively small, sparsely distributed population.

Alamy/The Natural History Museum

Figure 10.38 ▲ Mousterian tools associated with Neandertals

Mousterian technology

Ample archaeological evidence shows Neandertals were using relatively advanced stone tools. This **Mousterian** technology (named from a site in France) dates from ~300 000 years ago to ~30 000 years ago. Mousterian tools are mostly found throughout Europe but evidence for the technology also occurs in the Middle East and northern Africa. The technology appears to have evolved from Acheulean industry and is characterised by sharp, pointed blades (Figure 10.38) crafted by chipping flint, a type of dark quartz. Near the end of their history, Neandertal sites are found with flint-based serrated blades normally associated with modern humans, suggesting Neandertals were copying or trading the technology.

Neandertal lifestyle

The Neandertal diet was mixed, and varied according to what was locally available. Chemical analysis of fossil teeth residues and faecal deposits provides direct evidence for the consumption of starchy tubers, nuts, fruits, grasses and meat. Bone remains indicate Neandertals effectively hunted and butchered game, particularly reindeer, but also bigger prey such as bison and mammoths. Asymmetric anatomy and frequent broken bones suggest Neandertals hunted by thrusting spears at large game at close range. Archaeological evidence shows that Neandertals built hearths and controlled wood-fuelled fires for cooking and for warmth. Neandertals consistently took refuge in caves and rock shelters, a practice that contributes to the cave man stereotype. Indeed, the rich fossil record for Neandertals exists because a number of deceased Neandertals were buried in caves.

There is evidence that Neandertals buried their dead and occasionally marked their graves. Although disputed, there is no definite evidence that Neandertal burials were associated with rituals, nor is there any rock art firmly attributed to them. For these reasons, the prevailing if controversial view is that Neandertals were pragmatic and even altruistic but they displayed little of the symbolic expression that defines the art and ceremonies of *Homo sapiens*.

The African cradle: Evolution of modern humans

Towards the end of the 20th century there were two competing hypotheses for the evolution of *Homo sapiens*, modern humans.

The first was that *H. sapiens* evolved independently a number of times, descended from localised populations of *Homo erectus*. This is described as the **multiregional origin** hypothesis. The strictest interpretation of the hypothesis suggests that

individualised characteristics of local *H. erectus* populations evolved through genetic drift. These characteristics were transmitted to locally evolving *H. sapiens* that distinguish different populations of people in the world today. The idea was highly controversial as much for its racial as its scientific implications. A less restricted version of the hypothesis accommodates migration, interbreeding and gene flow between different evolving human populations. The core principle of the multiregional hypothesis is that modern humans evolved simultaneously across all colonised continents.

The competing hypothesis was that modern humans evolved initially in Africa and then migrated across the world, displacing earlier hominins as they advanced. This model is referred to as the **recent single origin** hypothesis, or more informally as the **Out-of-Africa hypothesis**. This hypothesis accommodates the possibility that *H. sapiens* interbred with localised populations of ancient humans. The distinction is that the migration of modern humans was a juggernaut. Older hominin populations were assimilated into a dominant modern human population, if they were not wiped out altogether.

In the 21st century, on the balance of fossil, molecular and cultural evidence, the Out-of-Africa hypothesis is more widely accepted. What is that evidence?

Fossil evidence

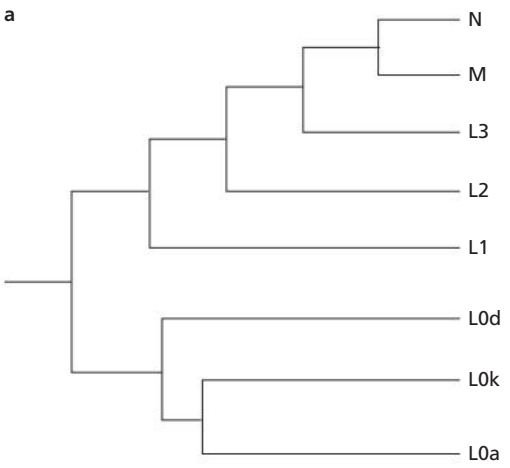
Anatomically modern humans first appear in the African fossil record ~160 000 years ago. Fossil evidence suggests modern humans evolved first in central Africa. As climatic conditions changed, drought in central Africa pressured modern humans to migrate to the eastern seaboard. A coastal existence may have contributed to cognitive evolution by supplementing the diet with seafood rich in omega-3 fatty acids. Modern humans then migrated northward and southward along coastal routes. There were at least two major migration waves north. The first occurred ~100 000 years ago but terminated after reaching the Middle East. The second wave occurred ~60 000 years ago and pushed on into Europe and Asia. Modern human fossils in Europe dating from ~40 000 to ~10 000 years ago are traditionally referred to as 'Cro-Magnon Man', after the French cave where the first specimens were discovered. The anatomical proportions of early Cro-Magnons' skeletons in Europe resemble those of modern Africans rather than modern Europeans, supporting the Out-of-Africa hypothesis. This second wave ultimately seeded the human population worldwide and brought the ancestors of the Aborigines to Australia.

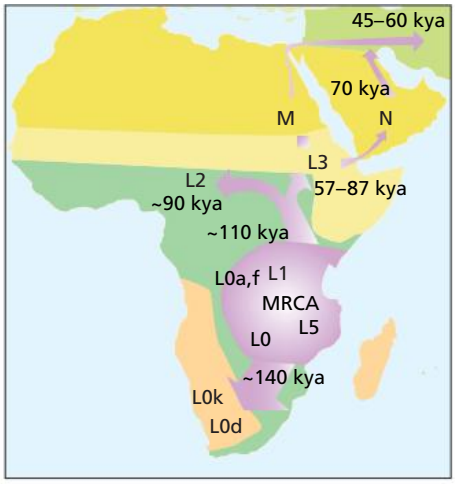
Evidence from mitochondrial DNA

See Chapter 9 for more on molecular homology and the application of mtDNA.

From the late 1980s, mtDNA was used to explore evolutionary relatedness among modern humans. The mtDNA was chosen because its pattern of maternal inheritance provided a relatively uninterrupted lineage of descent from ancestral populations. Global human populations were grouped according to the specific mutations in their mtDNA: members of a group that share the same mutations must be descendants of a common ancestor. These are described as **haplogroups**. Using molecular homology, phylogenetic trees were produced from the mtDNA haplogroups.

It was discovered that, among modern humans, most of the variation in mtDNA sequences occurs in African populations (L haplogroups, Figure 10.39). The mtDNA of Europeans, Asians and the Indigenous peoples of Australia, the Americas and Pacific islands represent just a subset of total human mtDNA diversity (M and N haplogroups, Figure 10.39). This provides further evidence for the Out-of-Africa hypothesis. Molecular clock estimates suggested diverse populations of modern humans evolved over 200 000 years in Africa, with the haplogroups that migrated out of Africa diverging ~70 000 years ago. Superimposing the phylogenetic tree on a map of Africa strengthens the case for migration (Figure 10.39). The two surviving mtDNA groups (M and N) that colonised the other continents are most closely related to the African L3 group located north-east of Africa and nearest to the Middle East.





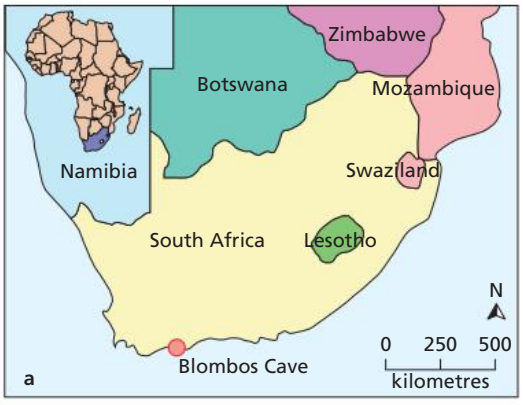
Sources: Mitochondrial eve tree by Wapondaponda, licensed under CC BY-SA 3.0 via Wikimedia Commons; Early Diversification map by Pdeliker; public domain via Wikimedia Commons.

Figure 10.39 ▶
(**a**) Phylogenetic tree generated from mtDNA sequences of global human populations. The labels at the tips of the tree represent the haplogroups into which human mtDNA mutations can be classified. (**b**) A map showing the location of each haplogroup and migration patterns inferred from them taking place thousands of years ago (kya). All haplogroup originate in Africa and the Middle East. Only the M and N haplogroup are found in Indigenous populations throughout the rest of the world. MRCA = most recent common ancestor.

Evidence of art and culture

Hominin evolution is associated with the evolution of tools. Evolution of modern humans, however, is associated with resources being used for artistic, as well as functional, purposes. Ancient art represents the most enduring record of symbolic expression during human evolution. The first convincing signs of art are associated with anatomically modern humans ~120 000 years ago in scattered sites in South Africa. Blombos Cave in South Africa provides evidence of continuous human occupation for over 100 000 years. It is a significant site for tracking the development of art and culture. Consistent use of particular artistic styles, or 'industries', appears there by ~90 000 years ago. The evidence includes artefacts such as perforated seashell 'beads', engraved ostrich eggs, and patterned engravings in stone tools, bone and ochre (Figure 10.40), as well

Figure 10.40 ▼
(**a**) Map showing the location of Blombos Cave. (**b**) Interior of Blombos Cave. Aretefacts found at the cave include (**c**) a stone block with patterned carvings and (**d**) an abalone shell used to prepare ochre. (**e**) A reconstruction of Blombos Cave bead work using modern shells



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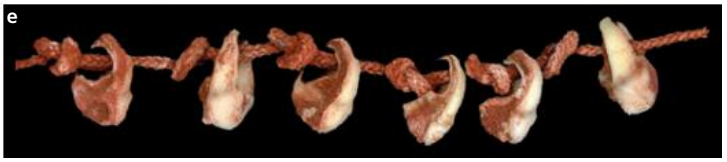


Copyright Courtesy of Marian Vanhaeren and Christopher Henshilwood, Cape Field School, University of the Witwatersrand

as evidence for symbolic burial practices. The appearance of industries is significant because it shows that human groups were organising according to shared beliefs, values and behavioural practices. That is, they demonstrate the establishment of culture. Furthermore, some resources at the excavation site, such as abalone shells filled with liquefied ochre, originated from distantly separated locations. This implies the cave inhabitants had the capacity to plan. They were identifying resources, relocating and storing them at their 'workshop', and combining them for later use.

The quantity, sophistication and geographical distribution of human art expands abruptly around ~40 000 ago. This is largely represented by cave art in Europe and Australia, including paintings, engravings and carvings. Australia's Aboriginal cave art represents the longest unbroken record of ancient art in the world. As modern humans cooperated in larger groups, art would have served to bond members and define group identities.

RECALL

- Neandertals were ancient hominins with distinctive anatomical features that coexisted for a time with modern humans.
- Neandertals demonstrated cultural and technological evolution but little symbolic expression or art.
- There are two competing hypotheses for the evolution and dispersal of modern humans: the multiregional origin hypothesis and the recent single origin hypothesis.
- Evidence from fossils, mitochondrial DNA sequences and cultural artefacts mainly support the recent single origin, or Out-of-Africa, hypothesis.

RECAP 10.8

1 Describe three differences between the skeletal anatomy of the Neandertal and the modern human.
2 Outline the key differences between the multiregional hypothesis and the recent single origin hypothesis.
3 Describe fossil evidence that supports the Out-of-Africa hypothesis.
4 What are 'haplogroups' and how do they support the Out-of-Africa hypothesis of modern humans?

The relationship between modern humans and Neandertals

It is unclear why the second wave of modern human migration was more successful than the first. It may be that modern humans in Africa developed better survival skills in the intervening period. Or perhaps they were cooperating in larger or better organised groups. Whatever the causes, it is certain that, during the second migration wave, anatomically modern humans coexisted with Neandertals across Europe and Asia. What was the outcome of this encounter?

A split in the tree

Ongoing speculation about interbreeding between Neandertals and modern humans was fuelled in part by discoveries of fossils of presumed anatomical hybrids. At the beginning of the 21st century, modern molecular methods enabled ancient DNA to be isolated and sequenced. This development offered an innovative approach to exploring evolutionary relatedness. Among the first extinct species to be studied was the mitochondrial DNA of sufficiently preserved Neandertal fossils from Asia to western Europe spanning

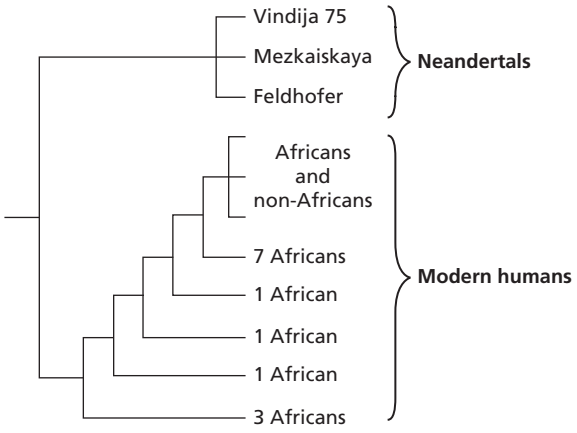


Figure 10.41 ▲
A cladogram for mtDNA sequences derived from ancient DNA of fossil Neandertal bones (Vindija, Mezkaiskaya and Feldhofer samples) and DNA from modern humans. The tree shows that the Neandertal and modern humans samples diverged into separate branches of the tree.

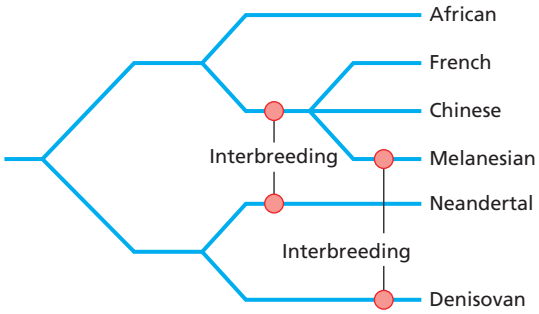
from 70 000 years to 30 000 years ago. These were compared with mtDNA sequences of modern human populations. The data showed that the degree of variation within Neandertal mtDNA was relatively narrow. Similarly, the variation within the mtDNA of modern humans was relatively restricted. The mtDNA sequences of modern humans and Neandertals were extremely different and shared no overlap (Figure 10.41). These early studies offered no proof of interbreeding between Neandertals and modern humans. Rather, the evidence suggested they diverged as two isolated populations.

The branches later cross again

In 2010, scientists published a draft nuclear genome sequence from the ancient DNA of Neandertal bones. This extraordinary technical feat overcame challenges posed by the size of the genome (over 3 billion nucleotides) and the age of the samples (~40 000 years old). The Neandertal DNA was severely degraded and heavily contaminated by bacterial DNA. The genomes of the scientists working on the project were also sequenced and compared to ensure their DNA had not contaminated the Neandertal samples.

The nuclear genome of the Neandertal was compared with nuclear genomes of various modern humans. The comparison revealed that 1–4% of the genomes of modern Europeans and Asians are uniquely identical to those of the Neandertal. However, these sequences are not shared between genomes of the Neandertal and sub-Saharan African populations. The simplest interpretation is that 1–4% of the nuclear DNA of modern humans living outside Africa was derived from Neandertals. This constitutes evidence for a limited amount of interbreeding between Neandertals and modern humans. Estimates suggest the Neandertal alleles entered the modern human population between 40 000 and 80 000 years ago. It is proposed that modern humans encountered and interbred with Neandertals as they migrated out of Africa and through the Middle East. This is the reason the signature for Neandertal DNA today is found mainly in descendants of Europeans and Asians but not those of African populations.

As no living human has been found to contain mtDNA of Neandertals, it is likely that individuals of those lineages have not survived to the present. In 2016, it was reported that chromosome 21 was sequenced from two European Neandertal specimens dating to ~30 kya. The sequences provided evidence for earlier interbreeding between Neandertals and modern humans. This was estimated to have occurred ~100 kya, most likely in the region around the Middle East. The evidence indicates multiple interbreeding events between Neandertals and modern humans.



Based on artwork by Jamie Kendrick/Science Brainwaves and Nature.

Figure 10.42 ▲
Patterns of divergence and subsequent interbreeding during human evolution. The tree is drawn from evidence revealed by mitochondrial and nuclear DNA sequences.

Another branch, another crossing

In 2010, scientists announced the discovery of bone fragments from a previously unknown ancient hominin in Denisova Cave in Siberia. The bone fragments and the few associated artefacts were dated to ~40 000 years old. The anatomy of the so-called **Denisovan** hominin remains a mystery but good quality DNA was recovered and sequenced from a single finger bone. The mitochondrial DNA of the Denisovan indicated it was more closely related to the Neandertals but was sufficiently different to be a distinct branch in the hominin evolutionary tree. Phylogenetic trees show that the split between the Neandertals and Denisovans occurred after their common lineage diverged from modern humans (Figure 10.42). Molecular clock estimates date the divergence between the Neandertal/Denisovan lineage and the modern human lineage at ~800 000 years ago. The Neandertals and Denisovans subsequently diverged ~640 000 years ago.

Analysis of nuclear genomes reveals that modern Melanesians share 4–6% of their DNA sequences uniquely with the Denisovans. No other modern humans carry this signature of the Denisovan nuclear DNA. The evidence suggests Denisovans interbred with ancestors of Melanesian populations as they were migrating through southern and south-eastern Asia. It is estimated this interbreeding occurred at least 44 000 years ago. The descendants are the Indigenous populations of Papua New Guinea and Australia, as well as representative populations of the Philippines and some south-east Asian islands. The majority of Chinese and south-eastern Asian populations, however, lack Denisovan DNA. This suggests multiple waves of modern human migration through eastern and south-eastern Asia, only some of which resulted in interbreeding with Denisovans. Figure 10.42 summarises the divergence and interbreeding patterns of modern humans, Neandertals and Denisovans. Our understanding of gene flow between prehistoric hominins and modern humans will continue to be re-evaluated whenever ancient remains are sequenced.

The evolution of domestication and civilisation

The most significant advance in the last 10 000 years or so has been the human practice of domesticating species by artificial selection. The development of agriculture depended upon the availability of domesticable species. The Fertile Crescent in the Middle East was the home range for a variety of suitable species, including wild wheats, barley, peas, cows, sheep, pigs and goats (Figure 10.43). This good fortune of biogeography is principally why the earliest farmers and herders were located in that region. The plants and animals they domesticated are still some of the most valuable species to humans today.

Alamy/Images of Africa Photobank

◀ **Figure 10.43**
(**a**) Map showing the location of the Fertile Crescent (green) (**b**) Artwork from Ancient Egypt depicting a cow being milked

Domestication also depended on there being advantageous genes and alleles in humans, as well as in wild plant and animal species. Just as humans selected for favourable traits in other species, the novel sources of nutrition applied selection pressures on modern humans. For example, modern dairying populations have higher allelic frequencies for the gene for lactase persistence. People who produce lactase in adulthood can digest the lactose sugar in milk derived from cattle or goats. Individuals in populations with a long history of agriculture tend to have more copies of the salivary amylase gene. This adapts people for a starch-rich diet of cultivated grains. The traits favoured by humans, however, tend to make domesticated species less capable of surviving if re-introduced into the wild. Domesticated species are therefore reliant upon humans for their dispersal and survival. In essence, humans and their domesticated species have co-evolved and become interdependent.

Traditional hunter-gatherers were nomadic, chasing the seasonal wild food supply. The transition to an agricultural lifestyle enabled humans to settle and eventually to specialise and develop heavy technology. Modern humans have advanced technology to an exceptional level. The tools of today enhance the speed, scale and efficiency of energy production, transportation (e.g. motor vehicles and commercial air travel), long-range communication (e.g. television, telephones and Internet), cognitive processing (e.g. computers and software), and humanity's capacity to perceive the imperceptible (e.g. microscopes, telescopes, subatomic particle accelerators).

Crops are grown at higher densities than they occur in the wild, and the enhanced supply of kilojoules is a key driver of increased human population sizes. The rate of human population growth has accelerated with the pace of technological innovation and industrialisation (Figure 10.44). Today, the human population exceeds 7 billion people, and humans have colonised every continent. The scale of the human population now affects every other plant and animal species. The impacts of human activities include depletion of wild species through overharvesting, habitat destruction, widespread pollution, the spread of invasive species and accelerated climate change.

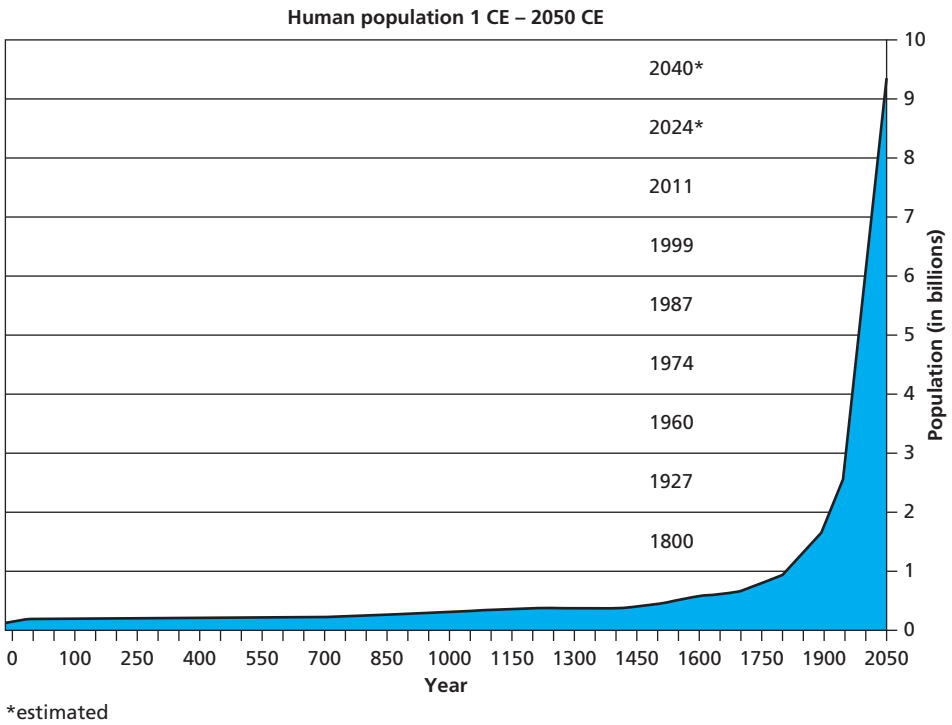


Figure 10.44 ▶
Growth in the human population during the last 2000 years

Homo sapiens is an extraordinary outcome of evolution: a conscious, social ape with an unprecedented capability to manipulate its environment. It is one species that can, to a considerable extent, shape its own future and that of countless other species. In the course of your lifetime, years from now, how will that future unfold?

RECALL

- Evidence from mitochondrial DNA suggests Neandertals and modern humans diverged up to 800000 years ago.
- Evidence from nuclear DNA sequences suggests select groups of modern humans later interbred with Neandertals and another group of ancient hominins called the Denisovans.
- About 10000 years ago, the shift from hunter-gatherer to agricultural lifestyles accelerated cultural and technological evolution.

RECAP 10.9

1 Explain how mitochondrial DNA shows that the Neandertal and the modern human lineages diverged some 800000 years ago.

2 Which modern humans have the signature of Neandertal DNA in their nuclear genomes today, and which modern humans do not? How do these observations fit with the Out-of-Africa hypothesis?

3 Who are the Denisovans, and how do they help to explain patterns of migration and colonisation by modern humans?

4 Describe two aspects of the shift to agriculture that made possible the expansion of the human population.

CONCEPT SUMMARY

The taxonomy of modern humans

Phylum Chordata

Class Mammalia

Infraorder Eutheria (*placental mammals*)

Order Primata

Parvorder Catarrhini

Superfamily Hominoidea

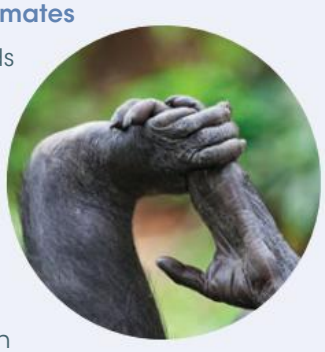
Family Hominidae (*great apes*)

Tribe Hominin (*bipedal*)

Genus Homo

Species sapiens

Characteristics of primates



- Five digits on hands and feet
- Innermost digit is opposable
- Hands and feet are prehensile
- Tips of digits have flattened nails on one side and sensitive touch receptors on the other
- Forward facing eyes allowing stereoscopic vision (in colour)
- Reduced snout and fewer teeth
- Flexible hips and shoulders allowing for modes of locomotion: brachiation, suspensory locomotion, quadrupedalism and bipedalism
- Enlarged brain

Characteristics of hominoids

- Dentition (eight pre-molars and molars with five cusps and a Y-shaped valley)
- No tail
- Flattened rib cage, broader chest and shortened spine between rib cage and pelvis – allows sitting upright
- Mostly have longer forelimbs than hindlimbs (not humans) and dorsally situated shoulders; orthograde locomotion

Characteristics of hominins

- Bipedalism: centralised foramen magnum, spinal curvature, pelvis shape, carrying angle, feet for propulsion rather than gripping
- Relatively hairless
- Expanded cranium and greater cognitive capacity, reduced brow ridges accommodating a larger prefrontal cortex
- Reduced canines and less prominent mandible
- Precision grip using hands
- Language, culture, technology, symbolism

The evolution of modern humans

Updated regularly as new evidence is discovered

Australopithecines

- Small bipedal apes inhabiting eastern and southern Africa, 4.2–1.4 mya
- Their evolution accompanied climate change that caused forested areas to become grassland
- Genus Australopithecus (gracile forms): five species including *A. afarensis*
- Genus *Paranthropus* (robust forms): three species, not considered to be ancestors of humans

Au. afarensis

- For example, 'Lucy'
- 3.8–2.9 mya
- A direct ancestor of the modern human
- Sexually dimorphic: Males were bigger and bore a sagittal crest at the top of the skull
- Bipedal features: Wide and shallow pelvis, femurs angled in towards the knees, strengthened weight-bearing knees, arched feet, wide heels, and its hallux aligned with the other toes; fossilised footprint impressions in Tanzania support this
- Was a good climber and ate mainly leaves and fruits
- Small cranial capacity – bipedalism came before cranial expansion
- Created Oldowan tools using a hammerstone – the beginning of cultural evolution

Genus *Homo*

Currently considered to be nine species, but this is subject to debate

Homo habilis

- 1.8 mya
- Fragments of skull, hand, and arm bones found in Tanzania
- Had increased cranial capacity and used Oldowan stone tools
- A transitional form

Homo erectus

- 1.6 mya–200 000 years ago
- Fossils found in Africa, Europe, and mainly in Indonesia and China
- First *Homo* species to migrate out of Africa
- Fine manipulation of objects and Acheulean technology, modern human dentition, fully upright posture, obligatory long-range bipedalism, and at least a middle-sized brain.

Homo floresiensis

- At least 95 000–12 000 years ago
- Liang Bua cave in Flores, Indonesia
- Coexisted with modern humans and may have evolved from *H. erectus*
- Reduced in size via insular dwarfism
- Alternatively, could have evolved from an australopithecine or *H. habilis* and this may have been the earliest hominin branch to have migrated out of Africa

Homo neanderthalensis

- 300 000–30 000 years ago
- Mousterian technology, flint-based serrated blades
- Stocky and muscular with larger cranial capacity than modern humans
- Ate a varied diet that included prey, probably hunted by throwing spears at close range
- Used fire for cooking and heating and often took refuge in caves and rock shelters
- Buried their dead
- Diverged from *H. sapiens* 800 000 years ago and from *H. denisovans* 640 000 years ago

Homo sapiens

- 160 000 years ago
- Modern humans
- Went from hunter-gatherer to agricultural lifestyles approximately 10 000 years ago, which accelerated cultural and technological evolution
  - Multiregional origin hypothesis: Modern humans evolved independently multiple times from *H. erectus* via genetic drift with gene flow occurring between populations.
  - Recent single origin hypothesis: Modern humans evolved in Africa and then migrated across the world, displacing more ancient hominins as they went. This is supported by fossils, mtDNA sequences and cultural artefacts.
  - Modern human mtDNA contains 1–4% Neandertal mtDNA, suggesting they interbred 80 000–40 000 years ago. Nuclear DNA sequence similarities suggest that Melanesian *H. sapiens* interbred with Denisovans at least 44 000 years ago.

CHAPTER GLOSSARY

Acheulean a culture defined by stone tools from 1.5 million to 150 000 years ago and associated with *Homo erectus*

arboreal related to, or living in, trees

australopithecine a member of a group of small, bipedal apes that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

bipedalism a type of locomotion in which an organism walks on two hind limbs

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

brow ridges the bony ridges located above the eye sockets

carrying angle the angle at which the femur is tilted in towards the knee

cerebral cortex the outermost layer of the brain

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions and apply new knowledge and skills

convolutions the folds on the surface of the brain

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

cultural evolution the way beliefs, social practices, skills and technology change over time

Denisovan a distinct, but undescribed, ancient hominin known only from bone fragments found in Denisova Cave in Siberia

foramen magnum the hole in the base of the skull through which the spinal cord passes

gracile of slender build

hallux the big toe, or innermost toe of the foot

hammerstone a hard stone used to chip off stone flakes to shape a tool stone

haplogroup a group of people that share the same genetic mutations and are descendants of a common ancestor through either the maternal or paternal line of inheritance

hominin a term for a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a term for a member of the superfamily Hominoidea; an ape, or tail-less primate

infraorder a taxonomic rank immediately above superfamily

insular dwarfism the trend for large mainland animals that colonise islands to evolve into smaller forms

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

mandible the lower jawbone of the skull

mirror neurons specific neurons that fire when an animal either performs an action or observes another animal performing the same action

Mousterian a culture defined by stone tools from 300 000 to 30 000 years ago and associated with *Homo neanderthalensis*

multiregional origin a hypothesis that modern humans evolved from more ancient hominins simultaneously on all colonised continents

Oldowan a culture defined by stone tools from 2.5 to 1.2 mya and associated with australopithecines

orthograde a type of locomotion in which fore and hind limbs move in opposition to one another

Out-of-Africa hypothesis See **recent single origin**

palaeoanthropology the field of study concerned with fossil hominins

postcranial all of the skeleton, except the skull

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manoeuvre an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of grasping

prognathism a condition in which the jaws protrude from the plane of the face

quadrupedalism a type of locomotion in which an organism walks on four limbs

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents

robust of sturdy build

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

sexual dimorphism the situation where males and females of a species have different morphologies, often in shape or size

stereoscopic describes vision that has a sense of depth

superfamily a taxonomic rank immediately superior to the traditional rank of family; a superfamily may contain multiple taxonomic families

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

tribe a taxonomic rank inserted between family and genus

CHAPTER REVIEW QUESTIONS

Remembering

1. A palaeoanthropologist excavated a primate mandible. He observed that it had six premolar teeth and the molars each had four cusps.
   - **a** Has he discovered a hominoid?
   - **b** What evidence supports your answer?
   - **c** If he excavated the rest of the skeleton, what other anatomical feature might he find that would prove it is or is not a hominoid?
2. Describe how the fingers and toes of modern humans differ from the digits of other great apes. What advantages do these adaptations confer to modern humans?
3. Summarise features of the postcranial anatomy of modern humans that adapt them for bipedal locomotion.
4. Describe at least five changes that have occurred during the evolution of the hominin skull and indicate likely reasons for why they came about.

Understanding

5. Sketch a cladogram that traces the evolutionary relationships among hominoids. On the cladogram, include the genera *Pongo*, *Gorilla*, *Pan*, *Australopithecus*, *Paranthropus* and *Homo*. Justify your positioning of each of the genera in the cladogram. Explain whether the hominins in your cladogram are a monophyletic or paraphyletic group.
6. Explain how three anatomical and/or behavioural features of *Homo sapiens* have contributed to cultural evolution.
7. Is cranial capacity enough to explain the cognitive capacity of modern humans? Explain your point of view and provide evidence, wherever available, to support it.

Applying

8. Discuss the advantages conferred on primates by at least three adaptations that enabled them to live in trees.
9. Describe key observations from mitochondrial and nuclear DNA of modern and ancient hominins that provide evidence for patterns of migration and interbreeding.
10. Draw an annotated timeline from 2.5 mya to 100 000 years showing:
    - **a** the appearance of stone technologies and other cultural artefacts
    - **b** which hominins the technologies were associated with
    - **c** what these artefacts say about hominin cultural and technological evolution.

Analysing

11. Alleles for lactase persistence occur in many human populations around the world. The alleles are different, for example, between North African, European and Indian populations, but the phenotype is the same: lactase production persists beyond infancy and adults can digest lactose in milk. Studies of ancient DNA show the alleles were present at low proportions in early dairying populations between 5000 and 10 000 years ago. Explain what has happened to result in higher frequencies of lactase persistence alleles in modern human populations. Your explanation should discuss:
    - **a** why the alleles differ in different populations
    - **b** which events represent natural selection
    - **c** which events represent artificial selection
    - **d** which events represent cultural evolution.
12. Social cooperation is observed in insect species such as termites and honey bees. Would you argue that this social cooperation is similar to or different from that observed in humans? Outline the evidence in support of your argument.

Evaluating

13. Consider the representative species of genus *Homo* discussed in this chapter. How satisfied are you with their designation as distinct species, and what reasons do you give in support of your response?

14 Humans exercise great control over their environment and often adapt the environment to their needs. Have humans therefore ceased to evolve? Provide evidence to justify your response.

Creating

15 Write an account of the changes in diet during hominin evolution from australopithecines to modern humans. Support your account with evidence from a range of sources, including the fossil record, comparative anatomy, biogeography and archaeological artefacts.

16 Sketch and annotate a drawing of what you predict the common ancestor of the chimpanzee and modern human might have looked like. Summarise the features you expect to see in the ancestor and list dot points to explain your reasoning.

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CHAPTER 11
DNA MANIPULATION

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

KEY KNOWLEDGE

DNA manipulation

- the use of enzymes including endonucleases (restriction enzymes), ligases and polymerases
- amplification of DNA using the polymerase chain reaction
- the use of gel electrophoresis in sorting DNA fragments, including interpretation of gel runs
- the use of recombinant plasmids as vectors to transform bacterial cells.

Biological knowledge and society

- techniques that apply DNA knowledge (specifically gene cloning, genetic screening and DNA profiling) including social and ethical implications and issues
- strategies that deal with the emergence of new diseases in a globally connected world, including the distinction between epidemics and pandemics, the use of scientific knowledge to identify the pathogen, and the types of treatments

KEY SCIENCE SKILLS

Analyse and evaluate data, methods and scientific models

- process quantitative data using appropriate mathematical relationships and units

Draw evidence-based conclusions

- draw conclusions consistent with evidence and relevant to the question under consideration

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Figure 11.1 ▶
Domestication is one of the earliest examples of biotechnology.

Most of the farm animals we know today were domesticated between 10 000 and 4000 years ago. Early humans used the principle that offspring of individuals presenting good traits (e.g. larger size, faster growth, better milk production and improved fertility) were also likely to express those traits. Animals were selectively bred in order to improve, over time, the quality of farmers' herds. The same is true for rice, wheat and other crops. Present-day varieties bear little resemblance to their wild ancestors. Today, new scientific techniques enable us to develop new breeds of domestic animals with enhanced phenotypes by changing their genetic sequence. In this chapter we will explore some basic techniques that have been borrowed from biology for use in a wide range of technological applications, such as changing the genetic sequence of organisms.

Biotechnology through the ages

The term **biotechnology** describes the use of living things to make new products or systems. While the term may be new, the concept is not. Traditional biotechnology has been with us ever since people began to grow crops and domesticate animals. Early Egyptian, Babylonian and Sumerian civilisations used micro-organisms to create bread, beer and wine. In recent times, increased knowledge of cell systems and molecular biology has revolutionised biotechnology, promising potential benefits for agriculture, the environment and medicine.

The gene revolution

Modern biotechnological techniques now enable scientists to manipulate the outcome of normal functioning genes to meet the needs of science and society in a more precise way than ever before. Because of this, much of modern biotechnology is called **genetic engineering**. This term simply means changing the genetic sequence of an organism through human use of modern biotechnology techniques. Such genetically engineered organisms are called **genetically modified organisms (GMOs)**.

The term genetic engineering applies to a range of techniques and processes for investigating and modifying DNA, genes and **genomes** of species. It is possible for scientists to use genetic engineering to switch genes on or off, remove genes and introduce genes from one species into another. For example, a US company created a genetically modified salmon that grows twice as fast as wild salmon. This was achieved by introducing a gene from a different fish species into the salmon genome.

Common genetic modifications include:

- Cutting out genes or gene segments to prevent expression or proper functioning of particular gene products (called **knock-out organisms**)

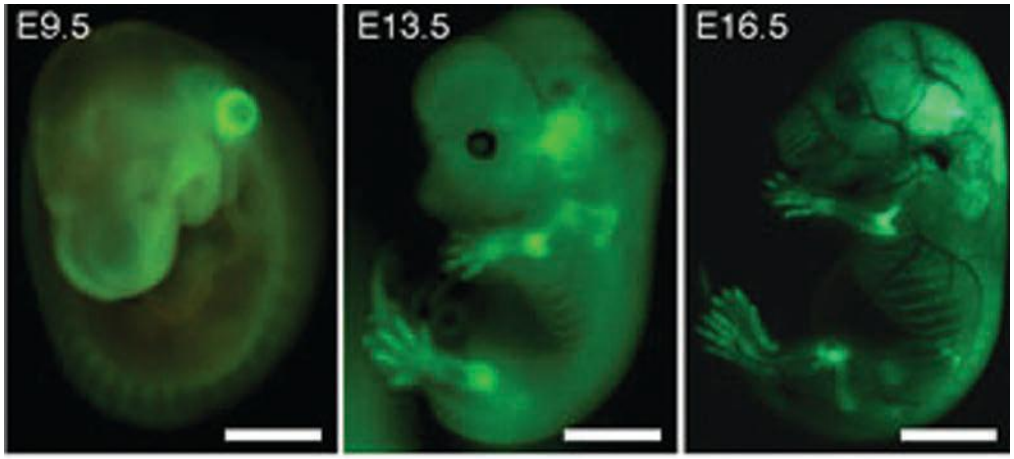
This technique is commonly used in research labs to study the functions of the knocked-out gene in animal models, particularly mice. Biological processes or disease models can be studied in the knock-out animals and the functions of the gene inferred from the differences between the knock-out animals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

- Inserting genes into a specific locus so that they are controlled by a particular promoter in the organism's genome (called **knock-in organisms**)

This technique can be used to insert a reporter gene, such as green fluorescent protein (GFP), into the locus of a gene whose pattern of expression is unknown. The reporter gene will be expressed under the control of the promoter of the gene of interest. The readout of the reporter gene, such as green fluorescence, can be measured in different cells and tissues to give information about the location and timing of expression of the gene of interest (Figure 11.2). Inserting the reporter gene can sometimes inhibit the activity of the gene of interest, and in this case the knock-in organism is also a knock-out organism for the gene.

- Inserting DNA from one organism into the genome of another unrelated organism in a non-specific locus (creating a **transgenic organism**)

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



Nakamura, Y. et al. Wwp2 is essential for palatogenesis mediated by the interaction between Sox9 and mediator subunit 25. Nat. Commun. 2:251 doi: 10.1038/ncomms1242 (2011). Figure 1.

◀ Figure 11.2 Expression of Sox9, which is important for normal skeletal development, in mouse embryos at different days of embryonic development (E9.5 = embryonic day 9.5, for example), measured by knocking GFP into the Sox9 locus

Case study box 1: Overview – Making insulin to treat type 1 diabetes

Insulin is a peptide hormone that promotes uptake of sugar from the bloodstream and storage in muscle or adipose tissue. It is essential for normal metabolism; without insulin, the body relies on fat as an energy source. This can result in the build-up of dangerous substances in the blood, which can be life-threatening. In type 1 diabetes the insulin-producing cells of the pancreas come under autoimmune attack and are unable to produce sufficient insulin. To survive, people with type 1 diabetes must take up to four insulin injections a day. With around half a million children affected worldwide, and incidence increasing by around 3% each year, how can enough insulin be made?

Bacteria can be grown quickly in controlled environments and in large batches. Bacterial cells can be lysed (ruptured) and proteins extracted from their cytosol. Genetic engineering of *E. coli* has allowed the human insulin gene to be inserted into bacteria, replicated to vast numbers as the bacteria replicate, and expressed by the bacteria. It is then ready for extraction, purification and distribution as the injectable therapy that is a lifesaver for many people worldwide.

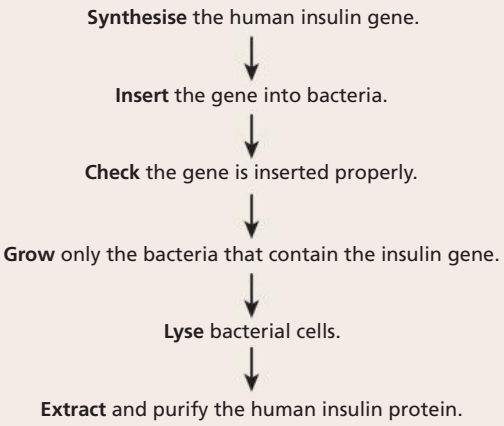


Figure 11.3 ▲
Steps involved in producing insulin in bacterial cells

Throughout this chapter, in case study boxes such as this, we will progressively explore how the production of insulin demonstrates a common sequence of biotechnology techniques used in a wide range of applications.

RECALL

- Biotechnology refers to the use of living things to make new products or systems. Modern biotechnology is called genetic engineering. It uses tools such as enzymes derived from living organisms.
- Organisms that are altered or produced using genetic engineering techniques are known as genetically modified organisms.
- Genetically modified organisms include knock-out organisms, knock-in organisms and transgenic organisms.

RECAP 11.1

1 List three types of genetic modification.
2 How has genome sequencing made genetic modification possible?
3 What type of genetically modified organism is the fast-growing salmon?

Genetic engineering techniques

In genetic engineering, just as in the construction of buildings, tools are used for specific purposes. Biotechnology has its own set of specialised tools, which are mostly derived from other organisms. These include tools for synthesising, cutting and pasting DNA, along with tools and techniques for viewing and analysing DNA. New biotechnological tools are continually being developed as understanding of biological mechanisms grows. An example is the recent development of the CRISPR genome

editing system, based on a bacterial defence mechanism. In this system, the bacterially derived Cas9 enzyme uses a short piece of RNA to guide it to a complementary target site in genomic DNA. Here, it creates a double strand break (which would naturally target bacteriophage DNA for destruction inside infected bacterial cells), which the host cell's machinery tries to repair. This often results in the insertion or deletion of nucleotides, causing a frameshift mutation that interferes with the translation of the targeted gene and results in a knock-out organism. If a donor DNA fragment is present, the DNA repair machinery may incorporate it at the target site, resulting in a knock-in organism. This technique has greatly simplified the processes involved in genetically modifying organisms.

While new technologies such as CRISPR continue to emerge and change the face of biotechnology, some tried and tested basic techniques have been in use for some time. These are described below.

Amplifying DNA: polymerase chain reaction

Each eukaryotic somatic cell has only two copies of a gene of interest and prokaryotic cells have only one copy. This small amount of DNA poses a problem for scientists wishing to work with it. Similarly, only a small sample of DNA may be available for analysis, for example, at a crime scene or DNA samples obtained from bones. The first step in any work sequence involving DNA analysis or use in genetic engineering is to make enough copies of the DNA of interest to be able to work effectively. To increase the amount of DNA of a particular sequence the biotechnologist has an important tool to work with: the **polymerase chain reaction (PCR)**. PCR makes use of the enzyme **DNA polymerase**, which catalyses the formation of new DNA molecules in the nucleus from free nucleotides. PCR is used to amplify, or make many copies of, a specific sequence of DNA.

A number of components are required: the DNA to be copied (the template), DNA polymerase, a buffer solution that contains salts and other chemicals that help the polymerase to function, a supply of the four nucleotides (A, T, C, G) from which to build the new DNA molecules, and two **primers**. The primers are short sequences (around 20 nucleotides) of single-stranded DNA, complementary to the nucleotide sequences at either end of the DNA section that is to be copied. These are necessary as a starting point from which the DNA polymerase can add new DNA nucleotides. DNA polymerase can only extend a DNA strand from an existing nucleotide; it cannot create a new complementary strand without primers to begin extending from.

PCR has three steps (Figure 11.4).

1 Denaturation: the double-stranded DNA is heated to 95°C, breaking the hydrogen bonds between the bases and thus causing the two strands to **denature**. This is sometimes called the melting stage.
2 **Annealing**: the temperature is reduced to 50–60°C, allowing the primers to anneal (join) to complementary sequences on opposite ends of each strand: either genomic DNA in the first cycle or PCR products generated during the previous cycle. The reduced temperature is necessary to allow base pairing and the formation of hydrogen bonds.
3 Extension: the temperature is raised to 72°C, the optimum temperature for the particular DNA polymerase used in PCR. Starting from the primers, new DNA strands are synthesised in the 5' to 3' direction using DNA polymerase and the available nucleotides. At the end of this phase there are two copies of the double-stranded DNA.

This cycle is repeated until sufficient quantities of the DNA are obtained to work with. Each cycle doubles the number of DNA strands; therefore, in just 20 cycles more than one million copies of target DNA will be produced. There are usually around 30–35 cycles in a PCR program.

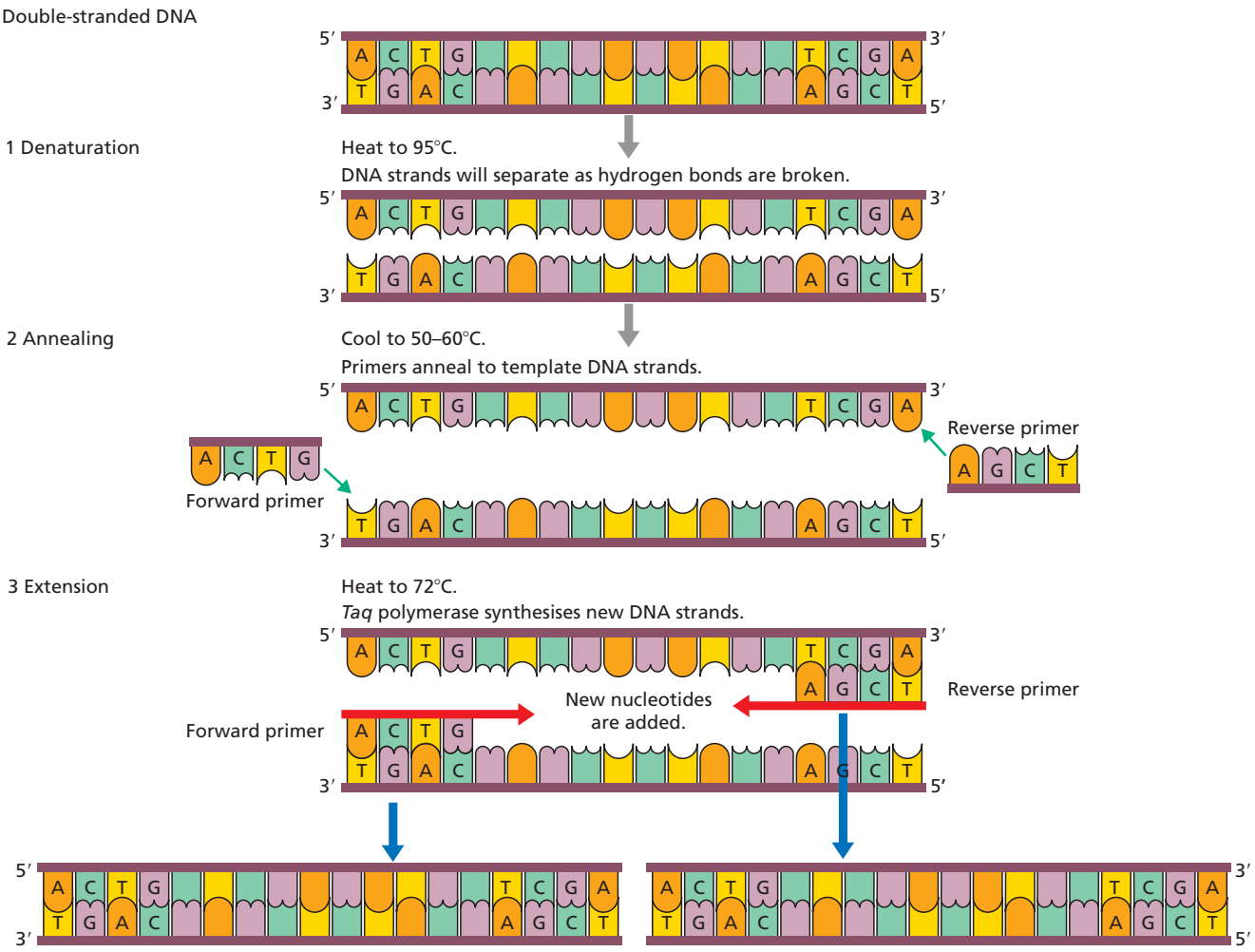


Figure 11.4 ▲
Amplifying DNA using PCR

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Figure 11.5 ▶
A thermal cycler, in which the PCR is carried out as an automated process

RECALL

- PCR is a process that amplifies a specific DNA sequence for analysis.
- The sequence of the primers determines the DNA sequence to be amplified.
- Steps involved in PCR include denaturation, annealing and extension. These steps are repeated many times to yield a large number of identical DNA molecules.

RECAP 11.2

1 State the components of a PCR reaction.
2 Outline the three main steps of PCR.
3 If you start with five copies of a DNA region, how many copies will be produced if your sample goes through 10 cycles of PCR?

Case study box 2: Synthesising the human insulin gene

Before DNA such as the human insulin gene can be used in genetic engineering, it usually needs to be copied, or amplified, to produce a quantity that is feasible to work with. Bacterial DNA does not contain introns, and bacteria do not have the machinery to splice them out of mRNA. For a eukaryotic gene such as the insulin gene to be expressed in *E. coli*, the DNA inserted must contain the exons (coding regions) only. In this situation the DNA template for PCR amplification is mRNA molecules (exons only). In a first reaction, these are converted back into DNA strands, now called copy DNA (cDNA).

Primers for PCR must be designed so that they bind on either side of the cDNA region to be amplified. One primer binds to the template strand and the other to the complementary strand. The nucleotide composition of the primer determines its annealing temperature because it determines the number of hydrogen bonds that form between the primer and template strand. Because G–C complementary nucleotides par with three hydrogen bonds, they require more kinetic energy (a higher temperature) to separate than do A–T nucleotides, which pair with two hydrogen bonds.

Synthesise the human insulin gene

PCR master mix:
- Template DNA (cDNA, which is a copy of the mRNA of the human insulin gene)
- DNA polymerase
- Buffer for DNA polymerase (includes magnesium ions and cofactors for DNA polymerase function)
- Primers
- Free nucleotides
- Water

PCR program on the thermal cycler:
1 Initial denaturation (5 min, 95°C)
2 Denaturation (30 s, 95°C)
3 Annealing (20 s, 50–60°C depending on the nucleotide composition of the primers)
4 Extension (30 s per 1 kb template, 72°C)
5 Final extension (5 min)

Return to step 2 ×35 cycles

▲ **Figure 11.6** Outline of the PCR reaction mixture (master mix) and the steps programmed into the thermal cycler

Cutting DNA: restriction endonucleases

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

Restriction enzymes occur naturally in bacteria, where they cleave (cut) 'foreign' DNA that enters from invading viruses, thus destroying any potential threat. In essence, they are the immune system of a bacterium. Restriction enzymes are named according to the bacterial strain from which they are derived. The first restriction enzyme was isolated from *Escherichia coli* RY13 strain and was thus named *Eco*RI. Table 11.1 identifies a number of common restriction enzymes and their source.

Table 11.1 Common restriction enzymes and their restriction sites

Enzyme	Bacterial source	Restriction site	After cutting
*Eco*RI	*Escherichia coli*	5′G ⋮AATTC3′ 3′CTTAA⋮G5′	5′G AATTC3′ 3′CTTAA G5′
*Hind*III	*Haemophilus influenzae*	5′A ⋮AGCTT3′ 3′TTCGA⋮A5′	5′A AGCTT3′ 3′TTCGA A5′
*Alu*I	*Arthrobacter luteus*	5′AG ⋮CT3′ 3′TC ⋮GA5′	5′AG CT3′ 3′TC GA5′
*Bam*HI	*Bacillus amyloliquefaciens H*	5′G ⋮GATCC3′ 3′CCTAG⋮G5′	5′G GATCC3′ 3′CCTAG G5′

To date, almost 4000 different restriction enzymes have been identified. Although each enzyme recognises a specific sequence of between four and eight nucleotide base pairs (bp) of the double-stranded DNA, multiple enzymes isolated from different organisms can recognise the same sequence. Restriction enzymes bind to their restriction site and cut the double-stranded DNA at that point. The cuts may form either overhanging steps, called **sticky ends**, which leave some nucleotides exposed (Figure 11.7a), or **blunt ends** (Figure 11.7b), in which the cut has occurred at the same position in each strand of the DNA and there are no overlapping strands. Spontaneous hydrogen bonding between overhanging nucleotides helps restriction fragments with sticky ends to bind to other DNA fragments with complementary sticky ends.

In molecular biology, restriction enzymes are used in **restriction digest reactions**, in which the substrate is usually a PCR product.

Figure 11.7 ▲
(**a**) Sticky ends produced by cutting DNA with the restriction enzyme *Eco*RI; (**b**) Blunt ends produced by cutting DNA with the restriction enzyme *Alu*I

RECALL

- Restriction enzymes cleave DNA strands at recognition sites that are specific for each enzyme.
- Restriction enzymes can generate blunt or sticky ends. In molecular biology, complementary sticky ends help DNA strands bind to each other via hydrogen bonding.

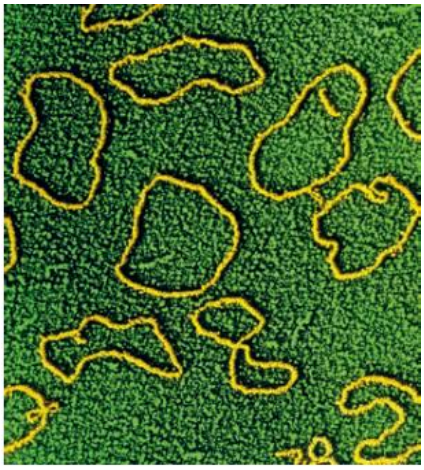
RECAP 11.3

1 Name the two types of restriction enzymes.

2 State the functions of the following enzymes:
- **a** DNA polymerase
- **b** restriction endonuclease.

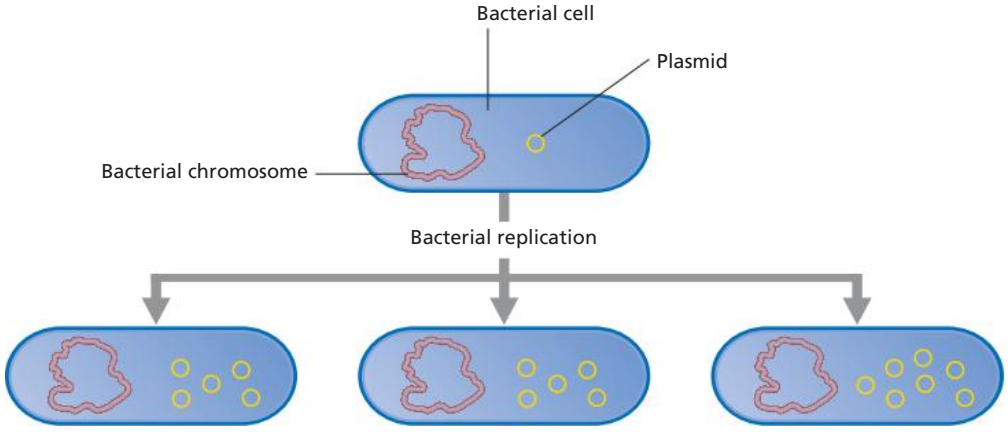
Manipulating DNA: plasmids

Short linear DNA fragments produced by PCR or cut out from a chromosome by restriction digest are unstable – they do not survive long in cells or in a test tube, and can lose base pairs from the ends through enzymatic or mechanical degradation. They are also often too small to manipulate in the lab. To get around these problems, scientists use bacterial **plasmids**. A plasmid is a circular piece of DNA that is found in bacteria and which reproduces independently of the bacterial chromosome (Figure 11.8). DNA fragments, such as PCR products amplified from a gene of interest, can be inserted into plasmids that have been cut open, then the plasmid can be closed up again, incorporating the DNA fragment.



Getty Images/SPL/Dr Gopal Murti

▲ **Figure 11.8** A transmission electron micrograph of bacterial plasmids from *Escherichia coli*



◀ **Figure 11.9** Cloning a gene using recombinant plasmids

In biotechnology, plasmids are ideal **vectors** (meaning vehicles or carriers) for DNA fragments. They are copied many times within the bacterial cells and are copied when the bacteria replicate, and this also copies any DNA fragments inserted into them (Figure 11.9). Because they are circular, they are much more stable than linear fragments. Their stability also allows them to survive the harsh conditions that are used to rupture the bacterial cells and purify the plasmid DNA. They are small enough to be able to be distinguished from the main bacterial chromosome, but large enough to be extracted and manipulated in the lab. They can also be easily engineered to carry a number of different genes or DNA elements such as promoters and restriction sites, making them ideal tools for manipulation of DNA fragments. Promoters on plasmids can allow genes to be expressed in prokaryotes and/or eukaryotes depending on the application. The use of plasmid vectors in DNA technology is discussed further on page 388.

Joining DNA: DNA ligase

At times molecular biologists may want to combine two samples of DNA, such as when they insert a piece of DNA into a plasmid. **DNA ligase** is an enzyme used to join different pieces of DNA together. DNA ligase acts by forming a phosphodiester bond between the two fragments of DNA. It joins the 3′ hydroxyl end of one nucleotide with the 5′ phosphate end of another nucleotide. DNA ligase requires magnesium ions and ATP for its activity, so DNA ligation is an energy-consuming reaction.

The success rate in joining two strands of DNA together using DNA ligase is vastly improved if the two strands are brought together somehow. If the restriction enzymes used to cut the DNA generate sticky ends, two DNA fragments that have been cut with

the same enzyme will have identical sticky ends and thus the complementary bases will be exposed. This means that if the ends of the two strands come into contact with each other by chance, their nucleotides will form hydrogen bonds at the sticky ends and remain in place, leaving just a nick in the DNA backbone to be ligated. DNA ligase can then be used to recombine these two fragments by creating a covalent phosphodiester bond between them, even if they are from two unrelated organisms. For example, *Eco*RI can be used to cut both human DNA and bacterial plasmid DNA, leaving sticky ends that are complementary and able to bond to each other (Figure 11.10). Fragments with blunt ends can also be joined by DNA ligase, but this process is much less efficient. Sticky end ligation also ensures the joined DNA fragments are the right orientation when joined. The technology that recombines DNA from different sources to modify the DNA sequence is called **recombinant DNA technology**.

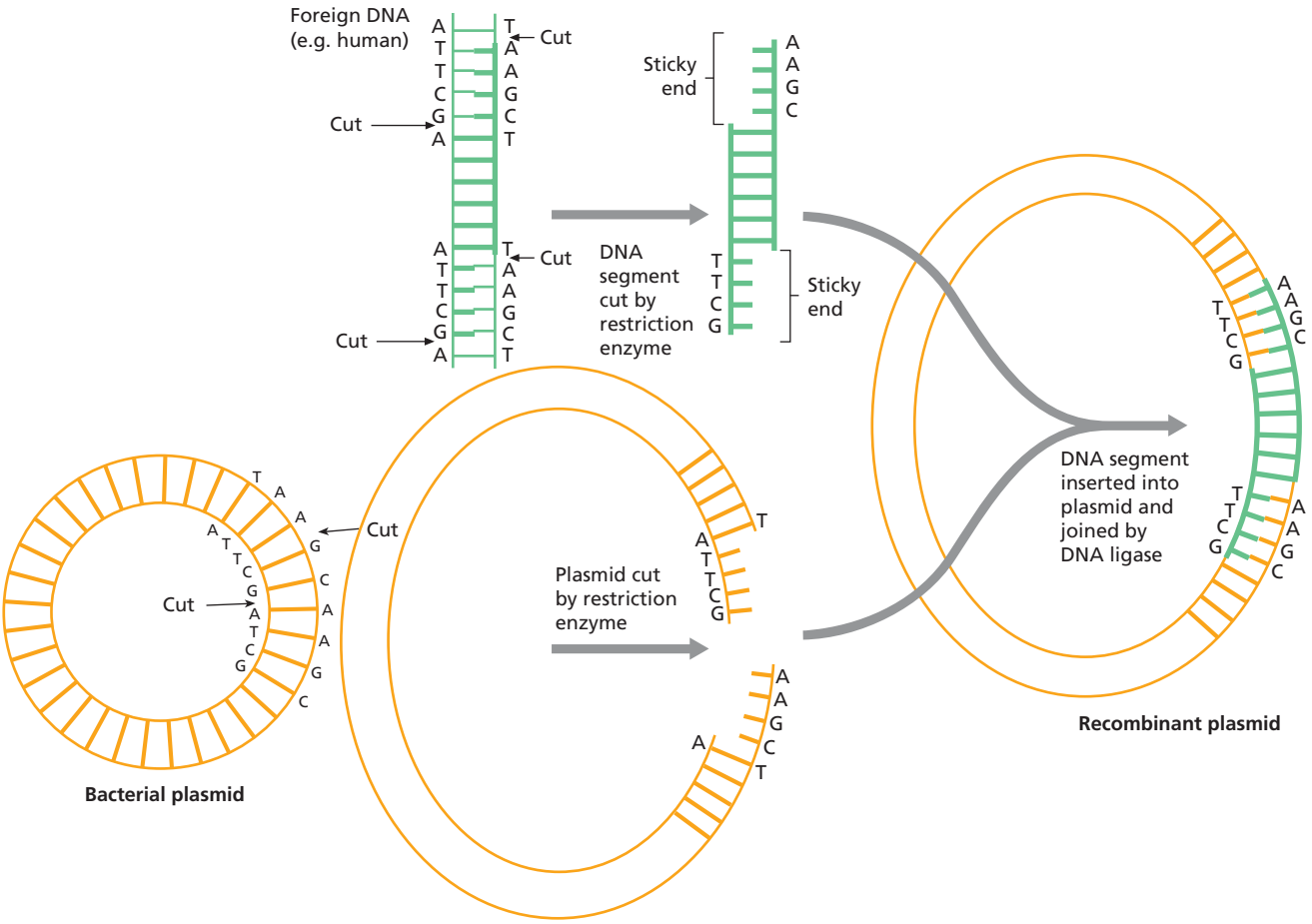


Figure 11.10▲
DNA ligases join DNA inserted from a foreign source that has complementary sticky ends.

RECALL

- Plasmids are circular pieces of DNA normally found in bacteria separate from the bacterial chromosome.
- Plasmids can be used as vectors for manipulation and replication of DNA fragments in biotechnology.
- DNA ligase is an enzyme used in biotechnology for joining two pieces of DNA together.

RECAP 11.4

1 List five factors that make plasmids useful tools in biotechnology.
2 Name five components that would be added to a DNA ligation reaction.
3 Why can DNA ligase join DNA from organisms of two unrelated species?

Case study box 3: Creating a recombinant plasmid with the insulin gene

Once the human insulin gene has been amplified by PCR, it is ready for insertion into a plasmid vector. To do this, the PCR product (the insulin gene) can be cut with two different restriction enzymes that give it different sticky ends at either side. The plasmid can be cut with the same restriction enzymes, and so the sticky ends of the insulin gene hydrogen bond with the complementary ends of the plasmid and the gene slots into the plasmid in the correct orientation. All that is left is for the DNA ligase to seal the nicks in the DNA backbone and then the recombinant plasmid is complete.

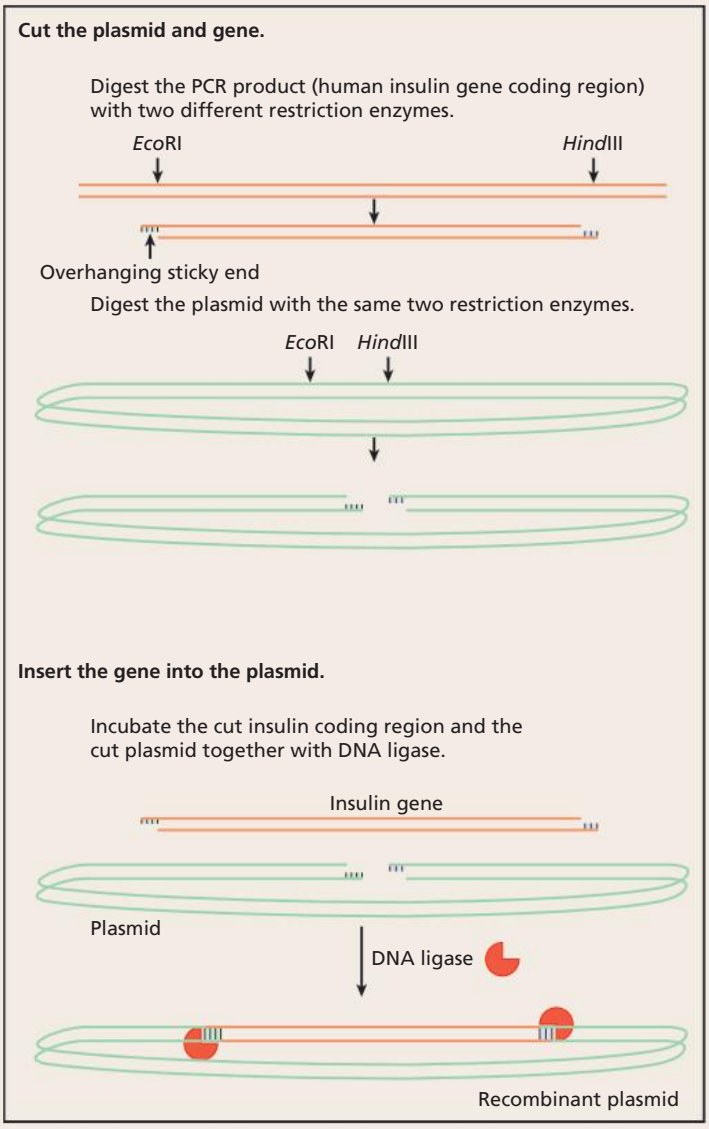


Figure 11.11 ▶ Inserting the human insulin gene into a plasmid

Visualising DNA

DNA molecules are far too small to see. One way to visualise them is to separate the fragments according to size, using gel electrophoresis. Alternatively, DNA fragments can be identified using a DNA probe, or the nucleotide sequence can be analysed using DNA sequencing.

Gel electrophoresis

Gel electrophoresis is a technique that separates fragments of DNA according to their size and charge. DNA has an overall negative charge due to the phosphate groups in its backbone. The technique of gel electrophoresis makes use of this property to separate DNA fragments within an **agarose gel**. The agarose gel is melted and poured into a flat mould to cool. Wells are created by placing a plastic comb into the gel as it sets, creating indentations into which DNA samples can be loaded.

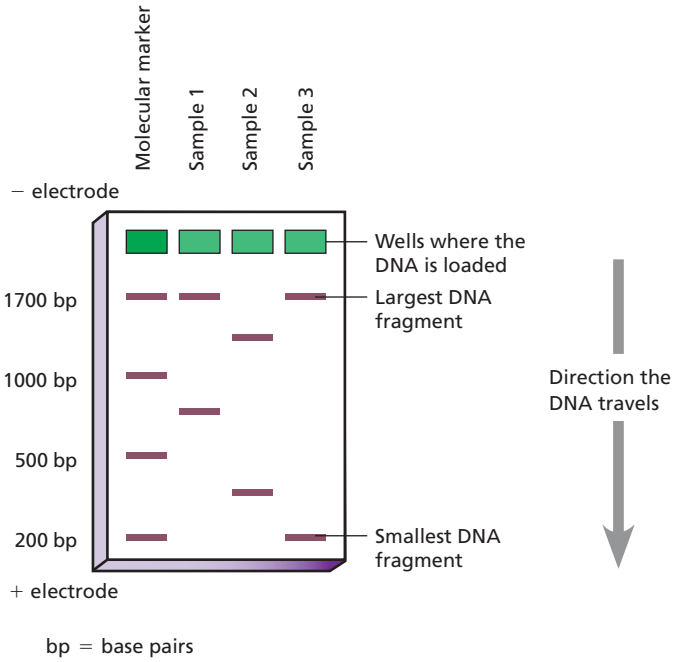
The gel is placed in a tray filled with buffer solution, and positive and negative electrodes are attached at each end of the gel. When the electric current runs, the DNA fragments are repelled from the negative electrode and move towards the positive electrode at the other end. The gel acts as a large sponge through which the DNA strands move while under the influence of the electric current. Smaller strands can wiggle through the gel matrix faster than the larger strands, which take longer to migrate through the gel. This method therefore separates DNA strands based on their size.

DNA itself will not be visible in the gel. To view the separated DNA fragments, a fluorescent DNA-binding dye such as **ethidium bromide** is added to the agarose gel before it sets. The dye binds to DNA and fluoresces under ultraviolet light, showing a pattern of bands that can then be photographed. Each band on the gel contains millions of pieces of DNA of the same size. The bands can also be cut out and the DNA purified to yield a solution of DNA fragments of the required size.

Getty Images/SPL/Simon Fraser

Figure 11.12 ▶
A researcher injecting genetic material from coral into an agarose electrophoresis gel apparatus

The position of bands on an agarose gel depends on the size of DNA fragments in each band; the smaller the fragments, the further they move in a given time. To determine the size of a given piece of DNA, molecular biologists use **molecular size markers**, sometimes called molecular weight ladders. These are pieces of DNA with a known number of base pairs. They are used to determine the size of the separated DNA fragments by comparing their location along the gel. Figure 11.13 shows four markers in the calibration lane: 1700 bp, 1000 bp, 500 bp and 200 bp, respectively.

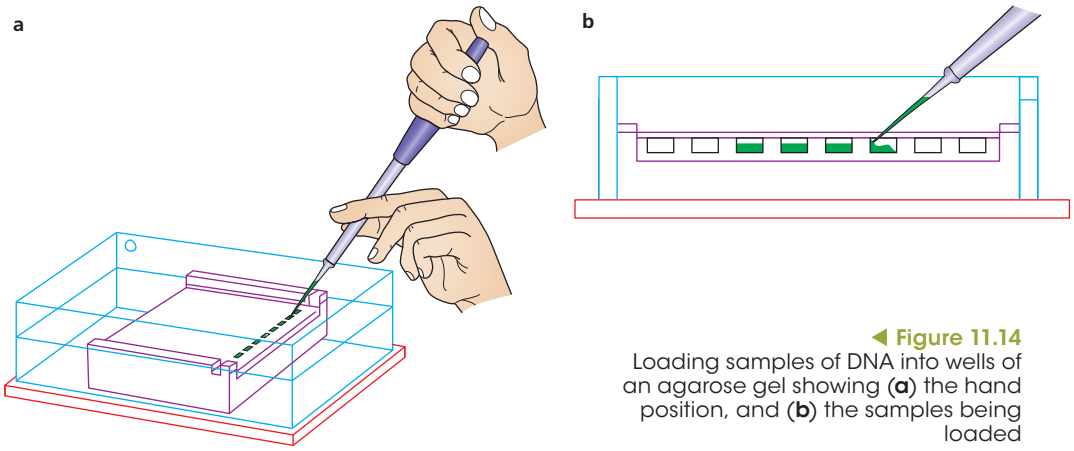


◀ **Figure 11.13**
Molecular markers of known size are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel.

EXPERIMENT 11.1

GEL ELECTROPHORESIS ANALYSIS

The DNA from all living organisms has the same chemical components and behaves in the same way in agarose electrophoresis. This makes analysis and comparison relatively easy. Scientists are able to read agarose gels and map the restriction sites of a DNA molecule. To prepare a run, molten and cooled agarose gel is poured with a well comb in place; when the gel is set, the comb is removed to expose the wells so the DNA fragments can be loaded (Figure 11.14).



◀ **Figure 11.14**
Loading samples of DNA into wells of an agarose gel showing (**a**) the hand position, and (**b**) the samples being loaded

DNA analysis is at the heart of recombinant DNA technology. In most cases when DNA is being cut for analysis, it is necessary to use a standard DNA fragment for comparison.

Bacteriophage λ-DNA is often used as a standard piece of DNA. It can be found as either linear molecules or circular molecules. At each end of the linear molecule are single-stranded sequences of 12 nucleotides that are similar to the sticky ends produced by restriction enzymes.

Figure 11.15 depicts the results of a standard test using λ-DNA. Three restriction enzymes, *Hind*III, *Eco*RI and *Bam*HI, have been used in a restriction digest. The following steps outline the procedure for obtaining the gel shown.

1 Each of the restriction enzymes was placed in a separate test tube containing a buffer solution and incubated with λ-DNA for 20 minutes.

2 A sample of DNA cut with *BamH*I was placed into the first well (B) of the agarose gel.
3 A sample of DNA cut with *EcoR*I was placed into the second well (E) of the agarose gel.
4 A sample of DNA cut with *Hind*III was placed into the third well (H) of the agarose gel.
5 A sample of DNA mixed with water was placed into the fourth well (–) of the agarose gel.
6 After electrophoresis and exposure to ultraviolet light, the banding pattern presented in Figure 11.15 was obtained. Using the gel, it is possible to determine the approximate base-pair size of λ-DNA.

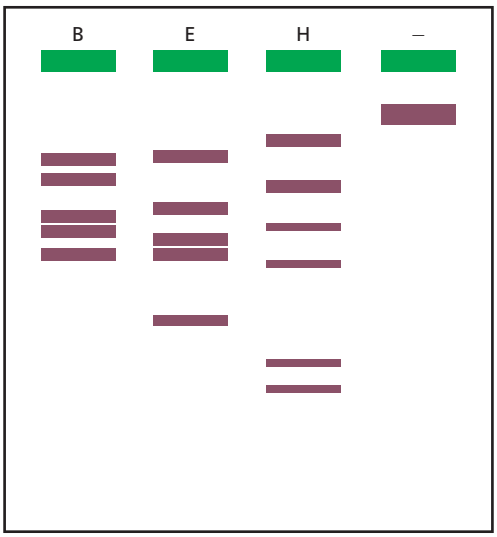


Figure 11.15 ▲
The DNA fragments separate according to length.

Aim

To analyse the results of a restriction digest experiment using agarose gel electrophoresis

Material

Each student will require:

- ruler

Procedure

1 Measure the distance from the lower part of the well to the lower part of each band on the gel in Figure 11.15.
2 Copy Table 11.2 and enter each of your measurements under the appropriate 'distance' heading.
3 The base-pair sizes for fragments cut with *Hind*III have already been included.
4 Calculate (approximately only) the base-pair sizes of the fragments cut with *Eco*RI and *Bam*HI, and complete Table 11.2.

Results

Table 11.2

*Hind*III		*Eco*RI		*Bam*HI	
Distance (mm)	**Size (bp)**	**Distance (mm)**	**Size (bp)**	**Distance (mm)**	**Size (bp)**
	23 130		21 226		16 841
	9 416				
	6 557				
	4 361				
	2 322				
	2 027				

Discussion

1 From the bands observed in the *Hind*III well, what is the total size of the λ-DNA?
2 It is known that λ-DNA has a linear length of 48 502 base pairs. Are the actual numbers of base pairs equal to the numbers shown on the gel? If they do not match, how can you account for the differences in base-pair numbers?
3 Why was one of the wells in the gel filled with DNA that was mixed with water?
4 Why does the band produced by the DNA that has not been cut with a restriction enzyme appear to have run the smallest distance through the gel?
5 Describe in your own words how the process of gel electrophoresis works.
6 Explain the role that restriction enzymes play in the process of gel electrophoresis.

Conclusion

Write a summary of this experiment.

DNA sequencing

Scientists often want to know the exact nucleotide base sequence of the DNA. Many mutations that cause genetic diseases are caused by single base substitutions or deletions, yet their effect can vary greatly. The process of **DNA sequencing**, that is, determining the exact nucleotide sequence of a gene, can help scientists identify individuals with deletion mutations, as in cystic fibrosis, or substitution mutations, as in sickle cell anaemia. DNA sequencing has many applications, including in biotechnology. For example, sequencing is often used to check the accuracy of a PCR product with respect to the template, or to check that a DNA fragment has been inserted into a plasmid in the correct orientation.

DNA sequencing can be done manually using gel electrophoresis, or automatically using an automatic DNA sequencer that can sequence a large amount of DNA in a very short time. In this process, the four nucleotides are labelled with four different coloured fluorescent dyes. As electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and consequently the base sequence. A computer can then automatically analyse the information from the gel to read the base sequence. This technique is called the Sanger method.

A large number of faster and cheaper sequencing technologies are now available for use by biotechnologists. These methods are collectively called next generation sequencing and they use whole genomic DNA as a template, resulting in much greater sequencing efficiency. For example, one million DNA fragments of 700 bp can now be sequenced in 24 hours, which is the equivalent of one full human genome every five days.

RECALL

- Gel electrophoresis separates DNA molecules based on their size. Negatively charged DNA travels through a gel matrix towards a positive electrode.
- DNA is visualised using a DNA binding dye. Sizes of DNA fragments can be estimated by comparing their movement through the gel with that of fragments of known sizes, referred to as molecular size markers.
- DNA sequencing can identify the exact nucleotide sequence of DNA fragments, which can be used to determine the genetic basis for particular phenotypes.

RECAP 11.5

1 What gives DNA its negative charge?
2 How can you estimate the size of a DNA fragment using gel electrophoresis?
3 Name two applications of DNA sequencing.

Case study box 4: Quality control of the recombinant plasmid

DNA ligation in the laboratory is not usually perfectly efficient. Following the restriction digest reaction and the ligation reaction, four possible DNA fragments may be found in the tube. These are:

1 The plasmid with the insulin gene incorporated into it. *This recombinant plasmid is the desired product*
2 The cut PCR product (insulin gene), which has not been inserted into the plasmid

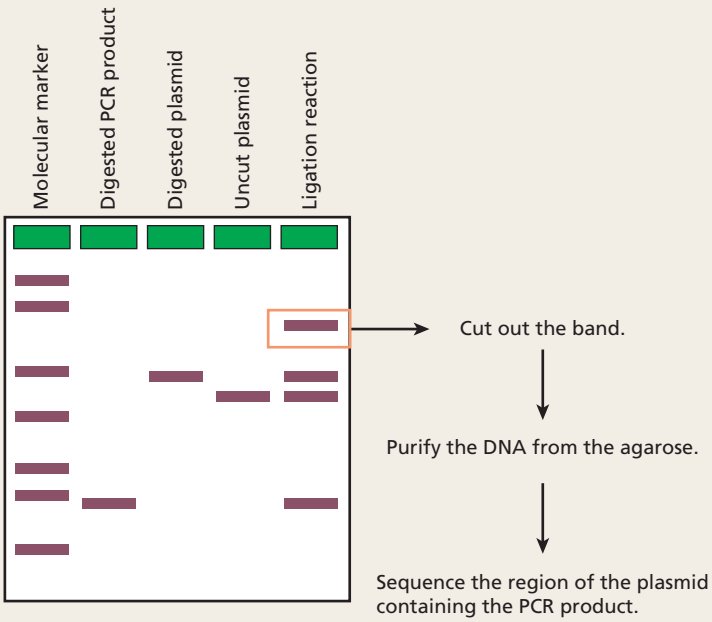


Figure 11.16 ▲
Isolating the recombinant plasmid using gel electrophoresis

3 The cut plasmid, in a linear conformation
4 The plasmid, with cut ends re-ligated to themselves.

The recombinant plasmid containing the insulin gene will be used to insert the gene into bacteria. To isolate this from the other DNA fragments, the contents of the tube containing the completed ligation reaction can be separated by DNA gel electrophoresis. To compare sizes, the digested PCR product, digested plasmid and uncut plasmid are run in parallel wells as controls. The circular plasmid runs slightly further than the linear plasmid because it is more compact. The recombinant plasmid is identifiable as the band that has travelled the smallest distance (since it is the longest fragment), and can be cut out of the gel and purified out of the agarose.

To ensure the insulin gene is inserted in the correct orientation and no mutations have arisen during the PCR reaction used to amplify the gene, the region of the plasmid containing the inserted insulin gene is usually sequenced. The sequence is compared to public genomic databases to check that it aligns and that there are no mismatches.

Sequencing the regions where the DNA fragment joins the plasmid can be used to show whether the DNA fragment has been inserted in the correct orientation; this is especially important when the ligation reaction has joined blunt edges.

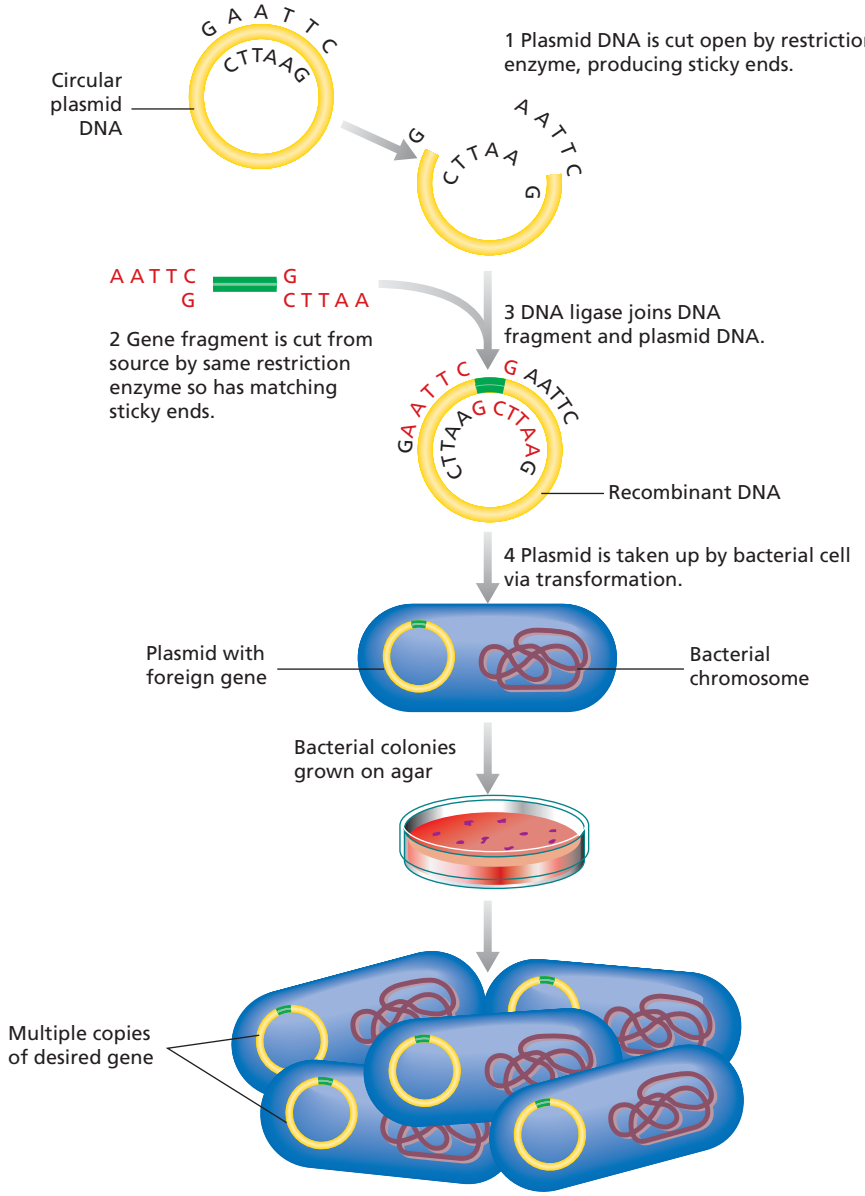
Together these steps ensure that the DNA being cloned is what is expected. Even a single base pair mismatch can completely change the function of a gene product.

Genetic cloning: copying DNA using plasmids

An alternative to using PCR to generate a large number of copies of a DNA sequence is to insert it into bacteria. This process is called **gene cloning** and it has multiple advantages. It allows replication of larger segments of DNA, and it permits the analysis of any gene and associated proteins encoded in the DNA sequence in an environment where they are active.

Plasmids are used to insert DNA into the bacteria. The key to using plasmids as DNA copiers lies in our ability to incorporate foreign genes into plasmid DNA and in their ability to replicate in bacteria. A number of steps are involved in this process (Figure 11.17).

1 Plasmids are extracted from bacteria by rupturing the cell walls.
2 The same restriction enzyme is used to cut the plasmid DNA and the DNA of the gene to be inserted so that both pieces of DNA have complementary sticky ends.
3 DNA ligase binds the 'foreign' DNA fragment into the plasmid DNA. After binding, the DNA fragment becomes a permanent part of the **recombinant plasmid**.
4 The recombinant plasmids are added to a bacterial culture. They are taken up by some bacteria, in which they replicate. In the normal process of growth and division, bacteria replicate the plasmid, and thus numerous copies of the incorporated foreign DNA are made.



◀ Figure 11.17
Transformation: a foreign gene is inserted into plasmid DNA to produce a recombinant plasmid. This is introduced into bacteria, where it can make multiple copies of itself. When bacteria take up the plasmid, they are transformed.

Only a small percentage of the bacteria take up the recombinant plasmids; others simply seal up without taking up the plasmid. The process of bacteria taking up the plasmid is called **transformation** (Figure 11.17). After transformation, the bacterial cells that contain recombinant plasmids have to be isolated from the majority of cells in the colony, which have not taken up plasmids.

Plasmid DNA often contains genes for resistance to an antibiotic; for example, ampicillin. Bacteria that have been transformed with the plasmid are able to grow and multiply on a medium that is supplemented with ampicillin because they are resistant to it. The bacteria without the plasmid do not grow as they are sensitive to the antibiotic ampicillin (Figure 11.18). This process is called **antibiotic selection** and is an important component of many biotechnology techniques.

Plasmids are very useful vectors in genetic engineering. Vectors in this context are agents that can deliver a piece of foreign DNA into a host cell. Other types of

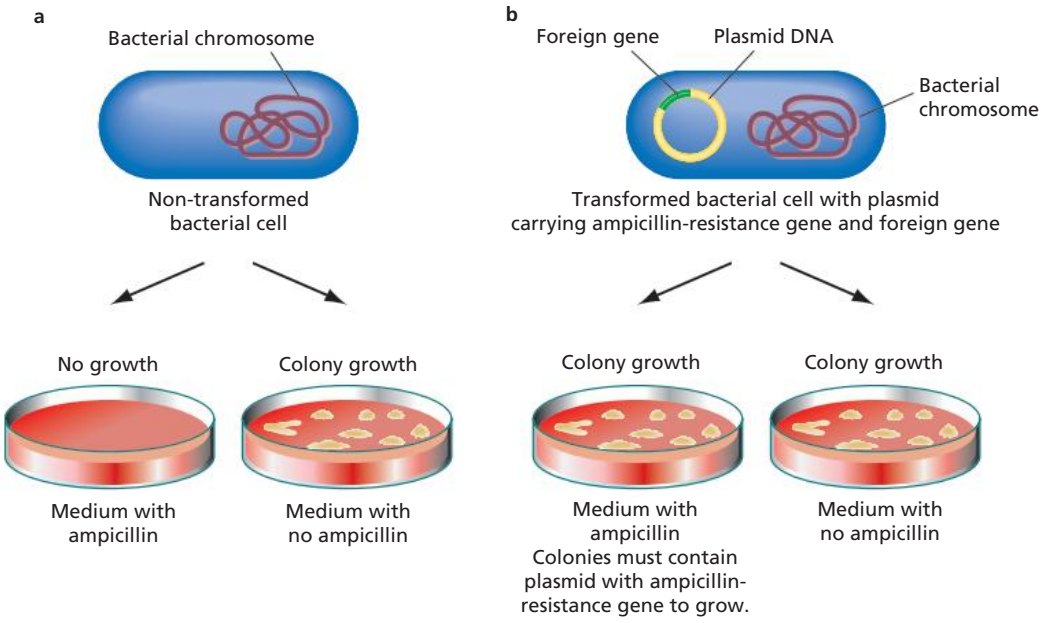


Figure 11.18 Antibiotic selection of transformed bacteria. (**a**) Non-transformed bacteria cannot grow on media supplemented with ampicillin, but grow well on normal media. (**b**) Transformed bacteria can grow equally well on either medium.

vectors include recombinant viruses and liposomes, which are synthetic spherical vesicles encased by a phospholipid bilayer that can encapsulate the DNA to be delivered.

The bacteria with antibiotic resistance are then selected and grown in culture. To study the gene of interest, the plasmids can be isolated and analysed. This technique of bacterial transformation is also used to insert genes that code for useful proteins into bacteria so that the bacteria will then make the protein for human use. As well as insulin, another example of this is the production of human growth hormone, which is used to treat people with a certain form of dwarfism. Prior to this technique, the hormone needed to treat these people was extracted from the pituitary glands of human corpses.

RECALL

- Gene cloning is an alternative to PCR for generating many copies of DNA. It utilises bacterial plasmids to produce many copies of a gene.
- A DNA fragment can be inserted into a plasmid and replicated as the bacteria carrying the plasmid divide. The plasmid is then called a recombinant plasmid.
- Recombinant plasmids usually have an antibiotic resistance gene added that allows them to be selected. Only the plasmid-carrying bacteria can grow in the presence of the antibiotic.

RECAP 11.6

1 List the steps involved in creating a recombinant plasmid.

2 Define the term 'transformation' in the context of genetic engineering.

Case study box 5: Scaling up production

Once the recombinant plasmid containing the human insulin gene is purified, it is ready to be inserted into *E. coli* cells. Bacteria are kept ice-cold to halt their metabolism, and their cell walls are compromised using chemicals or electrical pulses so that DNA can be taken up more readily. The cells are then 'shocked' using a rapid increase in temperature to close the cell walls and to kick-start the cells' entry into the cell cycle. The cells are put in a selection medium containing an antibiotic. Only cells that have taken up the plasmid are resistant to the antibiotic, and these selected cells rapidly reproduce in large fermentation tanks. While growing, the transformed bacteria containing the recombinant plasmid express the insulin protein. Once the cells reach an optimal density in the tanks, they can be filtered out and lysed to rupture their walls and release the insulin protein. The protein is then harvested and purified, then packaged into insulin pens for medicinal use.

In humans, the insulin protein consists of two polypeptide chains. In biotechnology, these are encoded by genes that are inserted into two separate plasmids, which are transformed into the same bacterial cells so that they can be expressed together.

iStockphoto/villiers

▲ **Figure 11.19**
A diabetes patient using an insulin pen to deliver a dose of insulin

Biotechnology applications

Gene technology has the potential to revolutionise the diagnosis and treatment of diseases such as cancer in humans, prolonging and improving the quality of life for patients. Cancer is a group of diseases that is defined by an uncontrolled proliferation of cells. These cells also have the ability to metastasise, or spread, to other tissues. Cancerous cells arise when multiple mutations occur in key genes that control or regulate cell division.

Gene technology allows researchers to identify some of the genes disrupted in individual cancers and tumours. Biotechnology can be used to investigate which genes are disrupted in certain tumours, thus enabling determination of which drugs would be most effective in restoring gene activity to normal. Genetic testing also facilitates a more accurate diagnosis of the type of tumour a patient has and which treatments will be most effective.

Gene therapies may also prove useful in the fight against cancer. Some therapies that are being researched involve injecting cancer cells with genes that give rise to toxic molecules when these genes are expressed, resulting in proteins that then kill the cancer cells. Another approach involves modifying the cells of the immune system of cancer patients so that they can recognise and destroy cancer cells. This process has been met with some success but is still very experimental.

The types of applications that scientists have developed that have potential use in medicine include:

- identifying alleles of genes responsible for serious medical conditions in an effort to speed up the discovery of a treatment for them

- screening for genetic diseases to determine if an individual or embryo is a carrier of a genetic disease
- developing transgenic organisms to produce large quantities of protein that can be used for commercial or therapeutic purposes
- gene therapy for humans with a genetic disease.

Biotechnology also has many applications for agriculture. Millions of people around the world are malnourished. Scientists have been trying to use their molecular tools and techniques to modify food crops, resulting in higher nutritional value and greater crop yields. Biotechnology has also been applied to reduce the impact of pests on crops, thus increasing the amount of food available in developing countries. This is achieved by inserting genes that confer resistance to pests, such as Bt cotton, which has genes from *Bacillus thuringiensis* that encode insecticidal proteins. Herbicide-resistant crops have been produced to allow spraying of crops against weeds, and the use of genetically engineered animals in agricultural applications is also on the rise. The process used for most of these applications is transformation: taking a gene from one species and inserting it into another to obtain a desired characteristic, producing genetically modified organisms. The use of GMOs is controversial and, prior to their widespread use, potential impacts on the environment, their potential toxicity and allergenic potential need to be investigated. Ethical considerations are an important aspect of the debate over the use of genetically modified organisms, particularly the consideration of animal welfare.

Biological knowledge and society: Outbreak to prevention: controlling infectious diseases

Plagues throughout the ages

Shakespeare wrote *Romeo and Juliet* in the late 16th century. In Act 3, as Romeo's friend Mercutio lies dying, he curses both the Montagues and the Capulets, crying 'A plague on both your houses'. During the 16th century there were repeated outbreaks of bubonic plague in London. In 1563 an outbreak of plague in London claimed 80000 lives. At the peak of this **epidemic** 1800 people were dying per week. This epidemic claimed a quarter of London's population.

When a person became sick a red cross was painted on the door of their house and no one was allowed to enter or leave. Watchmen were allocated to provide food and to alert the death cart when inhabitants died. Death cart workers came in the night to pick up bodies and take them to huge burial pits. It was a dangerous job. Eventually women and boys who had survived the plague were forced into the job. Only the rich could afford doctors, who visited wearing protective costumes: long dark robes, hats, leather gloves and boots, and a beaked mask filled with bergamot oil (Figure 11.20). Little could be done as no one knew the cause or an effective treatment. At this time, some people blamed invisible particles carried on the wind and others thought the water was poisoned.

It was not the first incidence of bubonic plague. In the 14th century this disease caused a **pandemic**, spreading from China to Europe and killing 25 million

amanaimages/AS400 DB

Figure 11.20 ▲
A 17th century plague doctor dressed for a house call

people, a quarter of the population. Society descended into panic and mayhem. The most recent pandemic, in 1855, lasted four years. Around this time a rod-shaped bacterium called *Yersinia pestis* was discovered to be the cause. It was also observed that rats experienced similar symptoms and that victims often had flea bites. A means of transferring the disease had been discovered. Fleas would suck the blood of infected rats and then transfer bacteria when they fed on humans. The infection also spread through body contact with infected individuals, living or dead. The infectious bacteria secrete toxins that kill off macrophages, so the bacteria can replicate unchecked. The bacteria are virulent. They can kill their host within 1–2 days, before the specific immune system has time to mount an effective response.

Today there are occasional outbreaks of the bubonic plague, usually in situations where people are forced to live close together in unsanitary, rat-infested conditions or where they come in contact with infected wildlife. The last serious outbreak was in the Democratic Republic of Congo when 50 people died, but there are sporadic cases in rural parts of the US. Outbreaks of the plague are now controlled using quarantine and pest-control measures, and the ill are treated with antibiotics.

Pandemics have decimated world populations throughout history; a few well-documented examples are listed below. The war on disease continues to challenge humanity and is a global issue as we tackle old and new disease-causing organisms.

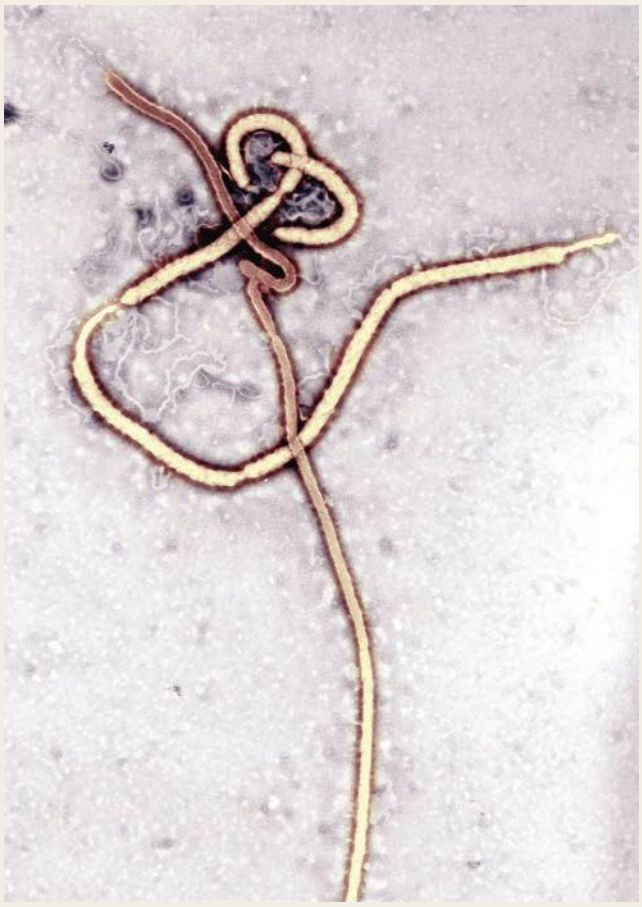
- The Antonine plague (165–180 AD) caused by the smallpox variola virus killed up to one third of those it infected. In the 20th century smallpox caused 500 million deaths. A worldwide vaccination program led to the disease being declared eradicated in 1980. Samples of the virus are still cultured in research laboratories, raising the fear of bioterrorism as we are no longer vaccinated against smallpox.
- The first cholera pandemic (1816–1826), caused by the bacterium *Vibrio cholerae*, spread from India to China and Indonesia, killing millions. Since this time tens of millions have died from cholera outbreaks. *Vibrio cholerae* causes diarrhoea. It spreads through infected food and water supplies. Hydration is the main treatment and antibiotics are not always used.
- The Spanish flu (1918–1919) was an influenza pandemic that spread to all continents, infecting one third of the world population (approximately 500 million people). Up to 100 million are believed to have died during the 18 months that this flu was prevalent.
- Measles killed around 200 million worldwide in the 19th century. It is caused by an airborne virus spread through talking, coughing, sneezing and sharing drinks and food. In the year 2000 almost 800 000 died, with 40 million reported cases. This disease is now controlled by immunisation programs.
- AIDS is a current pandemic caused by infection with human immunodeficiency virus (HIV). Infection rates are reported to be as high as 25% in southern and eastern Africa. In 2012 approximately 35.3 million people were living with HIV, with around 2 million deaths annually. Control measures include protected sex and not sharing needles.

Surveillance of diseases in the modern world

In Australia we can use a number of measures to help protect our citizens against pandemics. As an island nation we can monitor our airports and shipping ports using quarantine measures. Occasionally outbreaks of disease can occur that go unnoticed for some time. This can happen if the causative agent is not one that is tested for routinely and if symptoms are typical of other illnesses, such as diarrhoea, headaches, nausea and muscle pain. These symptoms often go unreported in the early stages. By the time people start consulting with doctors the spread of disease can be well under way. This has resulted in new methods of surveillance. Pharmacy sales databases are used to recognise spikes in the sales of particular medicines; emergency department databases record symptoms presented by patients to look for patterns; and particular diseases are monitored worldwide to predict and prevent outbreaks.

Over to you: Controlling the spread of disease in a globally connected world

Imagine you are working for the World Health Organization (WHO). There has been an outbreak of an infectious disease and you have been asked to lead a team to contain the outbreak. Use the following information and your research skills to advise officials on how to contain this outbreak.



Getty Images/BSIP/UIG

Figure 11.21 ▲
Scanning electron micrograph of the pathogen isolated from the blood of an infected individual

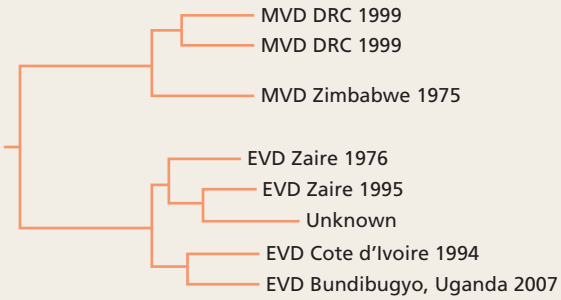


Figure 11.22 ▲
This phylogenetic tree was constructed by a computer program that uses algorithms to compare nucleotide sequences. The sequence from the pathogen causing the disease is labelled 'unknown'. The sequences used as comparisons are from various strains of Marburg virus disease (MVD) and Ebola virus disease (EVD).

Task 1: Identify the disease-causing organism

1. February, 2014: Cases of an unknown disease are presenting in the forests of Guinea, West Africa. Symptoms include fever, fatigue, muscle pain, sore throat, diarrhoea, vomiting, and occasionally blood in the stool. Suspected causes include malaria, typhoid fever, meningitis, Marburg virus disease (MVD) and Ebola virus disease (EVD). Conduct an Internet search to collect information about each of these diseases. Construct a table to collate the following information about each disease: name, causative pathogen, scanning electron microscope (SEM) image of pathogen, onset of symptoms, duration of illness, recommended treatment.
2. The SEM image in Figure 11.21 was taken of the disease-causing pathogen isolated from the blood of an infected individual. Compare this to your images of pathogens collected in step 1 to make a preliminary diagnosis.
3. The genome of the pathogen was sequenced and a bioinformatics computer program was used to compare this sequence to the genomes of related pathogens. Use the phylogenetic tree (Figure 11.22) to infer the strain of the pathogen causing this outbreak.

Task 2: Identify the natural host of this disease

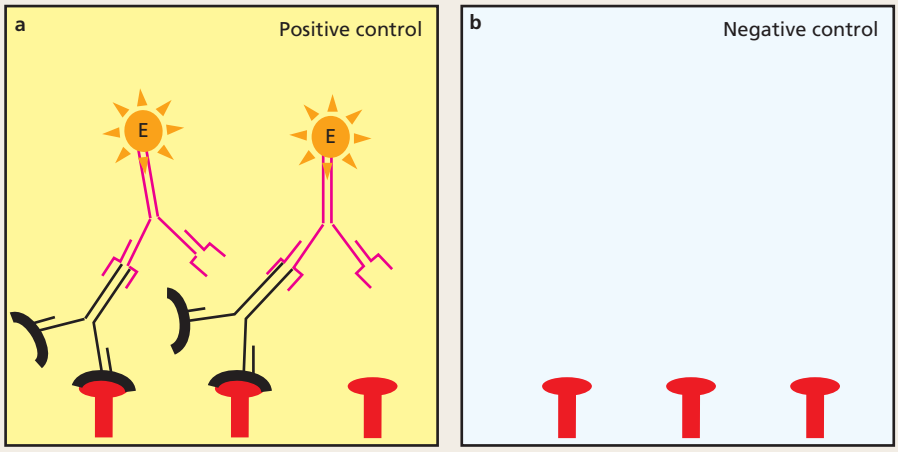
You suspect that this disease has emerged through contact with an animal that is a natural host of this disease. You ask for blood samples to be collected from mammals living in the forests of Guinea where the outbreak occurred. If the animal has been infected by the pathogen it will have produced antibodies against that pathogen and some antibodies will still be circulating in its blood. To check if the blood serum of mammals contains antibodies against the pathogen you order the laboratory to perform an enzyme-linked immuno-sorbent assay (ELISA) on the collected blood samples. Figure 11.23 reveals how an ELISA is used to detect specific antibodies in the blood of mammals. Use the results of the ELISA tests shown in Table 11.3 to determine which animals are a natural host of this pathogen. What recommendations could you make to local authorities to try to prevent new outbreaks of this disease?

1 Antigen from pathogen (shown as red) is stuck to bottom of plates.

2 Add serum from the blood of mammal being tested. If any antibodies in the serum (black) bind to the antigen, this means that the mammal has encountered the pathogen at some stage.

3 Add secondary antibody (pink) that will bind specifically to the end of any primary antibody that has bound to the antigen.

4 Add a substrate that will react in the presence of the enzyme (E) that is carried by the secondary antibody. The reaction causes a colour change from clear to yellow.



▲ Figure 11.23
An ELISA can be used to detect if a mammal has been infected with a specific pathogen in the past by revealing if the mammal has antibodies against that pathogen circulating in its blood. (**a**) The positive control shows the expected result if the mammal has produced antibodies against the specific pathogen. (**b**) The negative control shows the expected result if the mammal has not produced antibodies against the specific pathogen.

Table 11.3 ELISA results for mammals caught and tested for the presence of antibodies in their blood against the pathogen causing our disease outbreak

Mammal tested	Number tested	ELISA result
Pig	40	0
Fruit bat	35	15
Monkey	15	2
Striped ground squirrel	22	0

Task 3: Using a quarantine hospital to prevent local spread of the disease

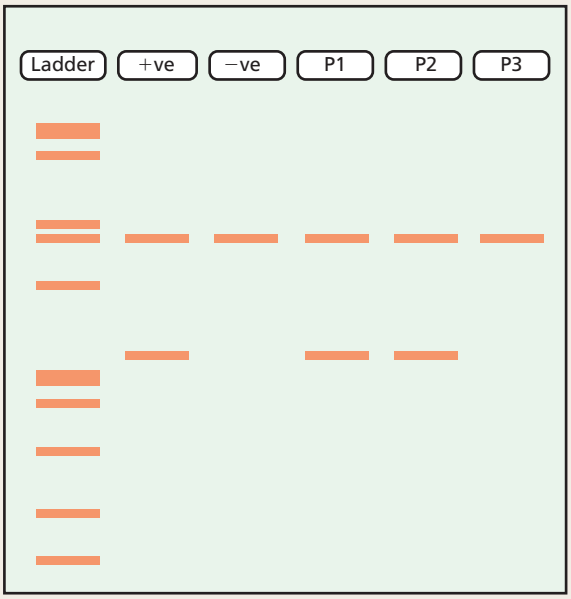
You set up a quarantine hospital to treat new cases of the disease. Quarantined individuals cannot leave until the pathogen has been cleared from their system completely.

1 A PCR reaction can be used to detect Ebola viral RNA in the blood of infected people. Use the PCR results shown in Figure 11.24 to determine which patients can leave your hospital.

2 Compile a list of guidelines that healthcare workers should follow to help protect themselves against disease when working at this hospital.

Task 4: Map the spread and percentage mortality of the disease

Use the information in Table 11.4 to map the spread of disease over 12 months since cases were first reported. Show where it is most prevalent and calculate the percentage mortality for each country. Calculate the percentage mortality by dividing the number of cases by the number of fatalities and multiplying by 100%.



▲ Figure 11.24
Results of a diagnostic PCR test for three patients (P1, P2 and P3). RNA was extracted from blood samples of patients and mixed with a PCR reaction mix containing primers to amplify a highly conserved Ebola virus nucleic acid sequence (seen in positive control lane) and primers for a nucleotide sequence common to all humans (seen in positive and negative control lane).

Table 11.4 Cases and deaths caused by this outbreak of disease between February 2014 and January 2015

Country	Cases	Fatalities
Guinea	3042	1990
Liberia	8818	3814
Sierra Leone	10905	3336
Mali	4	3
Nigeria	20	8
Senegal	1	0
Total	22790	9151

Task 5: Strategies to prevent global spread of disease

1. In September 2014 a case of Ebola was diagnosed in the United States. The man had been travelling in Liberia. He developed symptoms four days after re-entering the US and died eight days later. Design a questionnaire to be given to patients diagnosed with Ebola. Explain how each question can be used to help contain the outbreak of Ebola by providing possible responses and your risk-management strategies.
2. Provide a list of risk management strategies that could be used to control the spread of disease into new countries.
3. Project STRIVE is under way in Sierra Leone to trial a vaccine against Ebola virus. Navigate to the Centers for Disease Control and Prevention (CDC) website to find out more about this trial. Use a diagram to provide an overview of how this trial is being conducted and how it can help prevent the spread of this disease.

Task 6: How science understanding has improved control of infectious diseases

Make a list of measures that were being taken to prevent the spread of bubonic plague in 16th century London as reported at the beginning of this Biological Knowledge and Society section. Discuss how effective each risk management strategy was in containing bubonic plague. Finally, discuss how improved scientific knowledge about disease control and treatment has resulted in the eradication of bubonic plague pandemics since the late 19th century. Is it possible we could have a bubonic plague pandemic in the future?

Picture Media/Reuters/Andrew Kelly

Figure 11.25 ▲
A DNA bus in New York is popular with people who want to establish relatedness, including legal paternity.

Biological knowledge and society: Using DNA profiles to determine relatedness

Who's your daddy?

In New York as you walk down the street you may see a DNA bus similar to the one shown in Figure 11.25. This is a mobile unit for collecting DNA samples from citizens wanting to establish relatedness. Estimates place demand for tests in the US to establish parentage at 500000 per year. In one case two half-sisters who had grown up apart, parented by the same father but with different mothers, established their relationship to each other. In other cases men have discovered that they are, or are not, the fathers of their children.

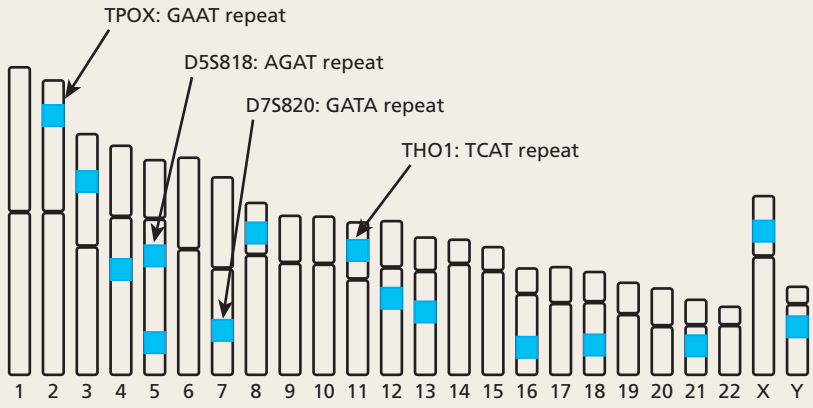
While we do not have mobile clinics in Australia, the number of people signing up for DNA tests to establish the parentage of their children is on the rise. Some men want peace of mind to establish that they are the father of their child. Some women want to ensure they came home from hospital with the right baby or to confirm the father of their child. Tests ordered from laboratories registered in Australia are reported to be in excess of 10000 annually. Seeking a test for personal information is easy. A DNA kit is ordered online so that DNA samples of parents and children can be collected in the home and mailed to DNA testing laboratories. The results arrive in the mail a few weeks later.

Concerns arise around contamination when collecting DNA to be sent in the mail, and often the interested party does not know how to interpret the results of the test. Another issue involves consent, as DNA can be collected without the child or one of the parents being aware this is being done.

Occasionally the issue of paternity can arise in cases where child support is sought from a man who claims not to be the father of a child. In such cases, the court will order a DNA test be conducted if they cannot determine paternity in any other way and if evidence places the paternity in question. Legal tests are required by law to comply with the Family Law Act, so samples must be collected and tracked from a registered collection centre and sent to specified testing laboratories. The results of non-legal tests are not admissible in a court of law.

Polymorphisms in DNA provide the variation required for DNA profiling

A DNA test to establish identity relies on inherited regions of our DNA that vary between individuals. These variable regions are known as polymorphisms. The polymorphisms used to construct a DNA profile for an individual are **short tandem repeats (STRs)**. STRs are segments of DNA that contain repeats of two to six nucleotides in tandem, such as TAGATAGATAGATAGATAGA. The human genome has many of these STRs found in fixed locations on our chromosomes (Figure 11.26). How we vary from one another is in the number of repeats we have at each STR region. Table 11.5 provides examples of the variation seen in selected STR regions used in DNA profiling.



▲ **Figure 11.26**
Chromosomal location of the 13 short tandem repeats used to construct a DNA profile in America. The names of four STRs and the nucleotide sequence repeated in that STR are shown.

Table 11.5 Variations found at STR loci used in DNA profiling

STR name	Locus	Repeat	Number of known alleles of this STR	Variation in number of repeats that can be found (known alleles of this STR)	Variation in length of DNA fragments (nucleotides)
TPOX	2p25.3	GAAT	15	4–16	12–64
D5S818	5q23.2	AGAT	15	7–18	28–72
CSF1PO	5q33.1	TAGA	20	5–16	20–64
D7S820	7q21.11	GATA	30	5–16	20–64
THO1	11p15.5	TCAT	20	3–14	12–56
D13S317	13q31.1	TATC	17	5–16	20–64

Over to you: Analyse DNA profiles and consider issues surrounding use of this technology

Task 1: Paternity case study 1

A legal paternity test was ordered by a judge to determine whether a man should pay child support. A DNA sample was collected from the mother, the child and the supposed father of the child. Each DNA sample was treated separately to produce a DNA profile for each individual. DNA was extracted from cells and polymerase chain reaction (PCR) was used to amplify chosen STR regions using sequence-specific primers. These primers are complementary to sequences of DNA that flank the STR region. Figure 11.27 reveals the THO1 alleles inherited by an individual. Each individual has two alleles for each STR, one inherited from each parent. This individual has inherited the alleles THO1-6 (THO1 allele with 6 repeats of TCAT) and THO1-5 (THO1 allele with 5 repeats of TCAT). This individual's genotype can be written as THO1-5,6.

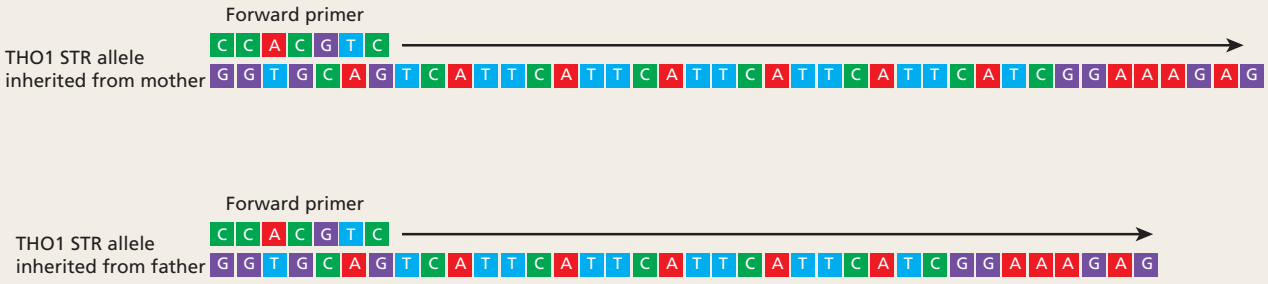


Figure 11.27 ▲
The THO1 alleles for an individual can be amplified using polymerase chain reaction (PCR). Forward and reverse primers are designed to complement sequences flanking the STR region so the specific STR region is amplified. Forward primers are shown here.

After PCR has been performed, the sample of DNA for each individual is loaded into individual wells of a gel and subjected to gel electrophoresis. This separates amplified fragments of DNA according to size. Table 11.6 provides results for the STRs inherited by the child, mother and possible father in this case study. Only three STR regions have been amplified for each individual. Use the data in Tables 11.5 and 11.6 to calculate the length of DNA fragments generated after PCR for each individual. Then construct an image of the resultant gel electrophoresis run for each individual. Be sure to label your gel to indicate which individual's DNA profile is shown in each lane.

What can you tell the judge presiding over this court case? Can you be certain of the results of paternity using this test? What might increase the certainty of these results?

Table 11.6 The STR alleles inherited by three individuals. Results are shown for three STR regions only.

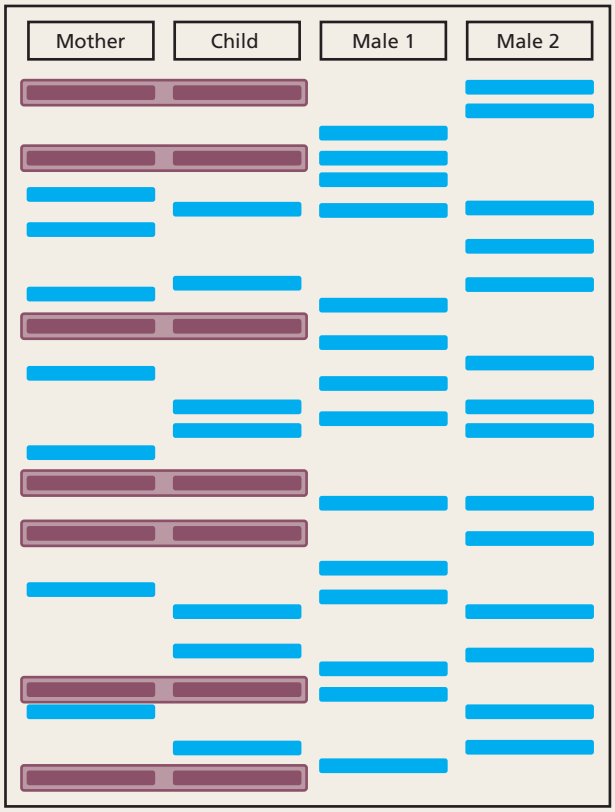
Individual	STR alleles inherited		
Child	TPOX–4,12	D5S818–7,7	THO1–5,14
Mother	TPOX–12,16	D5S818–7,12	THO1–3,14
Man who the mother claims is the father	TPOX–4,9	D5S818–7,10	THO1–5,6

Task 2: Paternity case study 2

A man (male 2) returned home to Australia after working overseas for 10 years. On his return he became aware that the girlfriend he had dated up until leaving had a 10-year-old son. He was suspicious that

he might be the biological father of this child. However, the mother was happily married to another man (male 1) who she claimed was the biological father of the 10-year-old. Male 2 decided to order a DNA testing kit. He managed to secretly collect a DNA sample from the child, mother and husband, and sent these off along with his own DNA for testing. A gel run of results for seven STR regions is shown in Figure 11.28. The STRs inherited by the son from his mother are indicated. Use the gel results to determine which male is the biological father.

Is it ethically sound to order DNA tests without the consent of all individuals concerned? Can you think of issues that may emerge given the results of this test?



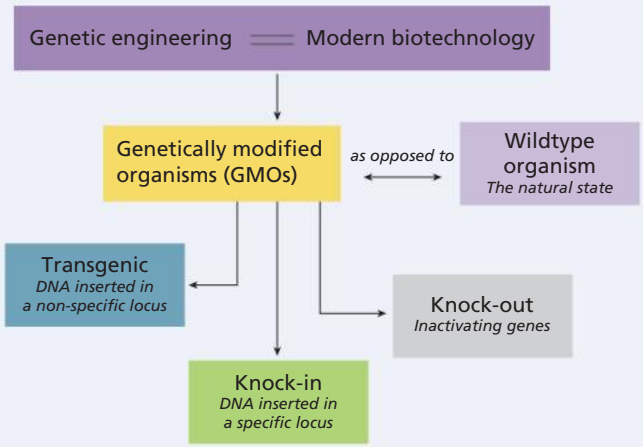
▲ **Figure 11.28**
This gel electrophoresis run reveals the DNA profiles of the four individuals in case study 2. Primers for seven STR regions were used to generate these DNA profiles.

Task 3: Exploring the ethics of paternity testing

The Men's Rights Agency is a lobby group who have made calls in the past for mandatory paternity testing of all babies at birth. They claim that up to 30% of men are living with a child that they mistakenly believe is their biological offspring. Such claims are not supported by social scientists, who believe the number is more likely to be 1–3%. Use the table of guiding principles that was introduced in the Chapter 6 Biological Knowledge and Society box (see Table 6.5, page 217) as a framework for analysing the issue of paternity testing. As you do this, conduct research to consider the perspectives of all interested parties. This might include children, parents, legal groups, the government, men's rights groups and paternity testing companies. Use this table to formulate an opinion about whether we should have mandatory paternity testing at birth.

CONCEPT SUMMARY

The plasma membrane



Amplifying DNA: polymerase chain reaction

Components

- DNA template
- DNA polymerase
- buffer solution
- nucleotides
- primers

Steps

1 Denaturation, 95°C: Melting the double-stranded template DNA into single strands.

2 Annealing, 55–65°C: Allowing primers to bind to their complementary sequence in the template strand.

3 Extension, 72°C: Extension of primers by DNA polymerase to form new strand with complementary sequence to the template strand.

Usually approximately 20–30 cycles are performed, with a long denaturation step at the start and a long extension step at the end.

Joining DNA: DNA ligase

DNA ligase is an enzyme that forms a phosphodiester bond between the 5′ phosphate and 3′ hydroxyl ends of two fragments of DNA, joining the fragments together. It requires ATP and Mg^{2+} ions. The success rate and correct orientation for DNA joining is greatly improved if the fragments have complementary sticky ends generated by restriction digest.

Cutting DNA: restriction endonucleases

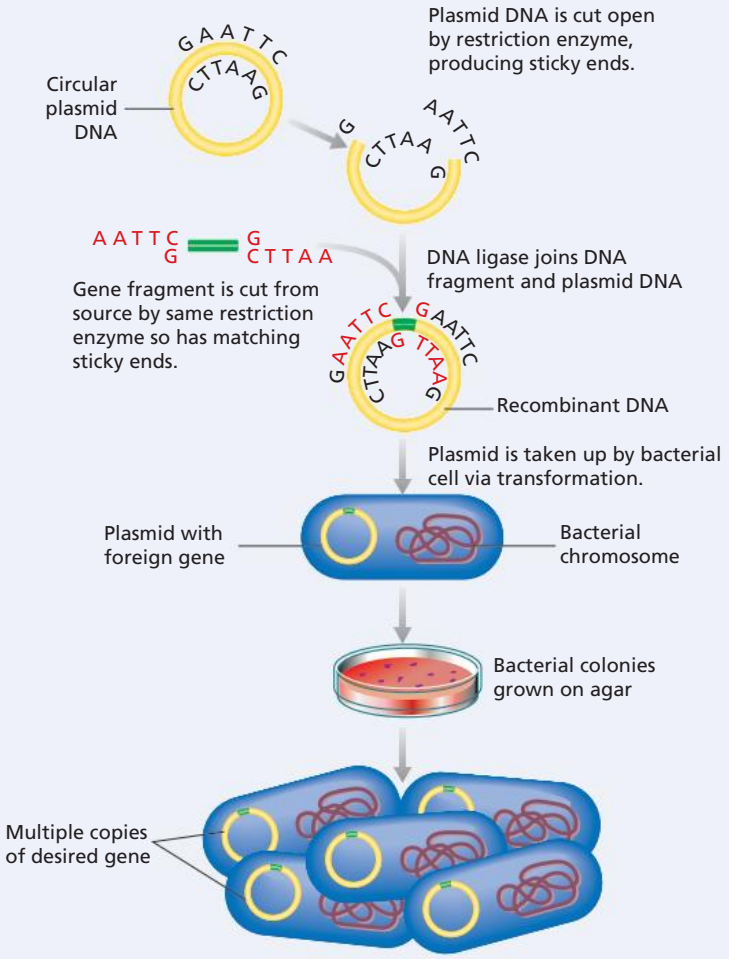
In a restriction digest reaction, restriction enzymes cut (digest) DNA at specific recognition sequences (restriction sites), generating restriction fragments with blunt or sticky ends, depending on the enzyme.

Manipulating DNA: plasmids

A plasmid is a circular piece of DNA (derived from naturally occurring bacterial plasmids) used as a vector for manipulating PCR products and restriction products. DNA fragments can be inserted if the fragment and the plasmid are digested with the same restriction enzymes, creating complimentary sticky ends or blunt ends that can be joined.

Useful features of plasmids

- Small, circular and therefore very stable
- Can be replicated in bacterial cultures to create many copies
- Easily engineered to contain restriction sites, promoters and other features that are useful in biotechnology
- Can contain genes for antibiotic resistance, making them selectable
- Plasmids are extracted from bacteria by rupturing the cell walls.
- The plasmid DNA and the DNA fragment are cut with the same restriction enzyme.
- DNA ligase binds the DNA fragment into the plasmid DNA generating a recombinant plasmid.
- The recombinant plasmids are added to a bacterial culture and are taken up by some (transformation).
- The bacteria containing the recombinant plasmid (which contains antibiotic resistance genes) survive in a culture medium containing an antibiotic and can therefore be selected.
- The bacteria containing the recombinant plasmid replicate and create many copies of the plasmid.



Visualising DNA: gel electrophoresis

DNA fragments in a solution are separated according to their size and an agarose gel is created with wells at one end. The DNA is loaded into the wells and a current applied to the gel, in a buffer solution. The DNA migrates towards the positive electrode. Smaller fragments can move more quickly through the gel matrix and appear to have migrated further through the gel than larger fragments within a certain time. DNA is visualised using a DNA-binding dye and sizes of fragments are determined by comparing them to molecular size markers.

DNA sequencing

Gel electrophoresis is conducted with a DNA fragment that contains nucleotides labelled each with a different fluorescent dye. A laser scans the sequence of fluorescence to determine the sequence of nucleotides.

CHAPTER GLOSSARY

agarose gel a gel matrix used for electrophoresis

annealing in PCR, a process of joining separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow

biotechnology the use of living organisms and biological systems and processes for human benefit

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

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

DNA ligase an enzyme used to catalyse the formation of a phosphodiester bond between two pieces of DNA

DNA polymerase an enzyme capable of making exact copies of fragments of DNA

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

epidemic an outbreak of infectious disease that spreads suddenly and rapidly through the population

ethidium bromide a chemical that binds to DNA and fluoresces when exposed to ultraviolet light; used to locate DNA in an agarose gel following electrophoresis

gel electrophoresis a technique that separates DNA fragments according to their size and charge

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organisms (GMOs) organisms whose genomes have been genetically engineered

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

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

pandemic a sudden outbreak of infectious disease that spreads rapidly across many countries

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

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

primer a single-stranded DNA molecule that acts as the start of the amplification process

recombinant DNA technology transferring a gene from a cell of a member of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

short tandem repeat (STR) a short non-coding region of DNA consisting of a sequence of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be use in DNA profiling

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

transformation the process by which DNA is taken from one organism and inserted into another organism using a plasmid

transgenic organism an organism that has been modified by incorporating a piece of foreign DNA into its genome

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

wild-type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

CHAPTER REVIEW QUESTIONS

Remembering

1 Match each item in the first column with a description in the second column. Each item can only be used once.

DNA ligase	Small circular self-replicating DNA molecule
Vector	Sorts DNA molecules based on size and charge
Primer	Joins two single-stranded sections of DNA together
Blunt ends	Specific site at which restriction enzymes cut DNA
Plasmid	Vehicle to introduce DNA into a host cell
Restriction site	An enzyme that catalyses the synthesis of DNA
Gel electrophoresis	Results from cleavage by a restriction enzyme in the middle of the recognition sequence (restriction site)
DNA polymerase	Synthetic short, single-stranded DNA molecule

2 Recall the features of a plasmid vector.

3 Outline how bacteria transformed with a plasmid can be discriminated from bacteria that have not taken in the plasmid.

4 State why the temperature is lowered to 50–60°C during the annealing phase of PCR.

Understanding

5 Predict whether the following cuts made by restriction enzymes will produce sticky or blunt ends. The lines show where the cuts lines in the double-stranded DNA.

a TCCGCGGA
AGGCGCCT

b A GGGCC T
T CCCGG A

c GC GGCC GC
CG CCGG CG

6 What would happen if the electrodes on a gel electrophoresis tank were accidentally swapped?

7 For how long would you apply a current to the gel during electrophoresis?

8 Agarose gels can be made with different concentrations of agarose. If increasing the concentration of agarose results in tighter gel matrix, what would be the impact on DNA migration speed?

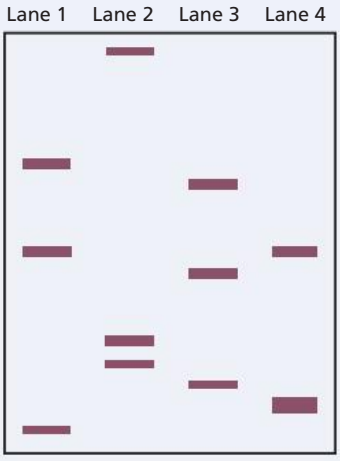
Applying

9 Predict the minimum band-sharing percentage in the DNA profiles of a mother and her baby.

10 Four samples, a, b, c and d, containing DNA fragments of the sizes given below, were accidentally swapped around while being loaded into the gel shown in Figure 11.29. Based on the figure, identify which lane corresponds to each sample.

- **a** 200, 250 and 900 bp
- **b** 150, 400 and 600 bp
- **c** 50, 450 and 650 bp
- **d** 100, 100 and 450 bp

11 Predict whether digestion of the human genome with *Alu*I or *Eco*RI would result in the larger number of fragments, and explain why.



▲ **Figure 11.29**
Gel electrophoresis

12 The section of DNA in Figure 11.30 shows a sequence of 120 bases in one strand of DNA. Refer to Table 11.1 on page 380 for restriction sites.

- **a** How many *Bam*HI and *Alu*I restriction sites are there in the sequence?
- **b** If the sequence is cut by *Bam*HI, how many fragments of DNA would be produced?
- **c** If the sequence was cut by *Alu*I, how many fragments of DNA would be produced?
- **d** If the sequence was cut by both *Bam*HI and *Alu*I, how many fragments would be produced?
- **e** If this piece of DNA was circular and not linear, how many cuts would have been made by *Bam*HI to get the number of fragments stated in part **b**?

```
ATATGTGT  GGATCCGT  CTTAGGTT  ATCGAATT  CTAGAGCT
ATGGCCTA  TTAGCTTC  CTGGATCC  AACCTGTA  TAGAGCTA
CTCGTCAG  CTATTGCT  ACGGGATC  CTAGCTGA  TTGGATTC
```

Figure 11.30 ▲
Nucleotides in a linear DNA sequence

Analysing

13 When conducting PCR, some unwanted DNA molecules are sometimes present.

- **a** Identify the possible consequences of having an unwanted DNA molecule in the PCR.
- **b** Identify two possible sources of this contamination.
- **c** Suggest what could be done to prevent this contamination from occurring.

Evaluating

14 A gene with unknown function, named *Taurin*, has just been cloned and used to generate a *Taurin* knock-out mouse strain. In mice, the *Taurin* gene contains three exons and two introns. The knock-out mutation was achieved by inserting a small region of irrelevant DNA into exon 1 of the *Taurin* gene, causing a frameshift mutation that resulted in a premature stop codon and prevented translation of *Taurin* into a protein. The knock-out mouse strain was analysed for any abnormalities by comparing the knock-out mice to wild-type littermate (sibling) control mice. It was noted that the knock-out mice developed tumours characterised by B cells proliferating in an uncontrolled manner (Figure 11.31).

- **a** What is the function of the *Taurin* protein that can be inferred from this observation?
- **b** Why are the knock-out mice compared with wild-type littermate controls?
- **c** Draw diagrams of the *Taurin* gene locus in wild-type mice and the same locus in the knock-out mice.
- **d** How could you use PCR to distinguish between wild-type mice and mice carrying the knock-out mutation? Draw PCR primer binding sites in the diagrams you drew for part **c**.
- **e** The knock-out mice were treated with rituximab, a monoclonal antibody used to deplete B cells. What can the survival curve of the treated mice tell you about your answer to part **a** above?
- **f** What important control is missing from the experiment represented in Figure 11.31?

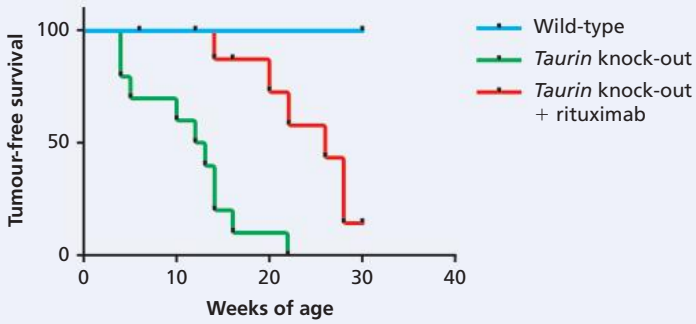


Figure 11.31 ▲
Tumour-free survival in *Taurin* knock-out and wild-type control mice

Creating

15 A new restriction enzyme has been discovered, called *Star*I. Design a strategy for large-scale production of *Star*I for use in research labs. Draw a flow chart outlining the strategy.

Reflecting

16 Many companies have started labelling their food products 'GM free'. Considering your knowledge of biotechnology techniques and applications, what do you think of the use of this type of labelling for consumers with a non-scientific background?

Getty Images/globestock

CHAPTER 12
SCIENTIFIC INVESTIGATIONS

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

KEY KNOWLEDGE

Practical investigation

- independent, dependent and controlled variables
- the biological concepts specific to the investigation and their significance, including definitions of key terms, and biological representations
- the characteristics of scientific research methodologies and techniques of primary qualitative and quantitative data collection relevant to the selected investigation, including laboratory work (biochemistry, cytology, immunology) and/or fieldwork (geomorphology); precision, accuracy, reliability and validity of data; and minimisation of experimental bias
- ethics and issues of research including identification and application of relevant health, safety and bioethical guidelines
- methods of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and limitations of data and methodologies
- models, theories and classification keys, and their use in organising and explaining observed phenomena and biological concepts including their limitations
- the nature of evidence that supports or refutes a hypothesis, model or theory
- the key findings of the selected investigation and their relationship to cytological, biochemical and/or evolutionary concepts
- the conventions of scientific report writing and scientific poster presentation including biological terminology and representations, standard abbreviations, units of measurement and acknowledgment of references.

Wellcome Images

Figure 12.1 ▲
Jenner vaccinating Edward Phipps

Performing practical investigations is your chance to experience what doing science is really like. Science is about finding things out through observation and experiment, which is what doing investigations is all about. This is why investigations are central to science, *and* why they are so much fun.

Sometimes an important advance in science begins when an observant person with an enquiring mind notices something that might be scientifically interesting. For example, after hearing from milkmaids that people who contracted cowpox (a relatively innocuous disease picked up after working with cattle) were protected from deadly smallpox, the British physician Edward Jenner effectively kick-started the science of vaccination. Jenner used samples from open cowpox sores on a dairymaid's hands to inoculate a young boy and protect him against smallpox. However, it would take another 200 years and a lot of carefully conducted research before scientists truly began to understand the biological basis for immunity. This sort of astute observation may begin a new field of research, which then proceeds by carefully planned investigation.

Scientific investigations can take years to complete and may involve collaboration among many scientists. They may require access to special equipment in Australia or overseas. They may cost a lot of money, sometimes millions of dollars, to complete. Hence scientists invest time in *planning* investigations before they begin. When scientists apply for grants to carry out investigations they need to show that they have carefully planned what they will do and how any money provided will be spent. Good planning is crucial to the success of the investigation.

Scientists then make careful *measurements and observations* and record their *results*. They *keep records* of all their experiments. This is a legal requirement. Typically, experimental results need to be kept for 5–7 years. There are also requirements for how and where data is stored.

Once data is collected it needs to be *analysed*. There are various ways this is done, but in the biological and biomedical sciences it typically involves constructing graphs and performing statistical tests to determine mathematical relationships within the data.

Finally, the results of the investigation must be *communicated*. Usually this involves publishing a scientific paper in either a journal or conference proceedings, or presenting the findings in talks or posters at conferences. If the result is funded by a grant then a research report must be submitted to the agency providing the funding. If the results are really exciting, then the scientists may write a media release. However the results are communicated, this step must happen for the investigation to be completed.

Planning your investigation

There are many things to consider when planning an investigation. You need to think about how much time you will have inside and outside class. You also need to think about the space and equipment you will need, and where you will go if you want to make measurements or observations outside.

You may be working in a group or on your own. Most scientists work in groups. If you can choose who you work with, think about this carefully. It is not always best to work with friends. Think about working with people who have skills that are different from your own.

Finally, and probably the first thing that most students think about, is the topic of the research. You will need to come up with a **research question** or **hypothesis**.

Choosing a research question

Obviously, it is a good idea to investigate something that you find interesting. If you are working in a group, try to find something that is interesting to everyone in the group.

A good way to start is by brainstorming for ideas. This is useful whether you are working on your own or in a group. Write down as many ideas as you can think of. Do not be critical at this stage. Get everyone in the group to contribute their lists and accept all contributions uncritically. Write down every idea.

Alamy/Jim West

◀ **Figure 12.2**
Brainstorm as many ideas as you can and then present them to your group.

After you have run out of ideas, it is time to start being critical. Decide which questions or ideas are the most interesting. Think about which of these it is actually possible to investigate given the time and equipment available. Make a shortlist, but keep the long list as well for the moment. Once you have your shortlist it is time to start refining your ideas.

Scientific terminology

Right from the beginning when you start thinking about your research question, it is important to use the correct scientific terminology. Using accurate scientific words to describe and communicate your ideas is essential for getting across their precise meaning. Such scientific language can be difficult for non-scientists to understand, and when it comes to communicating your research to a more general audience you will need to choose words and phrases that can be widely understood. However, now that you are embarking on scientific research of your own, you need to show that you can understand and describe the relevant biological concepts using the terminology that scientists use.

For every biological concept, there are certain key terms that should be used for scientific communication, instead of general language. There may also be scientifically appropriate ways of representing biological concepts that you should be aware of when planning, conducting and reporting your research.

Researching and refining your question

Having come up with a list of ideas, using appropriate scientific terminology, the next step is to find out what is already known about the ideas on your list. Use the Internet, your textbooks and the library to find out. Make sure you *keep a record* of the information that you find as well as the *sources*. You should start a **logbook** at this stage. You can write in **references** or attach printouts containing the source information to your logbook. This can save you a lot of time later on. Many research students forget to do this when they first start reading about their topic and then have to search all over again. See the section on referencing below.

Be critical of what you read. Do not assume that everything you read online or even in books is true. Try to find **reliable** sources of information. Textbooks and websites from universities and government research agencies are usually very reliable. Publications and web pages from professional associations, such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Academy of Science and equivalent international organisations are also good sources. Blogs and homepages of other students are not always reliable, although they are useful to give you ideas. Websites that are trying to sell you something or push a particular point of view should be treated sceptically. Talk to your teacher about sources of information as well. They will be able to help you assess whether a website is reliable and suggest sites that they know are suitable.

You may find examples of similar investigations to the one you are thinking of. It is a good idea to look at these so you can learn from the experience of other researchers. However, in general, it is better not to try to replicate someone else's investigation exactly. If you do decide to replicate someone else's investigation, even a part of it, then you need to acknowledge and carefully reference their work. If you do not do so, it is **plagiarism**. This is a very serious form of academic misconduct. Talk to your teacher about how original your research needs to be and how closely it can be based on someone else's work. It is much better to do this at the start than to be accused of cheating later on.

Finally, talk to your teacher about your ideas. They will be able to tell you whether your ideas are likely to be possible given the equipment available. They may have had students with similar ideas in the past and can make suggestions.

After you have researched your questions and ideas, you will hopefully be able to narrow the shortlist down to the one question that you want to tackle. If none of the questions or ideas looks possible (or still interesting), then you need to go back to the long list. You may need to read and gather more information on your topic of interest before you find an interesting, original and feasible research idea.

Proposing a research question or hypothesis

Once you have decided on what you will investigate, you need to turn it into a research question or a hypothesis. A good research question identifies the **variables** that will be investigated. Variables are factors or features that can vary or change during experiments, including factors that you vary on purpose or factors that you measure as part of your investigation. Usually you will have one **dependent variable** (whose value depends on another variable) and one **independent variable** whose value is changed by the investigator and will usually influence the dependent variable. For example, if a scientist is testing the effect of water temperature on seed germination, the independent variable will be the temperature of water poured onto seeds and the dependent variable will be whether the seeds germinate. In a lengthy investigation you may investigate two or more independent variables (such as water temperature and time of seed

exposure to the water), but you need to study them separately or you will not be able to tease out their effects. Variables are discussed in more detail later.

Research questions

A good research question is a question that is specific and can be answered by performing experiments and making measurements. A research question may be of the form 'What effect does a new fertiliser have on root growth?' The aim of your research is to then answer the question. It is important that you frame the question carefully. It needs to be specific enough that it guides the design of the investigation. A specific question rather than a vague one will make the design of your investigation much easier. Asking 'Does a new fertiliser increase root growth more than standard fertiliser?' tells you what you will be varying and what you will be measuring. It also gives a criterion for judging whether your results have answered the question.

Asking 'How can we make roots grow the best?' is not a good question. This question does not say what will be varied, nor does it tell you when you have answered the question. 'Best' is a vague term. What you mean by 'best' may not be what someone else means.

Finally, a good research question should be *feasible*: answerable with the time and equipment available.

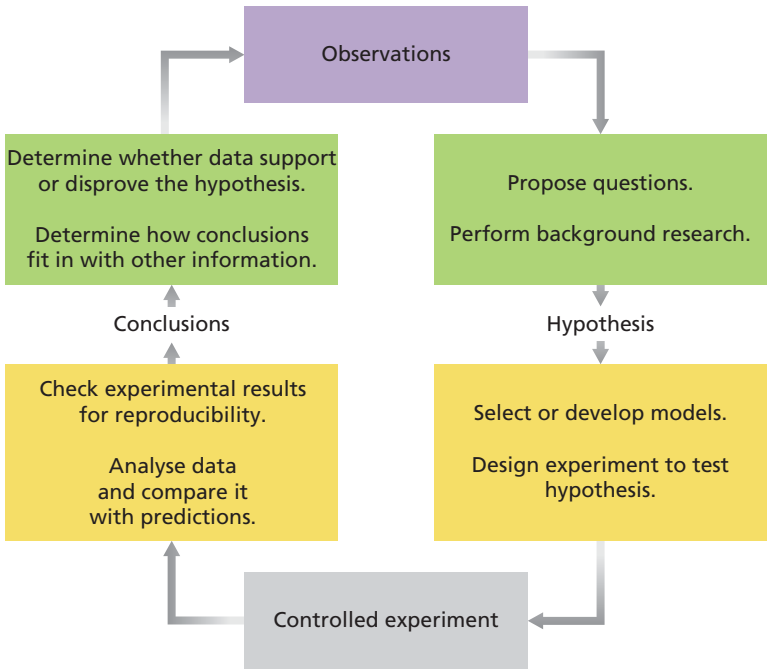


Figure 12.3 The scientific method. In this context, observations refer to the carefully considered overall conclusions of your study, based on reproducible and reliable results of controlled experiments.

Hypotheses

A hypothesis is based on the research question, but is not posed as a question. Instead, a hypothesis is a tentative explanation or prediction, not yet confirmed by experiment, such as 'The new fertiliser makes roots grow longer in two weeks than standard fertiliser'. A hypothesis is often based on some existing **model**, **theory** or **classification key**. It is a prediction of what will happen in a specific situation based on that model.

A hypothesis should give you a prediction that you can test by performing an experiment. This means it should at least be **falsifiable**; that is, it should be able to be disproved. However, you will *not* generally be able to claim that you have proved your hypothesis. Rather, you may be able to say at the end of the study that your results

See Chapter 5 of Nelson Biology VCE Units 1 & 2 *for more on classification (dichotomous) keys.*

support your hypothesis. Hence an aim for an experiment should not start 'To prove…', as it is not possible to actually prove a hypothesis, only to disprove it. If your experiments agree with predictions based on your hypothesis, then you can claim that they support your hypothesis. This *increases your confidence* in your model, but it *does not prove that it is true*.

If your experimental results disagree, then you may have disproved your hypothesis. This is *not* a bad thing! Some of the most interesting discoveries in science start when a hypothesis based on an existing model is disproved. This means that the model it was based upon is either not a good model or does not apply to the particular situation. You could then try to work out why the model does not apply or try to formulate a better model. What to do when your hypothesis is not supported is discussed further in the analysis section.

Do not be surprised if you change or modify your hypothesis during the course of your investigation. In scientific research, the question you set out to answer is often only a starting point for more questions. If you do modify your hypothesis, you will need to identify a new testable prediction and check that your experimental design will test this.

Designing your investigation

Once you have a specific research question or hypothesis, you need to design your investigation. It is fun to start making measurements or observations immediately, but it is also important to spend time learning how to use the equipment and experimenting to find the best way to set up your investigation. You may also discover that you need different or more equipment. This may save you time later on.

It is also important not to get distracted and forget the purpose of your investigation. At the end of the process, you need good data that answers your question or tests your hypothesis. Having a plan ensures that you make the measurements that you need. The longer the investigation, the more important it is that you have a clear plan. There are several things to consider.

- What data will you need to collect?
- What materials and equipment will you need?
- When and where will you collect the data?
- If you are working in a group, who will collect the data?
- Who will be responsible for record keeping?
- How will the data be analysed?

The data that you collect will always include **secondary data** and will usually include **primary data**. Secondary data is data that has been collected by someone else.

You will already have collected some secondary data when you investigated your research topic to formulate your question or hypothesis. You will probably want to collect more secondary data. If your topic is not one for which you can collect primary data, then you will need to rely on secondary data. Remember that when you collect secondary data it is important to use reliable, reputable sources. These sources will need to be correctly acknowledged as part of the references section of your report or poster.

Primary data is data that you collect yourself. You can collect data by performing experiments or making observations in the field. You should be able to measure parameters that are relevant to the biological question being asked (e.g. rate of cell division, body temperature, enzyme activity, size of population). You will have had practice at measuring some or all of these things already. You need to decide which variables you will measure and which variables you will control. Consider which variables you can control and which you cannot.

Consider how you will analyse the data. Will you need access to specific software such as a graphing or statistics package? If so, make sure that you know how to use it. If you are using software to draw graphs then you need to know how to produce a **scatter graph** and fit a **line of best fit** and add **error bars**. Note that a line of best fit is *not* the same as joining the dots. You should *never join the dots*, even though this is may be the default setting in spreadsheet software. You should consult a reference guide, the 'help' menu for your software or ask your teacher. Graphs are discussed in more detail in the analysis section below.

Keep a record of your planning. This should go in your logbook. Writing down what you plan to do, and why, will help you stay focused during the investigation. If you are working in a group, then a record of what each person agrees to do during the investigation will be very important.

Variables and measurements

Anything that can vary in an experiment is a variable. An independent variable is one whose properties you control. For example, if you were doing an experiment to measure the activity of an enzyme at different pH, then you would control the pH of the reaction and measure the rate of catalysis of the substrate. In this case the pH is the independent variable and the rate of catalysis, which varies with pH, is the dependent variable.

In the question 'Does the new fertiliser increase root growth more than standard fertiliser?', the type of fertiliser is the independent variable. The dependent variable is the root length (or change in root length). Other independent variables not mentioned in this question include soil composition, amount of water, light and temperature. These should all be kept constant (controlled), so they are not variables in the investigation. If it was a long investigation, the air pressure could be another controlled variable. If you decide to have two independent variables then it is important to keep one constant while you vary the other, if possible. Then you take multiple sets of measurements, keeping one variable at a fixed value for each set of data while you vary the other.

When variables have a numerical value, you make **quantitative measurements**. You measure the numerical value in the appropriate units. For example, you may measure root length in centimetres or mass of roots in grams.

Continuous variables may take any possible value, usually within some range. Length, time and mass are continuous. In the root growth example, root length is a continuous variable. A variable that may take only fixed values is called a **discrete variable**. Often these are whole numbers of things that cannot be broken into smaller parts, such as number of teeth or students. In the fertiliser example, the number of roots is a discrete variable.

Your measuring equipment will sometimes restrict you to measuring only discrete values. This is always the case with digital equipment. A set of digital scales that measures in grams gives you discrete values. It does not, however, mean that mass itself is a discrete variable. The mass of the roots is a continuous variable, but digital scales will only give you discrete measurements of the mass.

In some investigations you may use **qualitative measurements** or data. For example, a chemical reaction may lead to a colour change. You would usually describe the colour in words, such as 'pink' or 'green', rather than using a number. Sometimes you use a combination of qualitative and quantitative data. For example, you may describe the length of roots as reaching a maximum in centimetres (quantitative) but growing in a particular direction or pattern (qualitative).

Once you have decided on the variables you will be measuring, you will be able to identify the equipment and other resources you will need.

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Figure 12.4 ▶
Keeping conditions as consistent as possible helps to control the independent variables.

Experimental controls

When planning your experiments, it is usually necessary to include experimental controls. These show that the experimental conditions are appropriate and that no unexpected independent variables are influencing the results. A **controlled variable** is a variable that is kept constant during the investigation so it does not impact upon the interpretation of the relationship between the dependent and independent variables. In the root growth investigation example, controlled variables would include seed type, seed freshness, length of exposure to the temperatures being investigated, growing medium, planting depth, light exposure, water available to seeds, air circulation and the temperature of the growing medium. These are all potential confounding factors that would need to be controlled, i.e. kept constant.

A **negative control** is a condition in which no independent variables are altered. Essentially, in the negative control condition, you expect little to no change in the dependent variable. If a substantial change is observed, it may indicate that an additional independent variable is acting in your study that needs to be identified and controlled in a repeated experiment. In a root growth experiment, the negative control may be a condition in which no fertiliser is added, which will give you a baseline of root growth in the absence of fertiliser.

A **positive control**, on the other hand, gives you some certainty that the experimental set-up will yield the expected results. For example, the standard fertiliser is a positive control as you can expect it to enhance root growth. If it does not increase growth, the growth conditions may be insufficient and you will need to reconsider your experimental set-up. Positive controls are not always included in experiments but they can be very valuable in assessing the reliability of data generated in your investigations.

Identifying the resources required

If you are going to collect primary data, make a list of all the equipment that you need. Consider how precise the measurements will need to be. If your hypothesis predicts a temperature change of 0.1°C, but you can only measure to a precision of 0.5°C, then you will not be able to test your hypothesis. You may need to think carefully about how you measure some things. For example, in a root growth

experiment, you may need to measure the dry weight of the roots, which means finding a consistent way to dry them.

Consider who will supply the materials and how much they might cost. Scientists generally have to work within tight budgets. Also, the equipment you plan to use must be safe. Will you need special protective equipment, such as lab coats, safety glasses or gloves? There is a section on risk assessment below. Make sure that you include any safety equipment needed in your equipment list.

When you have your list, talk to your teacher about what equipment is available. You might find that you need to modify your question or hypothesis at this stage.

Consider where you will perform your experiments or observations. Can you use normal classroom space, or do you need to be outside? If you are outside, what provisions can be made for ensuring you can work without interference? Will you need to consider the convenience or safety of others? Talk to your teacher about what space is available.

Planning the experimental procedure

The most common problem that students have when doing research is time management. It is important to plan to have enough time to perform the experiments, *and* to analyse them, *and* to report on them. You also need to allow time to learn how to use the equipment if you have not used it before.

In any investigation you will need to collect reliable and precise data. You cannot do this if you do not know how to use the equipment. Always ask if you are unsure. Reading the user manual is also a good idea. It will usually specify the precision of the device and let you know of any potential safety risks.

Whenever possible you should make repeat measurements, so allow time for this. This allows you to check that your measurements are **valid**. Valid results are affected only by a single independent variable and only experiments with the correct negative and positive controls can produce valid data. If the results are similar each time, then your results are likely to be valid and *reliable*. If a result is not *repeatable* by you or **reproducible** by others, it is probably not a valid result. A result is repeatable if you or others make exactly the same measurement more than once and get the same result, within the limits of experimental uncertainty. A result is reproducible if another investigator, following your method, obtains data that leads them to the same conclusion as yours, even if there is some small variability between your results and theirs (for example due to the different equipment used to take the measurements). If a result is not repeatable or reproducible, then a variable other than the one you are controlling is affecting its value. If this is the case, you need to determine what this other variable is and control it if possible.

Think about how you can minimise uncertainties. Minimising uncertainty is not just about using the most precise equipment you can find; it is also about clever experimental technique. Important discoveries are possible using simple equipment and techniques.

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

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

With these things in mind, you may need to consider the number of **replicates** to include in your experiments. These are independent samples that allow you to take

multiple measurements, increasing the reliability of your data. In the root growth example, having several plants grown in each experimental condition allows you to calculate an average value as well as the variation between values in your sample set. If the variation is small, it is likely that there is only one independent variable acting in your experiment and your results are probably reliable. Read more on the statistical analysis of your results in the section below.

After you have analysed your results, you need to write your report or communicate your findings in some other form, such as a poster. You need to plan ahead how this will be done. If you are working in a group, who will write each part of the report, and when? Who will proofread it? Who will be responsible for making sure all the parts fit together?

You may find a timeline useful. A timeline helps keep you on track and reminds everyone of their responsibilities. If you are working in a group, get everyone to agree on it.

You can use the following table as a template.

Date and place	What will be done	By whom	Outcomes

Minimising error

Error occurs when experimental subjects are not randomised, when there are errors with the equipment being used, or when the person making the measurements would like to get particular results. These factors can cause bias, and when an experiment is biased the results are not valid and no conclusions can be made from the investigation. To minimise error, you first need to ensure that all equipment you are using has been calibrated and tested. Calibration ensures that the equipment gives the correct readings using known standards. For example, scales can be calibrated using known weights. The equipment should be calibrated at the top and the bottom of its range at least.

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

For every step of your investigation, try to identify possible sources of error, come up with ways of eliminating the error, and incorporate these into your investigation design.

Risk assessment

You may be required to complete a risk assessment before you begin your investigation. Even if this is not a requirement, it is a good idea to think about it. You need to think about three things.

1 *What are the possible risks* to you, to other people, to the environment or property?
2 *How likely is it* that there will be an injury or damage?
3 If there is an injury or damage to property or environment, *how serious are the consequences* likely to be?

A 'risk matrix', such as Table 12.1, can be used to assess the severity of a risk associated with an investigation. The consequences are listed across the top, from

negligible to catastrophic. Negligible may be getting clothes dirty or a very minor injury such as a scratch. Marginal might be a bruise from falling off a bike, or a broken branch in a tree. Severe could be a more substantial injury or a broken window. Catastrophic would be a death or the release of a toxin into the environment. In general, you need to ensure that your investigation is low risk. You can use a risk matrix either for individual identified risks, or for the investigation overall. If there were multiple experiments in your study, then you would use a risk matrix for each one.

All hazardous chemicals have an accompanying Safety Data Sheet (SDS, previously called a Material Safety Data Sheet or MSDS) that provides information on how the chemical affects health and safety. It gives guidance on safe handling and storage procedures for the chemical as well as emergency procedures for exposure, and considerations for the correct disposal of the chemical. The SDS for a chemical can usually be found by an Internet search or looking on the manufacturer's website. It is important to read the SDS when assessing the risk associated with the use of the chemical, and the precautions you should take in your experiments.

Table 12.1 Matrix for assessing severity of risk

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

Once you have considered what the possible risks are, you need to think about what you will do about them. What will you do to minimise them, and what will you do to deal with the consequences if something does happen? This may be as simple as 'Always wear a lab coat, gloves and safety glasses.' You can use a risk assessment table similar to the one shown or those included in experiments throughout the chapters of this book.

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

Safe use and disposal of biological material

When dealing with many biological materials, it is important to be aware of safe handling and disposal. For example, when growing known or unknown microbes on agar plates, it is important to use safe sterile techniques (discussed below) and to wear lab coat, gloves, safety glasses and, if required, face mask. Treat all microbes on agar plates as potentially pathogenic and **autoclave** used plates before disposing.

Ethics

Ethics in research can be controversial. More than one scientist has lost their job for unethical research behaviour. Being ethical in your research has two aspects. The first is about being honest as a scientist. This means recording data accurately and not ignoring, hiding or changing any data that does not support your hypothesis. It means acknowledging and referencing sources of information, including books, websites,

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Figure 12.5 ▲
The use of animals for research purposes is governed by State and Federal laws.

articles and people who have helped you. It means not using other people's ideas or data without their knowledge and permission. Put simply, it is showing integrity or 'doing the right thing'. A good rule is that if you would not want someone to know what you are doing, you probably should not be doing it. It is no different from behaving ethically in any other area of your life.

The other aspect to ethics is treating animals, other people and the environment with care and respect. If scientists will be using humans then they need to make sure they do not harm them, either physically or psychologically. If scientists are working with animals, then they need to make a strong case for any investigation that harms or could potentially harm them. When scientists want to use humans or animals in their research, they need to be able to show that the benefits to the environment, other animals or humans significantly outweigh the negative effects on the animals or humans used.

The use and welfare of animals for the purposes of research is legislated by State and Federal laws, and respect for all animals (vertebrate and invertebrate) used in research is of the utmost importance. When using animals for research, scientist must adhere to the '3Rs'. These are:

- *replacement* of animal research with other types of research where possible
- *reduction* of the number of animals used in research
- *refinement* of experimental techniques to minimise pain and distress.

The National Health and Medical Research Council (NHMRC) has guidelines on the ethical use of humans and animals in experimentation. If you are planning to collect live specimens in the field, be aware of specific State laws that may pertain to native species (including plants).

Experimental techniques

At an early stage of your investigation, you will need to plan your experimental procedure carefully, taking into account the minimisation of uncertainty, the risks involved and ethical considerations. Importantly, **feasibility** is something you will need to establish before you go too far with planning your investigation: can you realistically carry out the study with the resources available to you? Is it easy enough to acquire the reagents you will need? Do you need special equipment for the study?

There are many techniques available for scientific investigations. For example, in many laboratories, it is essential to use the aseptic technique (discussed below) to ensure that only the desired organisms grow in culture. This is an example of a general technique that is standard protocol in labs around the world. This technique is also adaptable for use in a school laboratory because it does not need specialist equipment. If you are able to use only techniques that do not require specialist equipment then your investigation will be much easier to carry out. However, if you have an idea of something you would like to investigate that does require specialist equipment, labs in research institutes and universities can be quite accommodating and may allow you to work in their lab, under their guidance, for a couple of days while you gather your data. This could also be an opportunity to see how a real lab works and to get some helpful feedback as you plan, conduct and analyse data. The process of gathering guidance and feedback from peers and more senior scientists is an important part of a research career.

Below are some examples of techniques that you may be able to use in your scientific investigation.

Aseptic technique

When dealing with cell culture (plant, animal and microbial), it is important to practise good **aseptic** (sterile) **technique**. This may mean working in a laminar flow hood – an enclosed workspace that prevents contamination of biological samples by maintaining positive pressure using filtered, sterile air. If a laminar flow hood is not available, a small sterile space can be created using a Bunsen burner, whose flame creates an updraft and kills airborne contaminants in the surrounding air.

Working in a sterile space must be combined with careful handling of all biological material and equipment and regular decontamination, typically with a solution of 70–80% ethanol.

Alternatively, when preparing microbial cultures on agar, utensils used to transfer the microbes may first be held over a flame to sterilise them, then cooled in a sterile environment before use. Holding the Petri dish upside down as much as possible minimises the opportunity for airborne contaminants to land on the agar.

Bioinformatics

While you are limited to 'wet-lab' techniques that are feasible using the equipment and reagents you have access to, there are many online databases and tools that allow you to conduct 'dry-lab' **bioinformatic** investigations. Bioinformatics is the digital storage, retrieval, organisation and analysis of genomic data. For example, most of the phylogenetic studies discussed in Chapter 9 are bioinformatic studies. The genome sequences of various organisms can be accessed online using the Genome Browser at University of California, Santa Cruz. DNA or protein sequences from an unknown organism or gene can be identified using a Basic Local Alignment Search Tool (BLAST), and multiple DNA or protein sequences, which can be found on the National Center for Biotechnology Information website, can be compared and organised into a phylogenetic tree using a Clustal algorithm. Internet searches can be used to find these tools as well as resources detailing how to use them.

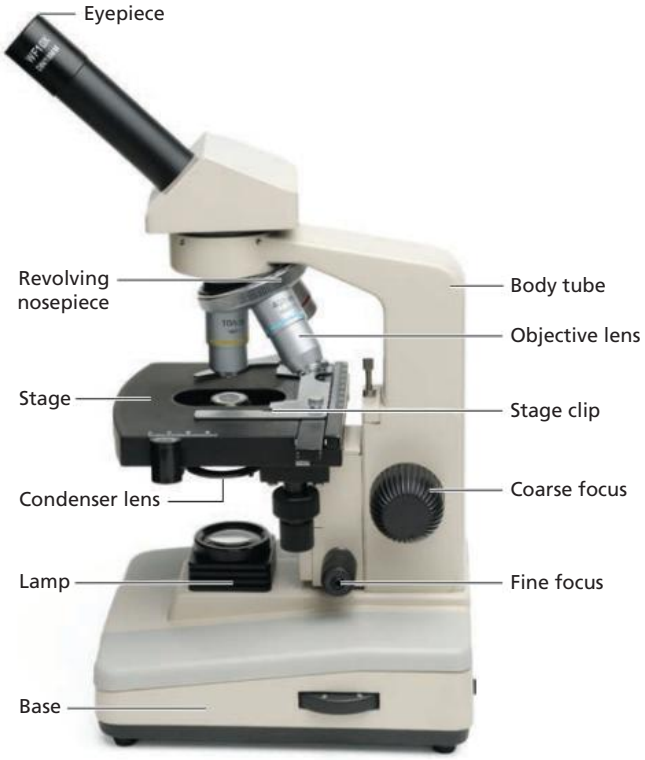
Using a microscope

Dutch scientist Anton van Leeuwenhoek changed our understanding of the biological world with his creation of a microscope that could observe bacteria, cells and other microscopic things. Since then, microscopes have become more powerful, widespread and easier to handle. You may choose to use a microscope as a way of making primary observations of microscopic objects as a part of your scientific investigation.

Use Figure 12.7 to identify the parts of the microscope. The microscope shown in Figure 12.7 has an inbuilt light source. If your microscope has a mirror, you will also need to have a light source. Check to see whether your microscope has an iris diaphragm or a wheel diaphragm.

▼ **Figure 12.6**
The use of a laminar flow hood can prevent contamination of biological samples.

Alamy/BSIP SA



Alamy/Martin Shields

Figure 12.7 ▲
The parts of a monocular microscope

To set up the microscope, move the nosepiece so the low power objective clicks into position vertically in line with the body tube of the microscope. If you are using a mirror, use the concave side of the mirror and move the mirror so light is reflected up through the stage.

Look through the eyepiece. If there are two adjustable eyepieces, move them together or apart so that the circles of light you see overlap, giving you a unified view. Adjust the iris or wheel diaphragm so that the circle of light first condenses and then dilates so that it is just outside the visible field of view.

You next need to find the size of the field of view. Place a minigrid or a slide with a piece of graph paper (marked with millimetre gridlines) on the stage. Without looking down the eyepiece, lower the body tube until it is almost touching the slide, then look through the eyepiece and start to raise the body tube using the coarse adjustment focus knob until the gridlines come into focus. The fine adjustment focus knob can help you focus more sharply.

Move the gridlines so that one sits at the leftmost edge of the field of view. While using the low power objective, you can estimate the diameter of the field of view (Figure 12.8). The eyepieces usually magnify 10× and the low power objective 10×. This means that looking through the eyepiece and the low power objective gives an overall magnification of $10 \times 10 = 100\times$. Other objectives used on standard light microscopes are 20× and 40× magnification. Use this information to calculate the size of the field of view.

Remove the grid and place a prepared microscope slide on the stage. Adjust the focus as before: not looking down the eyepiece, move the body tube down until it almost touches the slide, then look down the eyepiece and move the body tube upwards first with the coarse adjustment knob then the fine adjustment knob until you have a sharp focus.

Now rotate the objectives so that a higher power objective clicks into line with the body tube. You may need to increase the light intensity if possible and adjust the focus slightly.

Practise looking at some prepared slides first under low power magnification and then under higher power magnification. Can you distinguish different cellular features that you have learnt about? Can you estimate the size of cells based on your calculation of the size of the field of view under low power and your observation or estimation of the number of cells that span the field of view (e.g. Figure 12.9)? Practise drawing some cells and including scale bars on your diagrams.

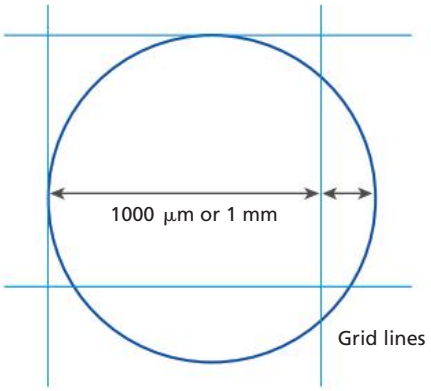


Figure 12.8 ▲
Estimate the field diameter using a minigrid.

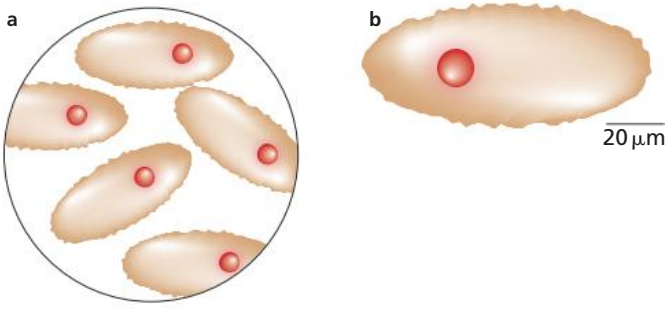


Figure 12.9 ▲
(**a**) Cells seen with the high power of a microscope. The field diameter of this microscope is 0.2 mm. (**b**) A cell with a scale bar. This cell is 100 μm long.

Notes

- The iris diaphragm or wheel diaphragm focuses the light from the light source onto the specimen on the stage.
- If you are going to visually represent anything you observe down the microscope, for example with a diagram or a photograph, it is essential to include a scale bar. This can be calculated as described above.
- Remember that the objectives have a scale of magnification (for example, 10×, 20× and 40×) but the overall magnification will be increased by ten times due to the eyepiece magnification of 10×.
- Scientists often stain samples so that they can distinguish features such as cell walls, organelles, starch granules and many other structures more clearly. Otherwise these features cannot be easily identified using a light microscope.
- Biological drawings of objects observed under a microscope should be made in pencil and have solid joined lines that outline features, with no shading or sketching. Features should be labelled with a ruled line and neat handwriting. A scale bar should be included (usually in the bottom right corner), and the diagram should have a title at the top. Images should fill the space available – ideally a whole page for each diagram – so that features are easily distinguishable for the viewer.

Biochemistry techniques

Biochemistry is the study of the chemical processes of living things. Understanding the structure and function of proteins, enzymes, lipids and organelles allows scientists to understand their activity better. Advances in biochemistry have the potential to change many industries. Applications including production of biofuels, improving photosynthesis to enhance crop yields, investigating mitochondrial DNA sequences and isotope profiles of hominid remains to advance studies of hominid evolution, and applications in medical research. For example, some genes and proteins that cause cancer have been identified and their structures solved, the signal transduction and apoptosis pathways they affect have been found, and drugs have been designed to block these pathways to treat cancer. All of these steps involve biochemistry.

Studying DNA

In Chapter 11 you learnt about various techniques for manipulation of DNA, including the polymerase chain reaction (PCR), gel electrophoresis and DNA sequencing techniques. You may be able to undertake an investigation that involves DNA manipulation if you are able to work in a lab that has the equipment needed for these techniques. Designing primers for PCR will allow you to choose which DNA fragment you amplify, and likewise specific primers allow you to sequence a specific region of DNA. Figure 12.10 shows an example project involving these techniques.

Studying enzymes

While it takes expensive equipment and specialist techniques to work with some enzymes, others are much easier to use. Some plant hormones are commercially available for example, and several enzymes are easily isolated and studied. Table 12.2 shows some examples of enzymes, their sources, the reactions they catalyse and the ways you can measure their activity without expensive or specialist reagents and equipment. Just be aware that you will not be able to get pure enzyme from these sources, which may mean there are more independent variables that need to be controlled for. Be careful to conduct your risk assessments before doing the study. You will also need to find more information on each of the techniques you will use in the study.

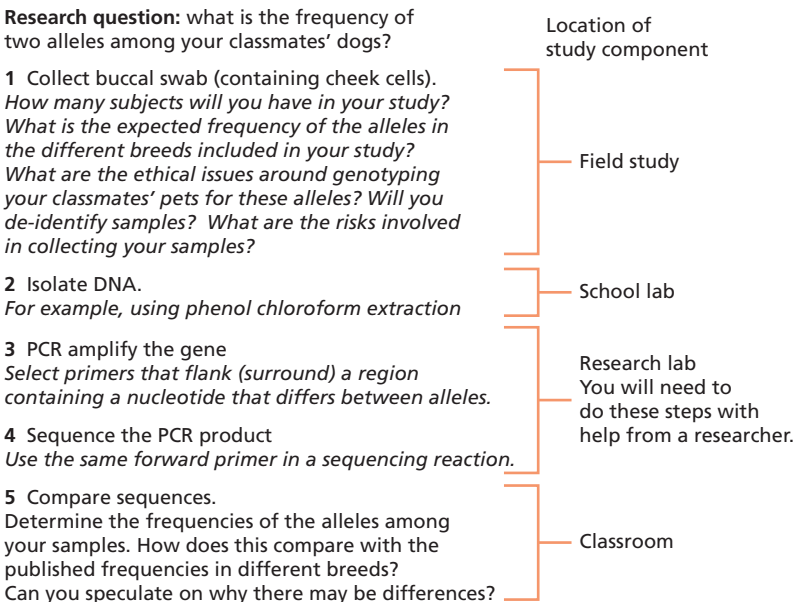


Figure 12.10 ▶
An example investigation involving DNA manipulation

Table 12.2 Common enzymes that could be used in an investigation

Enzyme	Source	Reaction	Applications
Lysozyme	Hen egg Tears Saliva (only work with your own tears or saliva)	Antibacterial – breaks down peptidoglycans in bacterial cell walls. This results in reduced bacterial growth in liquid culture or agar plates.	This enzyme is often used to lyse bacteria to isolate plasmids for biotechnology purposes.
Catalase	Liver (it is in most tissues of multicellular organisms and all aerobic micro-organisms)	$2H_2O_2 \rightarrow 2H_2O + O_2$ If catalase is present and active, bubbles of oxygen gas will develop if hydrogen peroxide is provided.	Some bacteria are catalase-positive and this test can help identify bacterial strains. This is also an easy assay to measure whether a protein (catalase) has been denatured. Heavy metal ions are non-competitive inhibitors of catalase.
Lipase	Can be bought	Converts lipids to fatty acids. This causes the pH of the solution to decrease, which can be detected using an indicator dye.	Used to diagnose cystic fibrosis, coeliac disease and Crohn's disease
Amylase	Can be bought Saliva (only work with your own saliva)	Breaks starch (such as from rice) into smaller sugars including maltose and glucose. Starch degradation is detectable by iodine staining.	Used in bread making, brewing beer, in detergents for washing clothes and dishes, and as a reporter gene in biotechnology. The gene for salivary amylase, *AMY*1, exists in different numbers in different human populations and may have been selected for during early human evolution.
Trypsin	Can be bought Pancreas	Hydrolyses proteins. Photographic film for laboratory work is coated with a mix of gelatine (protein) and silver halides. Digestion of the protein by trypsin releases the silver salts, clearing the film. Also breaks down casein in milk, causing milk to become translucent.	Trypsin is a digestive enzyme that has many commercial applications. It must be handled with particular precautions because it can also hydrolyse itself.

Biochemistry assays

More and more biochemistry assays are becoming available as scientists discover new ways of detecting and observing biochemical processes. However, you will be limited by several factors in the types of biochemical assays you are able to use in your investigation. Many tests use expensive reagents and assume access to specialised equipment to read the results. It may be difficult to understand many tests without having studied specific aspects of biochemistry. These factors mean you will have to be more creative when designing your study. Some examples of biochemical tests are listed in Table 12.3. You can find out more about each of these tests, whether they might be useful in a study, risks involved in the tests and how to perform them, by researching them online.

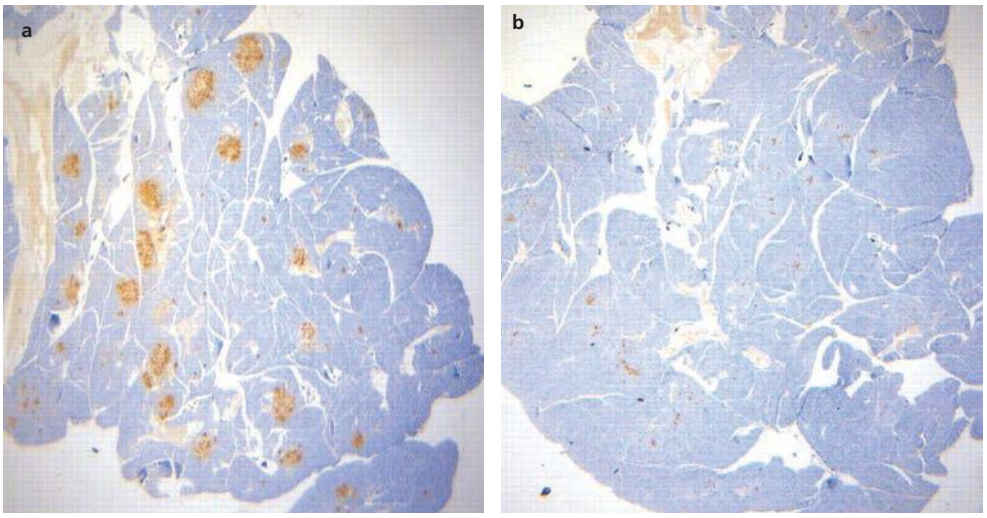
Table 12.3 Some biochemical tests

Test	Uses
Bicinchoninic acid (BCA) assay Lowry test Bradford assay	Determining protein concentration in a solution
Clinistrip (Diastix)	Detection of glucose (mainly in urine but can be used for other applications)
Enzyme-linked immunosorbent assay (ELISA)	Uses monoclonal antibodies to measure the concentration of a specific molecule (see page 424)
Gel electrophoresis	Used for separating DNA fragments of different lengths
Liquid chromatography	Separates components of a solution
Luminol	Luminescent reagent for detection of blood, or copper or iron in cellular assays
Lysochrome	A dye used to stain for lipids
Phenol-chloroform extraction	For isolation of DNA

Cytology techniques

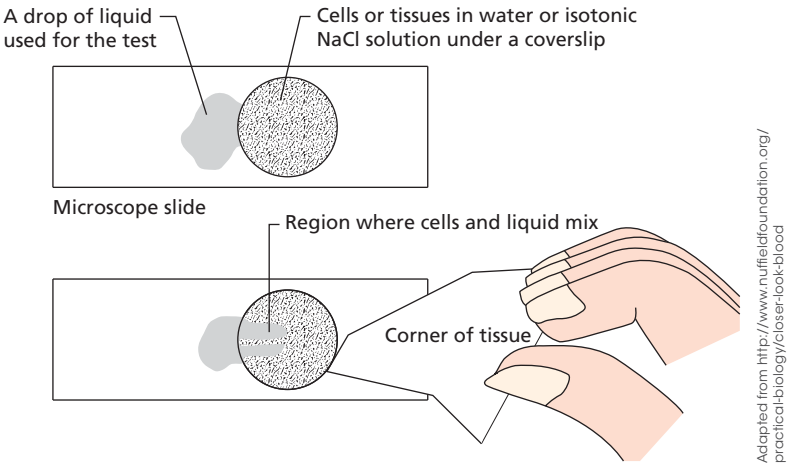
Cytology concerns the study of living cells. This includes the study of normal cell anatomy and behaviour, as well as the examination of tissue samples for diagnostic purposes (cytopathology). An example of a common cytological test is the Pap test, where cervical cells are examined for any abnormalities that are associated with cervical cancer. The sampled biological material is usually spread on glass slides and stained, then studied using a microscope. **Immunohistochemistry** is a staining technique used to detect molecules on cells or tissues on a microscope slide. It involves incubating the sample with a solution of antibodies that are specific for epitopes on the molecule. The antibodies used are labelled with fluorescent markers or enzymes that convert a substrate to a coloured product, and the slide can be viewed under the microscope for areas that contain the coloured substrate.

If you are preparing tissues for cytological examination, be careful not to let the cells or tissues dry out while you are handling them if you want them to stay alive – you can use water or an isotonic NaCl solution (9.0 g in 1 L distilled water) to keep them moist. You may wish to study the effects of solutions on cells using the technique shown in Figure 12.12. Fix them onto a microscope slide and stain them for viewing under a microscope using the method shown in Figure 12.12. Fixation kills the cells and essentially takes a snapshot of the state of the cells at the time of fixation, rather than allowing the cells or tissues to break down while they are being stained, viewed or stored. Choose an appropriate staining protocol depending on what you are measuring.



Development and characterization of a novel rat model of type 2 diabetes mellitus: the UC Davis type 2 diabetes mellitus UCD-T2DM rat', Bethany P. Cummings, Erin K. Digitale, Kimber L. Stanhope, James L.Graham, Denis G. Baskin, Benjamin J. Reed, Ian R. Sweet, Steven C.Griffen, Peter J. Havel. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology Dec 2008, 295 (6) R1782-R1793; DOI:10.1152/ajpregu.90635.2008. Figure 4

Figure 12.11 ▶
Immunohistochemical staining of insulin (brown) in islets of Langerhans of (**a**) healthy and (**b**) diabetic rat pancreas.



Adapted from http://www.nuffieldfoundation.org/practical-biology/closer-look-blood

Figure 12.12 ▶
A method for mixing liquid with cells on a microscope slide

Sources of cells for cytology

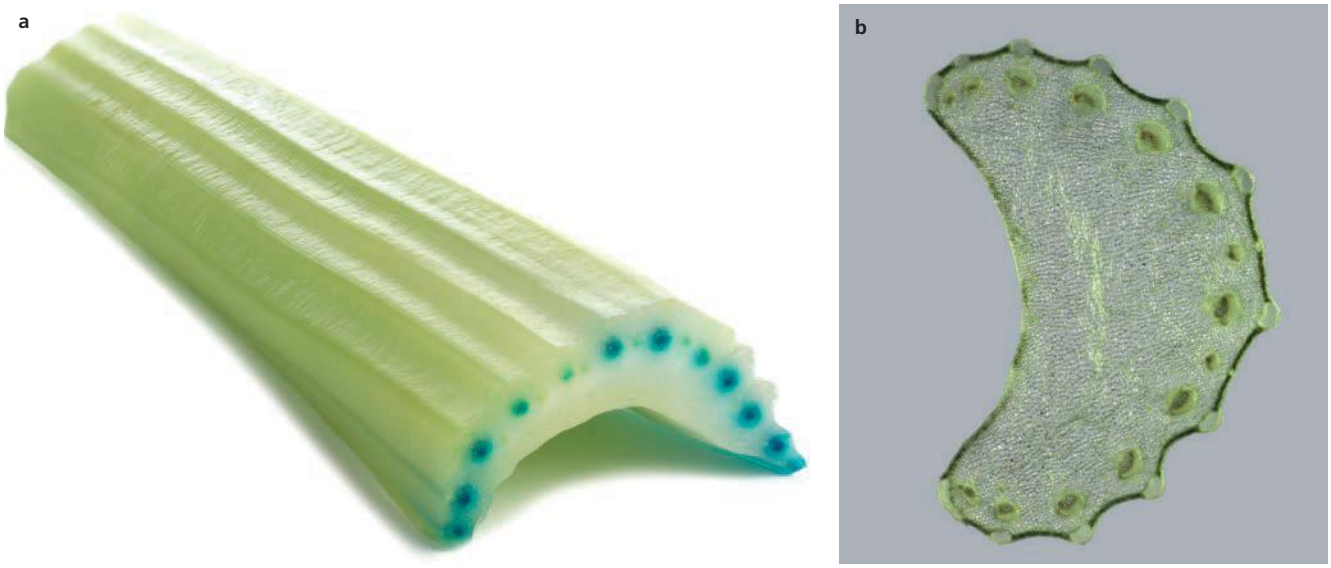
In your investigation, you will not be able to work on human cells due to health and safety considerations and ethical issues. However, you could examine prepared slides of fixed (dead) human tissue or animal cells. You could also design an investigation that involves the study of plant cells and tissues, or microbes such as yeast or bacteria. Before working with biological samples, conduct a thorough risk assessment. Ask your teacher for guidance at an early stage of planning.

Elodea is a water plant that has very thin leaves that are ideal for viewing plasmolysis and cytoplasmic streaming. You can make a whole wet mount of an *Elodea* leaf simply by placing it on a drop of water on a slide, then placing a coverslip over the top. To observe plasmolysis, put a drop of a salt solution on one side of the coverslip and draw it through by touching the edge of a tissue to the fluid on the edge of the other side of the coverslip.

To look at plant epidermal cells, take a piece of rhubarb or red beetroot leaf stalk and scrape the side of the stem with your finger or thumbnail until it just breaks the surface, then peel off some of the epidermis. You could also use your nail or tweezers to scrape a piece off the cut end near the surface and peel it up, keeping it tight. Although it may begin as a thick piece as it peels off, sometimes it becomes thin. Then use the razor blade to cut across the thinnest piece so that it is no longer than about 10 mm. Place a drop of water on a microscope slide and spread out the epidermal section over the water. Put a coverslip over the top and examine under the low power

using the microscope. You should see parallel lines of cells. If there appear to be criss-crossed lines, then you have at least two layers on top of each other and should remove the coverslip and spread the specimen out until it is one layer thick, or start again with a new piece. View the slide under high power to make a diagram of the cells, remembering to add a scale bar to the diagram.

To observe cells in a plant leaf or stalk, you can make a transverse section. Leaves are made of an outermost layer of cells called the epidermis. Epidermal cells produce a waxy substance that forms a cuticle impermeable to water. The pores (stomata) in the epidermis that allow for gas exchange are formed between specialised epidermal cells called guard cells. Vascular bundles (veins) are embedded in the mesophyll, the tissue that includes all of the cells between the upper and lower epidermis. The cells of the mesophyll contain the photosynthetic pigments. To cut a thin transverse section, you can hold the leaf down with a finger and use a razor blade with the flat side pressed against the supporting finger, cutting the leaf carefully towards you. Try several cuts with your finger in the same position or rolling very slightly downwards. If the leaf is very fine you can put it into a slit cut in a fresh carrot and use the carrot as a support for cutting the leaf. Use the corner of a razor blade or forceps to pick up some sections and tip them onto their sides on a drop of water on a slide, which you then cover with a coverslip. Movement of fluids through plant vascular tissue can be observed by cutting transverse sections of celery stalks that have been soaked in a solution containing food dye (Figure 12.13).



Science Photo Library

Science Photo Library/Eye of Science

▲ **Figure 12.13** (**a**) Xylem in a celery stalk stained with food dye (**b**) A cross-section of a celery stalk viewed under low power on the microscope

In plant root and shoot tips there is new material being produced relatively quickly and it is here we look to find examples of mitosis. Onion roots are ideal. You first need to stimulate new root growth by suspending the onion over a beaker of water placed in dull light for a couple of days. Use toothpicks to stabilise it with the water covering the bottom of the bulb. You can then follow established protocols for squashing the root tip and staining it ready for viewing the chromosomes at different stages of mitosis.

You need not limit your study to plant tissues. For example, you can observe budding in yeast or hydra by putting a couple of drops of a solution containing the organisms onto a slide then placing a coverslip over the top. Microscopic observation of cells will usually be the method used to read the results of an experiment that manipulates the cells, for example incubating cells in different growth conditions for a couple of days before cytological analysis.

Immunology techniques

Immunological investigations may study what frequency of the population has immunity to a particular pathogen, how different cells behave in a situation of infection, how cells interact with each other, and how signalling molecules such as cytokines affect the behaviour of different types of white blood cell. It is difficult to conduct immunological investigations on human tissues because sampling tissues other than blood is challenging. For this reason, mice are often used as a source of immune tissues, and much of our knowledge of immunology has been gathered from mouse studies. The *Australian code for the care and use of animals for scientific purposes* governs the care and use of all live non-human vertebrates and cephalopods for the acquisition, development or demonstration of knowledge or techniques in any area of science. Any studies on animals must adhere to this Code and so it is unlikely that you will be able to perform investigations involving animals. However, you can study plants, or conduct 'dry-lab' immunological investigations using online tools such as OMIM (Online Mendelian Inheritance in Man) to investigate the genetic basis of autoimmune diseases, for example. The Gene Expression Omnibus at the National Center for Biotechnology Information allows you to search publicly accessible gene expression data sets from other researchers' studies for expression of genes in different settings, such as during infection.

Cell culture

Many immunology assays involve stimulation of cells under various conditions followed by measurement of a cellular response, such as the production of a cytokine that could be measured by ELISA (below). One source of cells on which immunological experiments are conducted is a cell line. There are many different cell lines to choose from, depending on the research question being investigated. For example, scientists might use a macrophage cell line to microscopically examine how phagocytosis of microscopic synthetic beads changes in response to treatment with a bacterial preparation. There are many different types of cellular immunology assays, including assays for cell activation, proliferation, gene expression and apoptosis.

ELISA

The enzyme-linked immunosorbent assay (**ELISA**) is a common immunology technique for detection of a molecule, or detection of antibodies that recognise a particular antigen. For example, an ELISA can be used to quantitatively determine the concentration of antibodies in the blood plasma of an individual that recognise a specific pathogen, or to detect the presence of a pathogen in a tissue sample, such as potato leafroll virus (PLRV). PLRV is one of the most important pathogens of potato plants, responsible for around 20 million tonnes of lost potato crops annually. Affected plants become yellowed and dwarfed with curled leaves and reduced yield of around 50% (Figure 12.14).

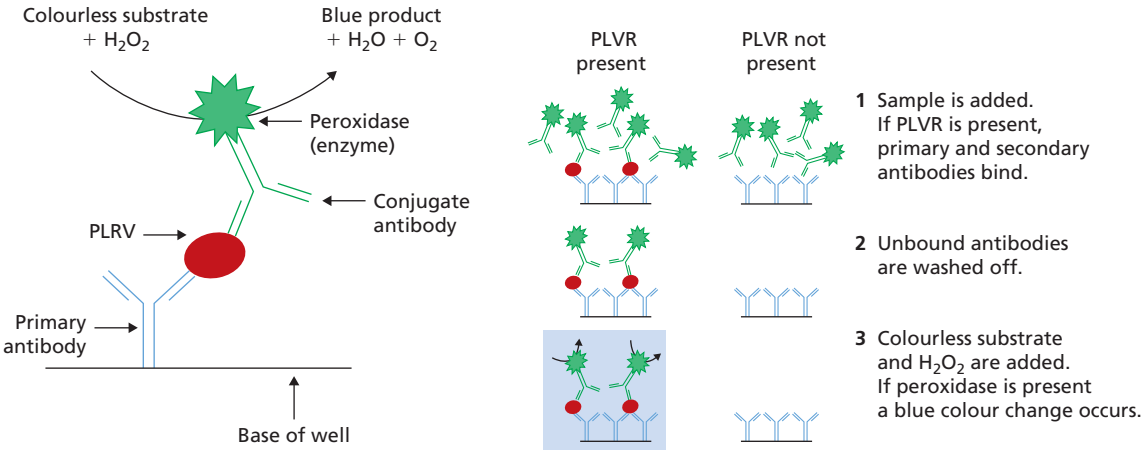
Figure 12.14 ▼
The plant in the middle is affected by potato leafroll virus.

FN3PT/RD3PT

To detect PLRV, a homogenised (finely ground) sample of potato leaf diluted in a solution is incubated in a well that has been coated with monoclonal antibodies that are specific for an epitope of the virus. The sample is then washed off and any virus in the sample will remain, bound by the antibodies on the bottom of the well. The viral particles can be detected by adding a secondary monoclonal antibody that binds to a different viral epitope. The secondary antibody is conjugated to an enzyme (peroxidase) that converts a colourless substrate to a coloured product. For a well to

have this colour, it must contain PLRV particles that allowed the secondary antibody to stick down (Figure 12.15). The degree of colour change reflects the concentration of PLRV particles in the leaf sample.

ELISA kits are commercially available for most molecules that may be of interest, including on-site agricultural kits for detection of PLRV and other agricultural pathogens. You may have to go to a lab that has an ELISA plate reader to quantitatively measure the results. Alternatively you could devise a scoring method to visually score the degree of colour change in each well, although this should be conducted in a blinded manner to reduce bias. You could also search for an ELISA-based method such as a dipstick test that gives qualitative rather than quantitative results and does not require specialist equipment to obtain results.



▼ **Figure 12.15**
The sandwich ELISA method to quantify the concentration of a molecule or particle such as PLRV

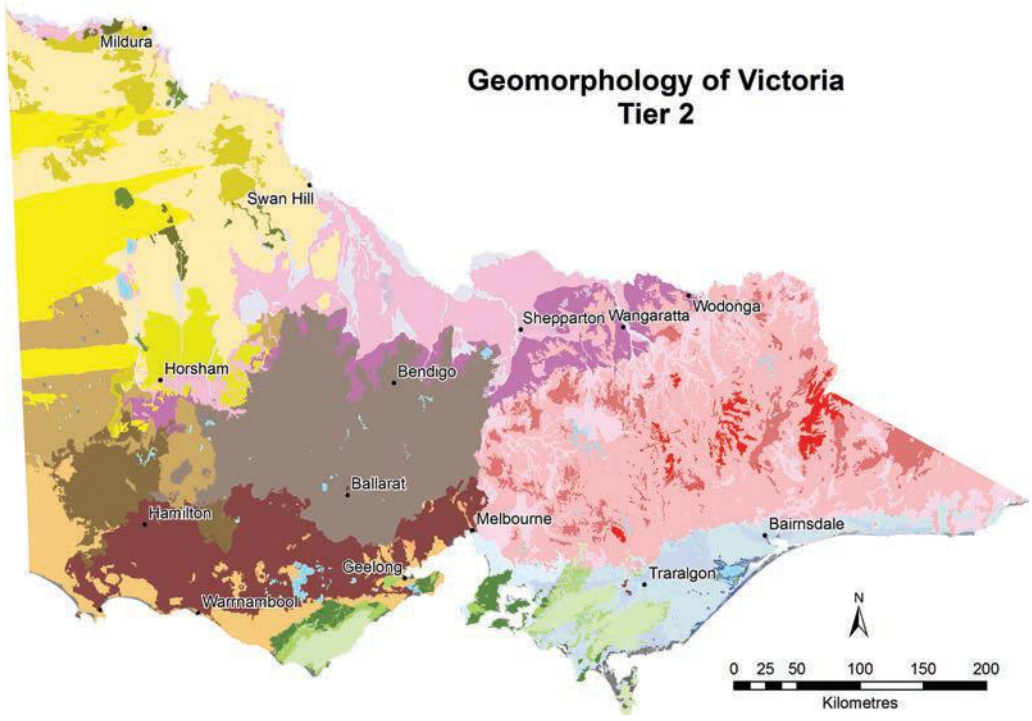
Geomorphology

Landscapes are archives of the past, and hidden within them is evidence of past distributions and behaviours of many organisms, including historical humans. Fossils and artefacts such as tools and remnants of fire pits are preserved within sedimentary layers, revealed at the surface as they become exposed, or are excavated in fieldwork investigations. The investigation of past organisms and human cultures benefits from being combined with **geomorphology**, the study of the history of the landscape itself. Geomorphology aims to interpret observations from landscapes to understand how they form and change – shaped by the movement of rock, sediment (soil) and water caused by weathering, erosion, transportation and deposition by gravity, ice, wind or water. Movement of land mass is complex and dynamic, influenced by tectonic processes, geology, climate and ecological factors, and acts on many different scales of time and space.

Governments usually collate region-specific data, collected by a group of geomorphologists and made available online. On the Victorian Government website, the Victorian Geomorphological Framework maps regionally distinct geomorphological characteristics in Victoria (Figure 12.16). These reveal interesting information about the history of familiar locations.

For example, Mt Dandenong and the Cathedral Range are remnants of hard Devonian sandstone, towering above surrounding land. This same type of resistant stone forms the impressive features of the Grampians, with the valleys between them carved out in soft shale or weathered granite. Ballarat and the area extending north and west is characterised by hills, valley slopes and plains on Palaeozoic rocks (coloured grey-brown in Figure 12.16). Glacial movement in the Permian period has scoured out scrapes in rocks in the Werribee Gorge near Bacchus Marsh. Western Victoria was once a large volcanic plain, leaving behind fertile soils and impressive lava formations, such as Hanging Rock near Woodend. In the east, basalt plains laid down by Palaeogene volcanic activity cover

Figure 12.16 ▶
The different colours show regions of Victoria with different geomorphological characteristics.



As published on Victorian Resources Online website, Department of Economic Development, Jobs, Transport and Resources. http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/landform_geomorphology

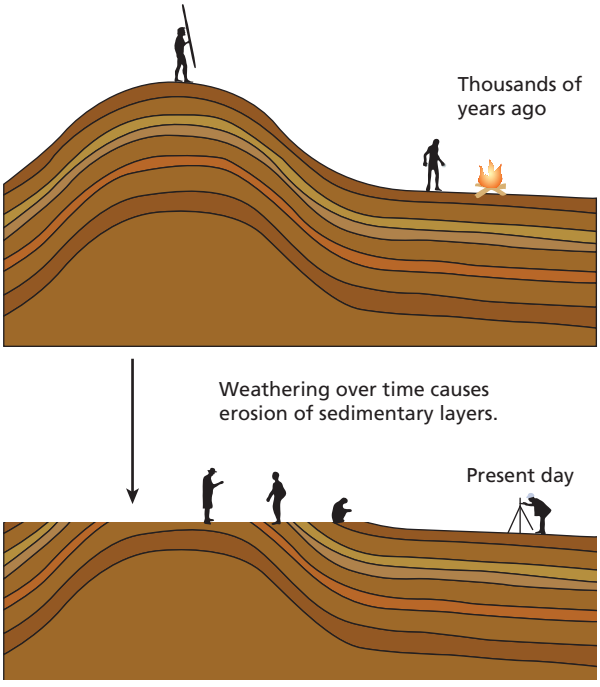
sediments that contain early Eocene macrofossils. In the high country of eastern Victoria, ski slopes now adorn folded Ordovician sedimentary rocks, and peaty soils on the high plains formed from fern and bog vegetation contain fossilised pollen that is useful for the study of the late Quaternary environment in the area.

Dating of fossils and artefacts

Studies of the fossil record can give insight into species diversity and distribution during different historical periods. Fossils can be found in some road and rail cuttings, exposed rock and sediments along the coast and in riverbeds, and in quarries. Sites that are dangerous should not be used – check with local authorities if you are unsure. There are many books and online resources that can give you more information about specific fossil sites and how to identify fossils. You may choose to study the range of species present at a single site during a single period, or the persistence of individual species over a period of time at one site. You could sample the fossil record at different locations, limited only by your ability to travel to different sites or gather secondary data, previously gathered by other fossil hunters.

For studies of human distribution and behaviour in the late Holocene period, geomorphological information needs to be gathered on a small scale. For example, the archaeological record of Australian Aboriginal people is mainly contained in surface deposits of stone artefacts, but the record is difficult to determine because of the complexity of geomorphic processes that have exposed the artefacts on the surface. Different sedimentary layers become exposed at different rates even in a small region (Figure 12.17) and this can make it difficult to understand the age of artefacts found within those layers.

Figure 12.17 ▼
Geomorphic processes can expose different sedimentary layers at different rates



Different sedimentary layers are now exposed at different sites.

European pastoralists have had a major effect on Australian geomorphic processes. For example, sheep grazing has sped up changes in the landscape and essentially excavated sediments at certain sites. Because of these many factors, when conducting archaeological studies in the field it is necessary to combine an assessment of the landscape with newer methods to make an accurate estimate of the age of the fossils or artefacts.

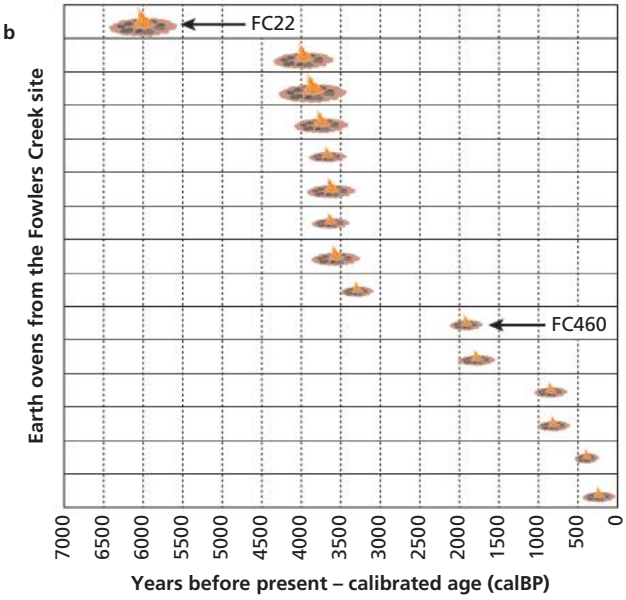
High resolution surface topography data is publicly available online in resources like Google Earth, and region-specific data such as the Victorian Geomorphology Framework provides a wealth of information on local sites. Absolute dating methods such as radiometric dating, electron spin resonance dating and luminescence methods have greatly increased the accuracy with which artefacts and the surrounding sediment can be dated.

See Chapter 7 for a discussion on absolute dating methods.

Several techniques are usually combined to get the most accurate estimate of age of artefacts. Sediments in the region are analysed for their geologic history. Optically stimulated luminescence (OSL), which determines the length of time since the sediments were last exposed to light, provides a more accurate measurement of the age of the sediments on which the artefacts rest. Radiocarbon dating can be used to measure the age of artefacts such as charcoal contained in Aboriginal earth ovens. For example, a site in Fowlers Gap in western NSW was studied to determine when Aboriginal people used fire for cooking in the area (Figure 12.18a). The radiocarbon data from heat retainer hearths, or earth ovens, in the site is shown in Figure 12.18b, which shows the oldest oven (called FC22) to be around 6000 years old. This oven was at a depth of 32 cm below the surface, and OSL data from surrounding sediments shows them to have been deposited between 6740 and 4340 years ago, which matches the radiocarbon dating of charcoal in the oven. Sediment at a different nearby site at a depth of 28 cm was determined by OSL to have been deposited between 10 920 and 7630 years ago. An earth oven at this site, FC460, was found to be less than 2000 years old (Figure 12.18b). While the OSL and radiocarbon dating information match for the FC22 site, they are quite different for the FC460 site, demonstrating the need for multiple approaches to estimating the age of archaeological artefacts.

▼ Figure 12.18
(**a**) The cluster of fire-cracked rocks in the centre is the remains of an Aboriginal earth oven at Fowlers Creek in western NSW. (**b**) OSL dating of charcoal in an Aboriginal earth oven at Fowlers Gap in western NSW. Peaks represent estimates of the age of charcoal at different oven sites.

Source: Holdaway S.J. and P.C. Fanning 2014 Geoarchaeology of Aboriginal Landscapes in Semi-arid Australia. CSIRO Publishing, Melbourne. Plate20



Source: Holdaway S.J. and P.C. Fanning 2014 Geoarchaeology of Aboriginal Landscapes in Semi-arid Australia. CSIRO Publishing, Melbourne. Figure 4.14

Geomorphology and biodiversity

The diversity of species is strongly affected by geomorphology and this can be an interesting basis for an investigation. An example is changes in river ecology when river systems change their course, magnitude or pattern of flow. Human activities that affect geomorphological processes, with impacts on biodiversity, occur in many instances and present opportunities for new and potentially very important investigations. Methods for sampling of populations are covered in Unit 2 Biology.

Collecting your data

Once your experiment is under way, it is time to start collecting data. This is usually the fun part of any investigation. Do not forget you have a question to answer or a hypothesis to test. To do this, you need to make sure that you think carefully about what you do and keep good records.

Record keeping

You will need to keep a record in a logbook of what you do during your investigation.

Scientists keep a logbook for each project that they work on. It is a record of what they did, why they did it, and what they found out. A logbook is a legal document for a working scientist. If someone's work is called into question, or there are disputes over patents or ownership of data, then the logbook acts as important evidence. Every entry in a scientist's logbook is dated, records are kept in indelible form (pen, *not pencil*), and entries may even be signed. Scientists' logbooks include details of experiments such as methods and results. They include comments and ideas, thoughts about the experiments, and analysis. They frequently include printouts of data, photocopies of relevant information, photos and other items. The logbook is the primary source of information when a scientist writes up their work for publication. It is also important for assessment and for authentication purposes.

Some scientists keep their research records electronically, but most experimental scientists still keep a hardcopy logbook. There are several advantages to a hardcopy logbook over an electronic one. First, electronic records are easy to make changes to, and it is hard to track what was changed, when and by whom. Second, if you are working in a group, it can be hard to keep track of who has the most recent version of the files. Third, files can easily be deleted or corrupted. It takes much more care and discipline to maintain a good electronic logbook than a good hardcopy. Remember that the purpose of a logbook is to record and maintain evidence of what you did. Electronic evidence is not as reliable as a signed hardcopy document.

You should talk to your teacher about what form of logbook records they require you to keep and any specific formatting requirements the logbook needs to adhere to. Make an entry in the logbook *every* time you work on your investigation. At the start of each session you should record the date and the names of all the people with whom you are working at the time. If you are working in a group and all working in the same place at the same time, you may record tasks that another group member is performing in your own logbook (noting down in your book who did what) and the measurements being made. If you are working on your own, for example doing independent fieldwork, you will need to jot down what you do and the measurements you take as you go along.

Write down what you do as you do it. It is easy to forget what you did if you do not write it down immediately. An accurate record is important if you need to repeat any measurements or if you get unexpected results. The more detail you include, the easier it will be to prepare your report at the end of the study.

Include large, clear diagrams of any experimental set-up and include details of equipment used. You can also include photos of experiments.

Record the results of *all* measurements *immediately and directly in your logbook, in pen*. *Never* record data on bits of scrap paper instead of your logbook. Results must be recorded in indelible form. This means using a pen. Never write your results in pencil. Never use white-out or scribble over anything in your logbook. If you want to cross something out, just put a line through it. It is also a good idea to make a note explaining why it was crossed out.

Conventions for presenting your data

When collecting your data and communicating your results to a scientific audience (including your classmates and teacher), ensure you use the correct scientific terminology and the key biological terms to describe your study and your findings. Be aware of any *standard abbreviations* that may be applicable (you might have come across these during your background reading in the planning phase of your study), and use only the *International System* (SI) units of measurement or other units recognised for use with SI units (Table 12.4).

Table 12.4 Some examples of SI units

SI unit	Measurement
Kilogram (kg)	Mass
Metre (m)	Length
Second (s)	Time
Kelvin (K) Degree Celsius (°C)	Temperature

There are also conventions of scientific writing to be aware of when reporting your findings. For instance:

- All unit names are written in lower case letters except Kelvin and Celsius.
- The unit symbol should be in lower case unless it is derived from a proper name (e.g. Kelvin, Celsius). The exception is L for litre.
- A Non-standard abbreviations should never be used (such as sec for seconds or kph for kilometres per hour) and the unit symbol should not be altered in the plural (e.g. 1 kg and 5 kg, not
  5 kgs).
- A space should be left between the number and the unit symbol (e.g. 50 s not 50s)
- When writing large numbers, a space (not a comma) should be left between groups of three digits. This may be omitted in four-digit numbers, for example 12 250 for twelve thousand two hundred and fifty, 3000 for three thousand.
- Large numbers can be represented in scientific notation, such as 1.225×10^4 for twelve thousand two hundred and fifty.

Shutterstock.com/Africa Studio

Figure 12.19 ▲
Plan exactly what you will measure to collect your data. Do you want to test the length of roots or the mass of roots? Where do the roots end? Will you use fresh weight or dry weight?

Collecting raw data

If you have planned carefully and learned how to use the equipment, then hopefully your experiments will go smoothly.

The raw data should always be recorded directly in the logbook unless it is recorded using data loggers connected to a computer. In this case a printout of the data should be attached to the logbook and the file name and location recorded. Make sure that you measure and record everything you will need for your analysis. For example, if you are investigating root growth, you could record the amount of fertiliser used, the temperature and the starting length of the roots. It is much better to measure something and then discover that you did not need to, than to start your analysis and realise that you did not measure something that you do need.

Use appropriate units; for example, millimetres for lengths and grams for mass. If you are going to be collecting multiple data points, it is a good idea to draw a table to record them in. Label the columns in the table with the name and units of the variables. Do not put the units in the table cells. Note that the instruments you use will often restrict the accuracy of your measurements. For example, a ruler may only have markings down to 0.1 cm. Make a note of these restrictions as they may affect the accuracy of your final results, especially if the changes measured are very small.

If you have not made a mistake, then plotting and analysing as you go can allow you to spot something interesting early on. You then have a choice between revising your hypothesis or question to follow this new discovery, or continuing with your plan. Many research projects start with one question and end up answering a completely different one. These are often the most fun, because they involve something new and exciting.

Analysing your data

When you have collected all your data you will need to analyse it. Record all your analyses in your logbook. If you do your analysis on a computer, then record the file name and location and attach a printout of the analyses to your logbook. Many scientists have logbooks that are bulging with printouts of their analyses.

The first step is to organise your data. If you have more than a few data points, it is a good idea to display them in a table. You may have several tables for different experiments. You may also need to do some analysis of the data. For example, you may wish to show the change in root length over the course of the experiment in addition to the lengths at the beginning and end of the experiment. Will this be a simple difference (end length – start length) or will it be a fold change (end length ÷ start length)?

Plotting graphs is a useful way to begin the analysis of your data. Graphs are a way of representing data so that trends and relationships can be visually identified. There are many different sorts of graphs that can be used to organise and display data. These are described below.

You will usually need to do some calculations with your data to be able to answer your question or test your hypothesis. Remember to keep units on all quantities so that any derived values have the correct units. You will also need to calculate uncertainties on any derived quantities.

Identifying trends, patterns and relationships

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

Descriptive statistics

It is useful to provide a summary of your data and the observations that you have made in your investigation. You will have posed a hypothesis as part of the design of your investigation, such as 'The new fertiliser makes roots grow longer in two weeks than standard fertiliser'. To determine whether your study supports this hypothesis, you will want to know whether the average root length of your replicate samples treated with the new fertiliser is greater than the average of the sample replicates treated with the standard fertiliser. You will also want to know that if there is a difference, it is highly unlikely that the difference could arise due to chance alone. That is, the difference reflects a true effect of the new fertiliser on root growth and your results are not just a false alarm. Descriptive statistics allows you to present the main features of your results, such as the central tendency of your measurements (mean, median or mode) and a measure of the variability within the different groups (e.g. control or experimental groups) in your study. The variability may be presented as range, variance or standard deviation. These statistics are the first steps in analysing your data.

Table 12.5 Descriptive statistics

Name of statistic	What it describes	Formula or method	Notes
Mean	Central tendency	$\text{Mean} = \frac{\text{Sum of all measurements}}{\text{Number of measurements (n)}}$	The mean is the arithmetic average of the data set
Median	Central tendency	Central number in a sorted list of numbers, e.g. 34, 35, 38, 39, 40, 42, 45, 46, 48, 50 The median of this data set is $\frac{40 + 42}{2} = 41$	Useful when there are outliers in the data
Mode	Central tendency	The most common value in the dataset. e.g. 2, 2, 3, 3, 3, 4, 5, 6, 6, 7, 7, 7, 8, 9 There are two modes in this dataset – 3 and 7.	There may be more than one mode.
Range	Variability	Range = Highest number in the data set – Lowest number in the data set	The smaller the range, the closer the values in the data set.

(*Continued*)

Table 12.5 (*Continued*)

Name of statistic	What it describes	Formula or method	Notes
Variance	Variability	Calculate the mean of a data set, then calculate the difference from the mean for each value, then square each of the differences. Then, add all those squared differences and divide the result by the number of values minus 1. $\sigma^2 = \frac{\bullet (x - \bar{x})^2}{n - 1}$ where σ^2 = variance, Σ = sum of, x = each value, $\bar{x}$ = the mean, n = number of values	It is helpful to use a table when calculating variance, with rows for each value in the data set and columns for each progressive calculation.
Standard deviation	Variability	The standard deviation is the square root of the variance. $\sigma = \sqrt{\sigma^2}$ or $\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$ where σ = standard deviation	When comparing two populations or data sets, if the mean of one is greater than two standard deviations different from the other it is considered statistically significantly different.

Graphing your data

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

A graph should be large and clear. The axes should be labelled with the names of the variables and their units. Choose a scale so that your data takes up most of the plot area. The origin does not always need to be shown in graphs, but by including it you will provide an honest representation of the data without any exaggeration.

When you are looking for a relationship between variables, plot a scatter graph. This is a graph showing your data as points. Do not join them up as in a dot-to-dot picture. Usually the independent variable is plotted on the *x*-axis and the dependent variable goes on the *y*-axis, unless there is a good reason to do otherwise. For example, if you were measuring root growth in response to temperature, root growth (change in length) would be plotted on the *y*-axis against temperature on the *x*-axis.

To determine a relationship you need to have enough data points and the range of your data points should be as large as possible. A minimum of six data points (therefore, six replicates) is generally considered adequate if the relationship is expected to be linear, but always collect as many as you reasonably can, given the available time. For non-linear relationships you need more data points than this, so collect as many as possible.

A graph of the raw data is a good graph to start with. You will usually be able to tell by looking whether the relationship between the variables is linear. If it is, then fit a straight line using a graphing package. You can then use a

linear regression tool to check how good the straight line fit is. This will give you an R^2 number, which is a measure of 'goodness of fit'. The closer R^2 is to 1 (or –1), the better the fit. If it is not *very* close to 1 or –1, then the relationship is not linear. An R^2 close to 1 means there is a positive relationship, or correlation, and so as you increase one variable the other increases as well. An R^2 of close to –1 means there is a negative correlation – that is, as one variable goes up, the other goes down, or vice versa.

If it is a linear relationship, then finding the equation for the line of best fit may be useful. *Never* force a line of best fit through the origin. Often the intercept gives you useful information. It may even indicate a systematic error, such as an error in calibration of your equipment.

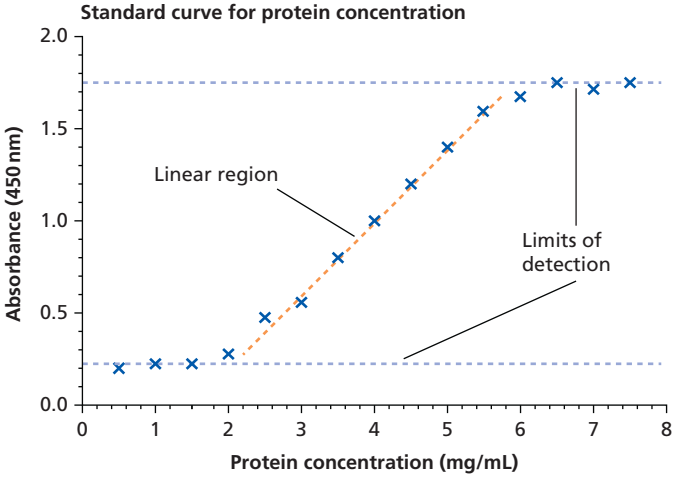
When you plot your raw data you may find that one or two points are outliers. These are points that do not fit the pattern of the rest of the data. These points may be mistakes; for example, they may have been incorrectly recorded or a mistake may have been made during measurement. They may also be telling you something important. For example, if they occur at extreme values of the independent variable then it might be that the behaviour of the system is linear in a certain range only. This is the case for many biological **assays**. You may choose to ignore outliers when fitting a line to your data, but you should be able to justify why.

When you extend a line of best fit beyond your measured points, this is called **extrapolation**. Any data that you read off a graph outside the range of your data points is extrapolated, and should be viewed with caution. You cannot say for sure that the system continues to behave in the same way beyond the bounds of your data.

Reading points, other than data points, from a line of best fit within the region in which you have data is called **interpolation**. You cannot be sure that this is exactly what you would find if you measured that point. However, if your line of best fit really represents the behaviour of the system, then you can use interpolated points in your analysis.

For example, an assay that measures the concentration of protein in a solution involves creation of a standard curve from which the concentration of samples can be interpolated (Figure 12.20). Because the assay has a minimum level of detection (its **sensitivity limit**) and a maximum detection limit above which the assay is saturated, it is not possible to extrapolate outside the linear region of the standard curve. To measure samples whose concentration is higher than the detection limits for the assay, the samples must be diluted so their concentration lies within the range of the assay (the linear region of the standard curve).

▼ **Figure 12.20**
Standard curves can be used to interpolate and extrapolate values, but only within the limits of detection.



In biological experiments, you are often comparing the effects of different independent variables on a single dependent variable. In the root growth example, you are comparing the effect of the new fertiliser with that of the standard fertiliser. In this case, you would typically plot a bar graph of the change in root length against the type of fertiliser used. If the data is taken from several different plants in each treatment group, you would plot the mean plus or minus one **standard deviation** (Table 12.5). These standard deviations around the mean are drawn as error bars, and may be drawn above or below the line of the mean, or both (Figure 12.21). The error is the same on both sides of the mean.

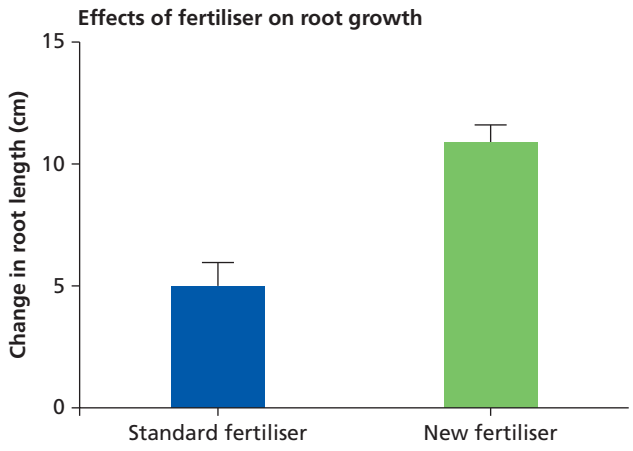


Figure 12.21 ▲
Bar graph of two data sets, showing error bars of one standard deviation from the mean

Interpreting your results

Once you have analysed your results you need to interpret them. This means being able to either answer your research question or state whether your results support your hypothesis. If you have performed statistical analysis, does this support your hypothesis? For example, if the new fertiliser induces statistically greater root growth than the standard fertiliser, with all other variables being equal, this would support the hypothesis that 'The new fertiliser makes roots grow longer in two weeks than standard fertiliser'. If there was no difference between the two, or if the new fertiliser induced significantly less root growth than the standard fertiliser, then this would argue against the hypothesis.

Statistical tests for significance

To be able to draw conclusions with any confidence, scientists use statistical tests to determine whether differences between data sets may arise through chance alone or if there is a significant effect occurring. If the means of two data sets are at least two standard deviations different from each other, they are considered statistically significantly different. On a bar graph that shows the mean with error bars of one standard deviation, you can visually estimate statistical significance: two standard deviations are approximately two times the length of the error bars on either side of the mean. If these two lengths do not overlap for the two populations, they are significantly different from each other. For example, in Figure 12.21, the new fertiliser produces statistically significantly greater root growth than the standard fertiliser. The error bar of the standard fertiliser (plus an extra length above it) does not overlap with the error bar (plus the length of the bar again) of the new fertiliser data set (which in this case is only shown above but can be imagined as being reflected below).

Alternatively, you could use a **t-test** to test for a **significant difference** between two data sets. Graphing software typically has functions for calculating standard deviation and for statistical analysis of the data. A t-test will generate a *P* value. *P* is a representation of statistical significance that describes the probability of the difference between two means arising by chance alone. It is essentially a measure of the rate at which an observed difference may be considered a false alarm. For a *P* value of 0.01, the probability that the populations are the same is only 1%, which is very low. They are therefore considered statistically significantly different. A *P* value of 0.05 or less is considered significant.

There are multiple different tests for significance that take into account complex relationships between data. For example, an ANOVA can test the effects of different doses of a particular treatment over time. Dose and time are two different independent variables and an ANOVA can determine whether there is a significant effect of either, or both, on the dependent variable, and whether they influence the effects of each other (for example, dose *x* causes a different growth rate over time than dose *y*).

The results from a statistical test for significance can help to support or refute your hypothesis.

If your hypothesis is not supported

If a test for significance does not show a difference in your data, depending on your hypothesis it may be that the hypothesis was not supported by the data. This may occur if the experiment was not able to show the effect posed by the hypothesis, for example, by not having enough replicates to reduce variability and produce statistical significance. Alternatively, the hypothesis may be wrong. However, it is not enough to simply say 'our hypothesis is wrong'. If the hypothesis is wrong, *what* is wrong with it?

It may be that you have used a model that is too simple, or did not take into account all of the other variables. For example, in the root growth experiments, it may be that the new fertiliser works best at a particular temperature, or over a longer time, or in conjunction with certain soil conditions. Or maybe it does not work with the type of plant you chose to use. It may be that the experiment was simply too limited to fully test the hypothesis. Thus, you might conclude that further experiments are required to test these other variables.

Before you decide that the model is at fault, however, it is a good idea to check carefully that you have not made any mistakes or ignored any variables.

Think carefully about any factors that you did not take into account but which might have affected your experiment.

Go through your method, results and analysis. Check that your equipment was correctly calibrated and that you were using it correctly. Check that data are recorded in the correct units and that units are correctly carried through all calculations during analysis. Check your analysis carefully. If you are working in a group, get another person to repeat the calculations.

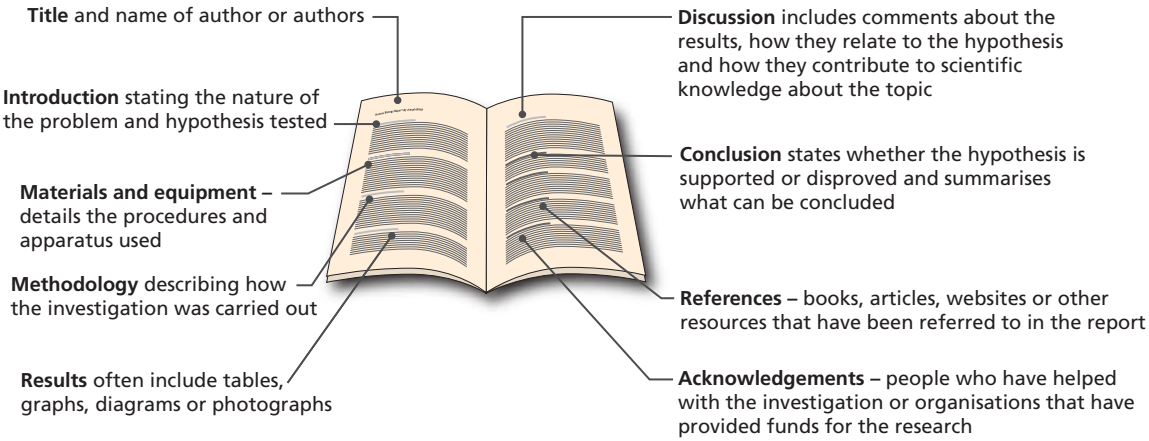
Limitations of descriptive statistics

Descriptive statistics provide a summary of the data derived from your investigation. However, because they do not show the whole picture, some information might be lost. The original data may be distorted or important detail may be lost. For example, consider a cricket match. You want to know which team is likely to win. If you tuned into the commentary partway through an innings and heard the score, you would not know whether the team batting is on a winning streak or in a slump. You would not know how they had performed in other innings. If you also heard the batting average, you still would not know whether the team had batted well at the start but were batting poorly now, or vice versa. For these reasons it is important to carefully examine your data as a whole, taking into account the limitations of the experimental design. Have you just got a snapshot of a phenomenon that does not tell you enough about how things change over time?

Communicating your results

If research is not reported on, then no one else can learn from it. An investigation is not complete until the results have been communicated. A report is usually written, and the results may also be presented in an oral format or a poster. If you are presenting your results in a visual format such as a slide show, follow the tips below, but break the sections up on different slides and remember that less is usually more on slides – reduce the text that you have on your slides and use the images and text on the slides to complement the words you will say with each slide in a way that does not distract your audience. If you are presenting your results as a report, you can include more text and

▼ **Figure 12.22**
Typical format of a scientific report



more detail, but most of the tips below will also be relevant for writing scientific reports. A concise, objective writing style using scientific terminology is important for conveying your key findings without losing the attention of the reader.

Presenting your work as a scientific poster

Poster sessions are common at scientific conferences and provide an important networking opportunity for researchers. Sharing your work and receiving feedback is a central part of being a scientist, and having the chance to see what other people are studying may help to guide your own research. At your poster session, you will usually stand by your poster and be ready to give an overview of your work to others who are interested. It is essential when creating a poster to consider your audience. Sometimes these might be judges for a poster prize, or eminent scientists in the field, and it is important to learn what makes a good poster early in your scientific career.

Table 12.6 Features of an effective scientific poster

An effective poster is...	An effective poster will...
Focused on a clear and concise message	Help you *communicate* your main points clearly
Graphic with figures telling most of the story and text used sparingly	*Engage* readers and advertise your results
Ordered so that readers are easily orientated and can follow the 'story' of the poster	*Encourage* readers to discuss the study with you

Scientific posters

Like scientific articles, posters usually have common components: a title, a list of contributing authors, an introduction, hypothesis and aims, methods used in the study, a results section with figures and legends, and a discussion and/or conclusions section. However, posters have much less text than an article or report, and the main points must be clearly and concisely made. It is important to keep these sections in this order because it orientates the reader and makes it easier for them to follow the 'story' of the poster.

Formatting

Formatting for a poster requires consideration and you should make sure you keep readability and accessibility in mind. You should have colour and font *themes* – that is, a restricted number of complementary colours and fonts used for background, section boxes (if using), section headings and so on.

All text, including figure labels (titles, legends, axes and other labels) should be large enough to be read easily from a distance of at least one metre, and large blocks of text should be avoided. Be consistent with font sizes in your body text and section titles, and always left-align text. Font size for body text may need to be at least 20 pt to be able to be read clearly, with section headings larger and highlighted somehow (e.g. using a different, easily readable colour). Too many different colours or fonts may distract the reader's attention and detract from the ability of the poster to clearly convey the scientific information it has been designed to present.

Keep sections in the order presented in this book and think of a poster as having two or three columns: readers' eyes will move down columns from top to bottom, then from left to right.

You may find it difficult to fit everything you want to include on your poster, but it is much easier to begin with more text (that you later cut down) than to have too little information and forget to include the key points. However, *text in posters should be used sparingly.* As a guide, aim for no more than 1000 words in total on your poster. Let the figures tell the story – include enough text to help the reader understand the figures and the parts of the story that the figures cannot tell.

Software used to create posters can vary. You can use any program that can create a document of the appropriate size and orientation (usually A0 in portrait orientation), which allows you to insert images and text boxes and move these around with fine enough accuracy. A good starting point is Microsoft PowerPoint but Adobe Illustrator or InDesign will give you much more flexibility if you have access to these programs.

Title

The title of the poster is the first thing that will attract the reader's attention and is a way of immediately conveying the key question and findings of your study. It should be informative, concise and clear and, for a poster, in large font at the top of your poster. Immediately below the title and in smaller font, you should list any contributing authors (in this sense, meaning people who contributed to the study) in the order of their contribution from most to least, and underline the name of the presenting author (this is probably you). Another approach, particularly when it is difficult to rank the contributions, is to list the lead author first and put the rest in alphabetical order. It is conventional to have the senior author, the person who oversees, advises upon and provides the equipment or infrastructure for the study, listed at the end. If you are preparing a report, the title may be on the first page together with the list of contributing authors or at the top of the report.

Introduction

Also called the *background* section, the introduction outlines the existing knowledge surrounding the research topic, in essence summarising the *state of the field* and providing any background information needed to understand the rest of the report. This is the place to summarise any existing theories, models, concepts and similar studies, all of which should be correctly referenced, as described in the section on referencing below. On a poster this section is not usually more than 200 words, depending on the space available.

The introduction is important for conveying the reason for your study and for justifying your hypothesis. The best introductions progress so that, by the end, the reader has a clear understanding of what the most important 'burning question' is (your research question, of course!), and what to expect from the rest of the poster. To make this even more clear for the reader, the introduction usually contains a stated *hypothesis* followed by the *aims* of the study. On a poster, and sometimes in a report, the aim or aims of your study are sometimes written as a single point or numbered points immediately below your hypothesis. The aims state clearly what you set out to test and will usually have already been concisely written when you were designing the study.

Scientific writing: choosing your words

However you communicate your work, make sure you know what the message is and who the audience is.

Language used in posters and in scientific articles is clear, accurate and technical, and not colloquial or conversation-style. Be careful to use correct units and symbols where appropriate and to use the correct scientific terminology for your topic. You should get ideas for this from your background reading. Past tense is used to describe your own findings and the findings of others, unless those findings have been repeated enough times to make the phenomenon a *scientific consensus*, in which case the observation is stated in present tense. When stating the findings of others, or a theory or hypothesis already proposed by another person or group, it is always necessary to reference the peer-reviewed report or article in which that finding or hypothesis is published. It is normal to have a short reference section at the bottom of the poster. This can be in a smaller font size – if the reader is interested in the detail, they can come close for this one!

Methodology

The main methods used in your study should be briefly but clearly described in sentences. It is not a recipe for someone else to follow. Write your method using sentences, not dot points. Remember that these need to be written in past tense. You are not commanding anyone to do anything. You are telling people what you did. For example, you would write 'root length was measured' not 'measure the root length'.

Sometimes it is helpful to include diagrams, flow charts or photographs that illustrate your methodology, particularly when it is complicated for the reader to understand, although this depends on the space you have available. Do not waste precious space on illustrations in this section if they do not provide helpful information. The diagrams in your logbook will usually be rough sketches, but the diagrams in your poster or report should be very neat and carefully labelled. Flow charts can be useful to describe any procedures in which a series of steps was followed.

If your study contains potential safety issues or ethical considerations, these should be identified in this section and the ways in which these issues were handled should be described.

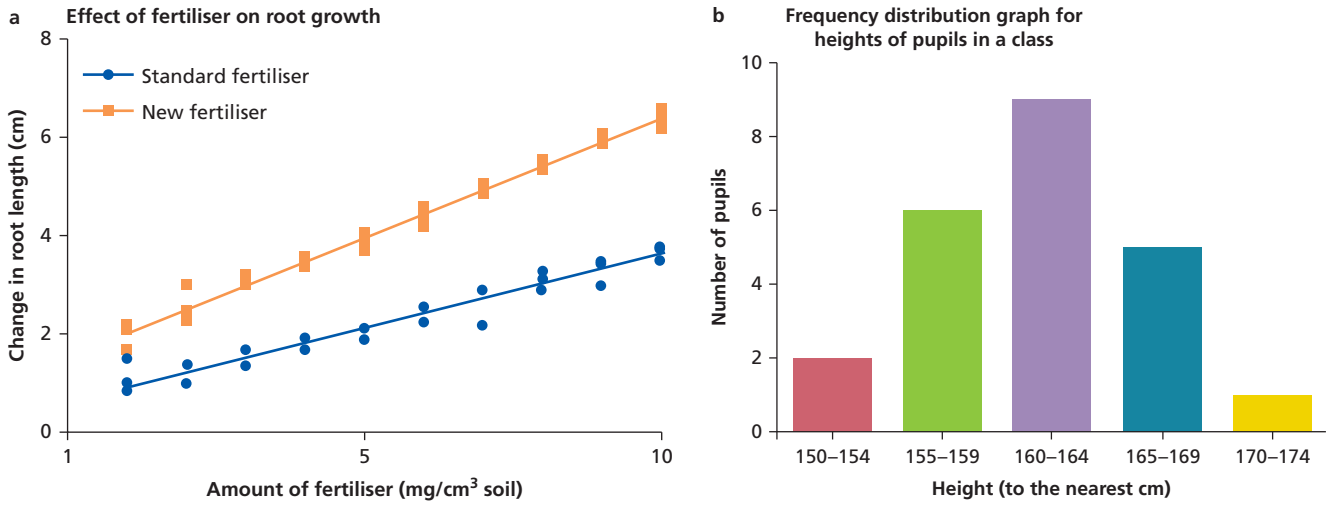
Results

The results section is a summary of your results together with graphs, tables or other ways of showing the data. Do not interpret your results in this section, unless it is a combined results and discussion section.

Avoid including tables of raw data in your report unless they compare the results of different experiments. Wherever possible use a graph instead of a table.

If a table has more than a few rows of data, it is better to represent that data in some other way. Usually this will be a graph.

Think about what sort of graph is appropriate. The type of graph you use should be the one that most clearly shows any trends or patterns, and the range of your axes is particularly important for this. If you want to show a relationship between two variables then use a scatter plot. Display your data as points with error bars and clearly label any lines you have fitted to the data. Always make sure you label your axes, including units. Choose an appropriate scale so that the data takes up most of the plot area.



▲ **Figure 12.23** (**a**) Scatter plot with linear regression demonstrating a mathematical relationship (**b**) Column graph comparing numbers between different groups

Column and bar charts are useful for comparing two data sets, such as average root length with different types of fertiliser. *Do not* use a column or bar chart to try to show a mathematical relationship between variables. Figure 12.23 gives examples of two types of graphs.

Any data and derived results should be given in appropriate units with standard errors of the mean or standard deviation, as appropriate. If you performed calculations or statistical analyses of the data, show the equations or describe the statistics you used. You might want to show one example calculation.

You may find it beneficial to have several results subsections, each one with a heading, a figure and some brief text clearly stating the result that addresses one of your aims. Most readers will focus mainly on the subsection headings and the figures under each of those headings.

When stating a finding of your study in the main text of the results section, refer immediately afterwards to the figure in which the finding is shown, for example 'The vertical growth of *Arabidopsis* seedlings was significantly greater following two weeks of new fertiliser treatment than with the standard fertiliser or water alone (Figure 1).'

Figures

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

Each diagram should have a figure number, and you should refer to it in the text of your report. Position the diagram close to where it is referred to in the text. You should take the time to learn how to position figures neatly using your chosen software. When including images taken on a microscope, a scale bar and magnification must always be noted.

Figure legends are essential. These are usually below the figure, and begin with the figure number followed by a title, then, if required, a brief description of the methods used to obtain the results. The figure legend does not contain any interpretation of the data; this is reserved for the discussion section. Important statistical information can be included in the figure legend, including the sample size (*n*), any statistical results presented, and the number of times the experiment was repeated (Figure 12.24). The figure legend uses up space and text, and you may be able to present the information by annotating the figure rather than writing out a figure legend (Figure 12.25).

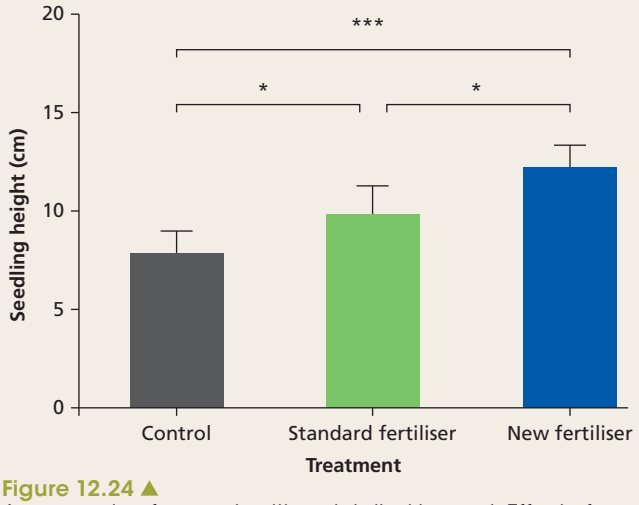


Figure 12.24 ▲
An example of a graph with a detailed legend: Effect of new fertiliser on seedling height. Seedlings were grown in standard conditions to a height of 2 cm, then separated and treated daily for two weeks with 50 mL 2% new fertiliser or 2% standard fertiliser in tap water. The control group was given water alone. Bars show mean ± standard deviation; $n = 6$ per group. $*P < 0.05$, $***P < 0.0001$. Results are representative of three independent experiments.

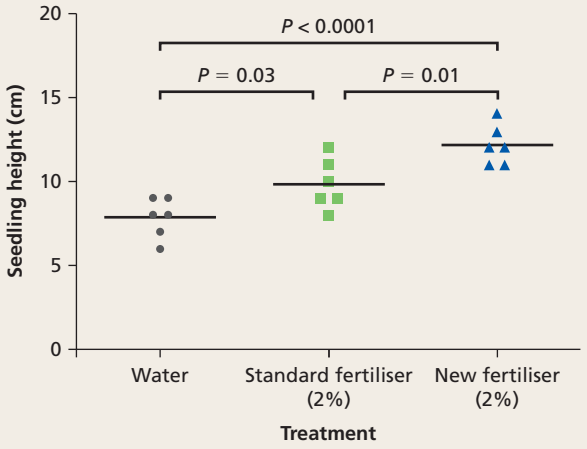


Figure 12.25 ▲
An example of a graph containing more detail and a less bulky legend: Effect of daily treatment with new fertiliser on *Arabidopsis* seedlings over two weeks

Discussion

The discussion section is the place for analysis and evaluation of your data. The discussion should explain *what your results mean*. Here, you can interpret your data and discuss its novelty (what is new about it) and significance. Identify the key findings of your investigation and outline how they fit with existing concepts. If you began with a research question, give the answer to the question here. If you began with a hypothesis, state whether or not your results support your hypothesis. If not, explain why. (You might only be able to say that the model was not suitable for the situation being investigated.)

This section also gives you the opportunity to *discuss and connect ideas* you have formed from your own study and the background research you conducted as part of the study. If there are any implications of your work, such as implications for better agricultural processes or the design of better medicines, discuss them here.

The discussion is also the place to briefly describe any difficulties that you had and make suggestions for improving the process. Remember that you should never say 'the experiment did not work' if you did not get the results you expected. You might choose to make some comments on possible further work that could be done.

Some questions you should consider when writing your discussion section are:

- Did your results support or refute your own hypothesis?
- Did you have any outliers in your data and, if so, how did you treat them?
- What were the limitations of your study, and how could you address these in (hypothetical or real) future studies?
- What do your results add to the current scientific knowledge of biological concepts?
- Do they agree with or contradict models or classification keys based on other published findings?
- How might your findings impact the scientific community, industry, medical practice or the community at large?

It will be difficult to address all of these questions in the limited space you have available. Start by writing all of the key points down and then read through several times, cutting

down unnecessary words each time. Do not remove your own connections and ideas – this type of *critical thinking* is often a significant part of what is assessed in scientific writing. Remember that concise, coherent writing is an important scientific skill to practise.

Conclusion

This short section allows you to draw conclusions *based on the evidence you have gathered* during your study. It is a *very* brief summary of the results and their implications. It should provide a response to your research question and directly address the hypothesis you proposed in your introduction. Say what you found out and what it means. A conclusion should only be a few sentences long.

If you are making a poster, some readers in a busy poster session may only look at the hypothesis and conclusion, and so these should make sense together and provide the key information about the study. Sometimes you may have enough space to be able to include a schematic (cartoon) diagram that summarises your results and how they fit into the current state of scientific knowledge.

Acknowledgements

You should thank anyone who helped you in your investigation. This includes people who supplied equipment or funding, as well as people who gave you good ideas or helped with the analysis. In science, as in other aspects of your life, it is polite to say thank you.

References

A reference list details all the sources of information that were actually used to write the text and figures for the poster. Wherever a piece of information or quotation is used in your poster it must be referenced *at that point*. This is typically done either by placing a number in brackets at the point [2], or the author and year of publication (Smith, 2014). The reference list is then provided in a single, complete list at the bottom of the poster. Referencing must be done in a consistent style. Check with your teacher what style is preferred. There are several good online guides to referencing.

A reference list is *not* the same as a bibliography. A bibliography is a list of sources that are useful to understanding the research. They may or may not have actually been used by the report authors. You should have a bibliography in your logbook from the planning stage of your investigation. The references will be a subset of these sources.

Poster sessions

Having completed your poster, you will want it to be seen. Poster sessions are included as part of most conferences and can be large affairs (Figure 12.26) or smaller, more intimate sessions, depending on the setting (Figure 12.27). It is customary to stand by your poster and to be ready to answer specific questions about your work, to explain and discuss the findings presented in your poster, or often to quickly talk through the poster from start to finish (3–5 min), pointing to sections or figures as you go. Make sure you use engaging conversation and do not overwhelm your audience with details.

If you practise this with your friends or colleagues, you will quickly realise whether you have included relevant figures that are easily interpreted by the reader and which you can refer to as you discuss your study. In fact, you may wish to ask your classmates to read your poster, provide feedback and summarise the main points of the poster. This could tell you whether you have included enough information, whether the poster is focused and clear, and whether your figures and text convey all of the information you are trying to present. If your reader cannot remember the main points of your study after reading it, it is time to go back and look at how you can improve the poster. If they can summarise the aims, findings and relevance of your study, and even ask you questions about your study, then your poster has done its job.

Dominique Naegele-Clifford

Figure 12.26 ▲
A poster session at a large international conference

Lascap

Figure 12.27 ▲
A poster session is a common way to present scientific findings at a conference.

CHAPTER GLOSSARY

aseptic technique the technique of working under sterile conditions to prevent contamination of samples

assay an experimental technique or procedure used to test a specific biological process or effect

autoclave a device used to sterilise equipment, reagents or contaminated waste; autoclaves work by subjecting contents to pressurised steam at 121°C for a set time

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

classification key a key for the identification of organisms based on a series of choices between alternative characters

continuous variable a variable that is able to take any value within a range; length, time and temperature are examples of continuous variables

controlled variable a variable that is held constant throughout the investigation so that the relationship between the dependent and independent variables can be observed

dependent variable the variable that changes as a result of changes to the independent or controlled variable

discrete variable a variable that may take only certain values; for example number of individuals, or number of legs on an animal

ELISA enzyme-linked immunosorbent assay to measure the concentration of a molecule or antibodies against a molecule

error bars bars drawn above and below and/or to left and right of a data point on a graph to indicate the size of the uncertainty in that point

extrapolation extension beyond the measured range of data to read or construct new data that has not been measured

falsifiable able to be disproved

feasibility capability of being done or achieved

geomorphology the study of landscapes, how they form and change in response to movement of rock, sediment (soil) and water, on different scales of time and space

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

immunohistochemistry a technique that can be used to detect the presence of molecules on cells or tissues on a microscope slide

independent variable a variable that is controlled by the experimenter, upon which another variable depends

interpolation reading or construction of a new data point that has not been measured but is within the range of measured data

linear regression a statistical tool used to model the dependence of one variable on another

line of best fit the line that most accurately fits the data, usually calculated using linear regression

logbook the record of an experiment or investigation kept by the scientist performing the experiment; it is a legal record of the experiments and their results

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

negative control a condition in which no substantial change is expected, providing a baseline to increase confidence that no unidentified independent variables are acting in the experiment

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

plagiarism presenting someone else's work, including their words or ideas, as your own

positive control a condition in which an effect is expected, giving confidence that the experimental set-up can yield the expected results

primary data data that you have measured or collected yourself

qualitative measurement measurement with descriptive or non-numerical results

quantitative measurement measurement with numerical values

reference the source of a specific piece of information or quotation

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

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

reproducible giving the same result, within uncertainty limits, when repeated measurements are made

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

scatter graph a graph or plot showing data points, without a line joining the points, and used to demonstrate or determine a mathematical relationship between variables; the axes are defined by the variables

secondary data data or information that has been collected by someone else

sensitivity limit marks the portion of a curve that is non-linear; data that falls into these non-linear regions cannot be extrapolated

significant difference a difference between data values that is statistically significant; that is, the probability (*p*) of the difference being due to chance is so small (usually less than 5%) that the result is considered true

standard deviation a measure of the dispersion of a set of data from its mean; expresses the variability of a population or set of data

t-test a statistical test commonly used to analyse differences between two sets of data

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

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

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

GLOSSARY

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

abscisic acid a plant hormone that is involved in many plant developmental processes including seed and bud dormancy

absolute dating the process of determining the age in years of rocks and their contained fossils on the basis of the physical or chemical properties of materials in the rock

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

acetylcholine a neurotransmitter in the human nervous system

Acheulean a culture defined by stone tools from 1.5 million to 150 000 years ago and associated with *Homo erectus*

action potential a brief change in the electrical potential on the surface of a nerve or muscle cell in response to stimulation, which results in the transmission of an electrical impulse

activation energy the energy required to initiate a reaction

activator a regulatory protein that binds to an enzyme or DNA, causing a change of conformation so that enzymes become active, or activating gene expression

active immunity the immunity formed by stimulation of the immune system with an antigen and the generation of effector and memory cells; it is contrasted with passive immunity

active site the place on the surface of an enzyme molecule where substrate molecules attach

active transport the process whereby cells actively transport substances across a membrane against a concentration gradient (from a low concentration to higher concentration of the substance); consumes energy

adaptation a developed characteristic that enhances an organism's survival in its natural environment

adaptive evolution changes in population of organisms that make that population better adapted to its environment over time

adaptive immune response an immune response that is directed against a specific antigen and retains memory of that antigen, responding with a secondary response on subsequent exposure to the same antigen

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

adenosine diphosphate (ADP) a low-energy compound composed of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound composed of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

adhesion proteins proteins on the surface of cells that are involved in binding with other cells or to an extracellular matrix in a process called cell adhesion

adjuvant a substance added to a vaccine along with an antigen that contains pathogen-associated molecular patterns to improve the immune response to that antigen

aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell

agarose gel a gel matrix used for electrophoresis

agglutination when antigens or pathogens become stuck together because of antibody binding

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

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

allergen an antigen that is normally innocuous but in some circumstances causes allergy

allergy an immune response characterised by IgE production

allopatric speciation speciation that occurs as a result of physical or geographic isolation

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

amine hormone a hormone derived from amino acids; examples include epinephrine, dopamine and thyroxine

amino acid a nitrogen-containing compound that is the building block of proteins

amino acid sequence see **polypeptide sequence**

anabolic reaction a reaction that builds up complex molecules from more simple ones

anaerobic without oxygen

analogous structures features of organisms that have the same function but not the same structure

anaphylactic shock a severe form of allergic reaction that causes widespread swelling, including of the face and neck, which can make breathing difficult

aneuploidy describes a genome that varies from the conventional by the loss or addition of one or just a few chromosomes

annealing in PCR, a process of joining separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

anti-apoptotic describes a gene or protein acting to prevent apoptosis and allow cell survival

antibiotic a naturally produced or synthetic compound that is toxic to bacteria

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow antibodies

antibody a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called immunoglobulin

anticodon the three nucleotides in tRNA that bind to mRNA following base-pairing rules

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antigen presenting cell (APC) a cell that displays peptides derived from processed antigens on MHC class II molecules for presentation to T_H cells; includes B cells, macrophages and dendritic cells

antiparallel parallel but orientated in opposite directions

antiseptic a substance that kills or inhibits the growth of micro-organisms on external surfaces of living things

apical dominance a growth pattern in which the central stem of the plant grows more strongly than (is dominant over) the side stems

apical meristem the tip of the shoot of a plant

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes, including caspases

aquaporin a type of membrane protein that facilitates the transport of water

arboreal related to, or living in, trees

artificial selection the breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

aseptic technique the technique of working under sterile conditions to prevent contamination of samples

assay an experimental technique or procedure used to test a specific biological process or effect

ATP synthase an enzyme that provides energy for the cell through synthesis of ATP

ATP-powered pump a type of membrane protein that uses the energy from ATP to move molecules across the membrane against a concentration gradient

australopithecine a member of a group of small, bipedal apes that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

auto-antigen (auto means 'self') a normal body component that activates an immune response in autoimmune disease

autoclave a device used to sterilise equipment, reagents or contaminated waste; autoclaves work by subjecting contents to pressurised steam at 121°C for a set time

autocrine a type of signalling in which the signalling molecule binds to receptors on the cell type that produced it and affects the function of that cell type

autoimmune disease when the body's own immune system attacks a normal body component

autotroph an organism capable of making its own food from inorganic substances using light (through photosynthesis) or chemical energy (through chemosynthesis); green plants, algae and certain bacteria

auxins plant hormones that have three main effects: apical dominance, root growth and cell elongation

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

B cell receptor (BCR) a surface-bound antibody that serves as a receptor so that B cells are able to detect antigens

B lymphocyte/cell a class of lymphocytes; once activated, they are characterised by the production of antibodies

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

bacteriophage a virus that infects bacteria

base pair a pair of complementary nitrogen bases linked by hydrogen bonding

basophil a circulating leukocyte that secretes histamines

beneficial mutation a mutation that increases an organism's chances of survival and reproduction

binary fission an asexual mode of reproduction in which a unicellular organism grows and then divides into two cells, forming two separate organisms

binding site a region on a protein, DNA or RNA molecule to which other specific molecules and ions bind through chemical interactions

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to form a final product

biogeography the study of the distribution of living things over a geographical area through geological time

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

biological functionality the function of a protein

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

biotechnology the use of living organisms and biological systems and processes for human benefit

bipedalism a type of locomotion in which an organism walks on two hind limbs

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population and its genetic diversity

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

brow ridges the bony ridges located above the eye sockets

bulk transport the transport of large quantities of materials into or out of the cytoplasm all at the one time

C_3 plant a plant that carries out a type of photosynthesis in which a three-carbon compound, phosphoglycerate, is the first stable product of carbon fixation; this occurs during the first step of the Calvin-Benson cycle. Most plants are C_3 plants

C_4 plants plants such as tropical grasses in which a four-carbon compound, malate, is the first stable product of carbon fixation; carbon dioxide is later drawn out of the malate and into the Calvin-Benson cycle rather than directly from air

Calvin-Benson cycle a biochemical pathway in which sugar molecules are produced from carbon dioxide

CAM plant (crassulacean acid metabolism) a plant that uses an enzyme to fix carbon dioxide at night, generating the four-carbon compound malate; this is broken down during the day to release carbon dioxide, which then enters the Calvin-Benson cycle

cancer a disease that arises when the signals that control apoptosis and cell division are disrupted, so cells survive and divide uncontrollably

carbon fixation the use of atmospheric carbon dioxide and its conversion into carbohydrates; this process occurs in the stroma of chloroplasts in eukaryotic cells

carrier protein a protein within a membrane that assists other molecules to cross the membrane in facilitated and active transport

carrying angle the angle at which the femur is tilted in towards the knee

caspases enzymes (proteases) that cleave, or cut, intracellular proteins and DNA in the process of apoptosis

catabolic reaction a reaction, such as cellular respiration, that involves the breakdown of complex molecules to simpler products

catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change

cell differentiation the process by which a less specialised cell develops or matures to have more distinct characteristics and functions

cell signalling a complex system of signal transduction pathways that governs basic cellular processes and coordinates cell actions

cellular metabolism the sum of metabolic reactions in a cell

cellular process any process that is carried out at the cellular level but is not necessarily restricted to a single cell; for example, cell communication occurs among more than one cell but occurs at the cellular level

cerebral cortex the outermost layer of the brain

channel protein a protein that forms a channel within a membrane to allow the passage of substances across the membrane

charged tRNA tRNA when linked to its appropriate amino acid

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) a chemokine

chemotroph an organism that synthesises its own food from inorganic substances using chemicals as the primary energy source

chitin a fibrous substance containing polysaccharides that forms the tough outer shell of insects and fungi

chlorophyll the green pigment found in chloroplasts; it is able to absorb light energy, making it available for photosynthesis

chloroplast a membrane-bound organelle containing the green pigment chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates

cholesterol a type of lipid found in animal cell membranes that stabilises membrane fluidity

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

cilia slender hair-like structures projecting from the cell surface that beat against fluid outside the cell

citric acid cycle see **Krebs cycle**

clade a branch of a cladogram that comprises a common ancestor and all of its descendants

cladogram a rooted phylogenetic tree that depicts a hypothesis about evolution

classification key a key for the identification of organisms based on a series of choices between alternative characters

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

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

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

coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction

cofactor an ion that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions, and apply new knowledge and skills

companion plant a plant that is grown together with another plant because one species improves the growth of the other

comparative dating the process of determining the age of rocks and their contained fossils relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

comparative genomics a field of biology concerned with comparing DNA sequences, gene arrangements and chromosome structure between different species

competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T and C pairs with G

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

conformation the proper or functional shape of a protein

conjugation the union between two bacterial cells enabling the direct transfer of genetic material from one to the other

conserved refers to amino acids of polypeptide sequences or nucleotides of DNA sequences that remain unchanged during the course of evolution

contact-dependent describes signalling that requires cells to be in direct contact

contact inhibition the cessation of cell growth and division upon contact with other cells

continental drift the relative movement of Earth's continental landmasses, which appear to drift or 'float' over Earth's mantle

continuous variable a variable that is able to take any value within a range; length, time and temperature are examples of continuous variables

controlled variable a variable that is held constant throughout the investigation so that the relationship between the dependent and independent variables can be observed

convergent evolution a process whereby unrelated organisms evolve similar adaptations in response to their environments

convolutions the folds on the surface of the brain

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

cristae the folding of the inner membrane into the matrix of a mitochondrion, thus increasing the total surface area of the inner membrane

cultural evolution the way beliefs, social practices, skills, and technology change over time

cyclic AMP (cAMP) cyclic adenosine monophosphate; a nucleotide derivative that can act as a second messenger in signal transduction pathways

cytochrome a membrane-bound protein that carries out electron transport; cytochromes are located in the mitochondrial inner membrane and in chloroplasts

cytokines signalling molecules that coordinate inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

cytokinin a plant hormone that promote cell division, chloroplast formation and growth of lateral shoots and leaves

cytoskeleton a network of filaments within a eukaryotic cell that provides structural support, anchorage, shape, motility and the capacity to move and arrange organelles within the cell

cytosol the fluid part of the cytoplasm surrounding the organelles

cytotoxic T (T_C or killer T) lymphocyte/cell a class of lymphocytes that destroys virally infected or cancerous cells by secreting proteins that cause apoptosis

damage- or danger-associated molecular pattern (DAMP) a body (or plant) component that is released during tissue damage, such as internal cellular components that stimulate innate immune responses

death receptors cell surface receptors that transmit apoptotic signals

defensin a small antimicrobial peptide secreted by virtually all plants and animals

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

degranulation a cellular process where the granules of the neutrophils, mast cells, basophils or eosinophils are emptied into the extracellular surroundings

deleterious mutation a mutation that decreases an organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

demyelination destruction or removal of the myelin sheath that surrounds the axons of neurons

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

dendritic cells antigen-presenting cells that phagocytose and present antigens to cells of the adaptive immune system

Denisovan a distinct, but undescribed, ancient hominin known only from bone fragments found in Denisova Cave in Siberia

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

dependent variable the variable that changes as a result of changes to the independent or controlled variable

descent with modification Darwin's terminology indicating that life today has descended and evolved from common ancestors that were generally different from their modern descendants

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

differentially permeable describes a membrane that allows some substances but not others to pass across it

diffusion the passive movement of molecules from a high to a low concentration of that substance

diploid (2*n*) describes a cell or organism that has a genome comprising two copies of each chromosome, represented by 2*n*

directional selection a form of selection that selects against one of two extremes and leads to a change in a trait over time

discrete variable a variable that may take only certain values; for example number of individuals, or number of legs on an animal

disinfectant a substance that destroys micro-organisms and their spores but is too strong to be used directly on skin

disruptive selection a form of selection that operates in favour of extremes and against intermediate forms

distance method a strategy for inferring evolutionary relationships based on patterns of evolutionary distance between pairs of species

disulfide bridge a strong bond formed between two sulfur atoms within a protein

divergent evolution evolution in which a single ancestral species diverges, or splits, to eventually become two or more descendant species

DNA barcode a nucleotide sequence from a representative gene that uniquely identifies a particular species, subspecies or variety of organism

DNA–DNA hybridisation a technique used to determine how similar the DNA of two species is

DNA fingerprinting also called DNA profiling; comparison of individuals or groups based on patterns of non-coding, repeating base sequences in the genome

DNA ligase an enzyme used to catalyse the formation of a phosphodiester bond between two pieces of DNA

DNA polymerase an enzyme capable of making exact copies of fragments of DNA

DNA sequence the order of the four possible nucleotides in a segment of DNA

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

double-strand break a mutation involving breaks in the sugar–phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

down-regulate decrease the quantity of a cellular component in response to an external variable

effector a muscle, gland, organ or protein that acts in response to a stimulus to bring about a particular outcome

electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the phosphorylation of ADP to yield ATP

ELISA enzyme-linked immunosorbent assay to measure the concentration of a molecule or antibodies against a molecule

endergonic reaction a chemical reaction requiring energy

endocrine the system comprising a collection of glands that secrete chemical signals directly into the bloodstream

endocytosis the movement of solids or liquids into a cell from the environment via vesicle formation

endoplasmic reticulum (ER) an organelle in eukaryotic cells consisting of an interconnecting system of thin membrane sheets

endospore a tough, dormant structure for asexual reproduction formed inside some bacterial cells; it is resistant to extreme temperatures, chemicals and drying out

endosymbiosis a mutually beneficial relationship between two single-celled organisms in which one of them lives inside the other

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

eon a division of geological time that can be divided into periods, epochs and ages

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular pathogens

epidemic an outbreak of infectious disease that spreads suddenly and rapidly through the population

epitope a small part of a larger molecule that binds to a receptor site such as B cell receptors and T cell receptors

epoch a division of geological time that is shorter than a period and is marked by one or more significant events

era a division of geological time comprising periods and epochs

error bars bars drawn above and below and/or to left and right of a data point on a graph to indicate the size of the uncertainty in that point

ethidium bromide a chemical that binds to DNA and fluoresces when exposed to ultraviolet light; used to locate DNA in an agarose gel following electrophoresis

ethylene a plant hormone involved in the ripening of fruit

evolution the process of gradual change in the characteristics of a population of organisms that results in new species

evolutionary developmental biology the study of the evolution of species and their developmental processes by comparing the embryonic development of different organisms

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

exergonic reaction a reaction that releases energy

exocytosis the movement of solids or liquids from a cell to the environment via vesicle fusion with the plasma membrane

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

extracellular occurring outside a cell or cells

extracellular fluid all fluid outside the cells in multicellular organisms

extracellular receptor a receptor embedded in the plasma membrane that binds hydrophilic ligands

extrapolation extension beyond the measured range of data to read or construct new data that has not been measured

extrinsic pathway (of apoptosis) apoptosis activated by binding of a ligand to a death receptor on the target cell

facilitated diffusion a form of diffusion that requires a substance to be attached to a specific carrier molecule to move across a membrane

falsifiable able to be disproved

feasibility capability of being done or achieved

feedback inhibition the cellular control mechanism in which an enzyme that catalyses the production of a particular product is inhibited by the product, therefore balancing supply and demand of a product for a cell

fever increased body temperature

fitness the capacity of an individual to survive and produce viable offspring

flagellum a helical filament that rotates to give bacteria locomotion

fluid mosaic model a model that explains the structure and function of the plasma membrane

foramen magnum the hole in the base of the skull through which the spinal cord passes

fossil the preserved remains or traces of an organism

founder effect a type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

frameshift mutation a mutation that dislocates the translational reading frame

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

gel electrophoresis a technique that separates DNA fragments according to their size and charge

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

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

gene duplication generating an extra copy of a gene within a genome as a result of mutational duplication of a chromosomal segment or because of polyploidy

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

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

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

gene sequence the order of the four possible nucleotides in a gene

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

genetic drift a change in the gene pool of a population as a result of chance; usually occurs in small populations

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organisms (GMOs) organisms whose genomes have been genetically engineered

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

genotype a specific combination of alleles for a particular gene locus belonging to an individual

geomorphology the study of landscapes, how they form and change in response to movement of rock, sediment (soil) and water, on different scales of time and space

germ-line the cell line in eukaryotic organisms from which the gametes are derived

gibberellin a plant hormone that promotes vertical growth of the plant

glycogen an energy-storage polysaccharide in animals that is composed of glucose

glycolysis an energy-yielding process occurring in the cell cytosol in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

glycoprotein a protein with an attached carbohydrate group

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

gracile of slender build

gradualism a theoretical model of the pace of evolution occurring as a steady, slow divergence of lineages at an even speed, irrespective of gaps in the fossil record

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

granulocyte a leukocyte containing intracellular granules

hallux the big toe, or innermost toe of the foot

hammerstone a hard stone used to chip off stone flakes to shape a tool stone

haplogroup a group of people that share the same genetic mutations and are descendants of a common ancestor through either the maternal or paternal line of inheritance

haploid (*n*) describes a cell or organism that has a genome that contains one copy of each chromosome, represented by *n*

helper T (T_H) lymphocyte/cell a class of lymphocytes that aids T_C cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling

herd immunity the concept that unvaccinated people are protected from diseases if almost everyone else (around 95% of people) is vaccinated

heterotroph an organism that cannot synthesise its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

histone a protein that binds and packages DNA in eukaryotic chromosomes

hominin a term for a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a term for a member of the superfamily Hominoidea; an ape, or tail-less primate

homologous in reference to genes or polypeptides, having sequences that are similar and indicate a shared evolutionary ancestry

homologous chromosomes a pair of chromosomes that have the same size, shape and genes at the same locations

homologous structures features of organisms that have the same general structure but different functions

homologues in reference to genes, genes that have similar nucleotide sequences; usually refers to genes from different species but may also refer to genes from the same species

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

hormone a chemical messenger secreted directly into the bloodstream, other body fluids, or into adjacent tissues, where they move to their target cells

host the organism in which a parasite lives

humoral immune response an immune response mediated by antibodies

hybrid offspring of parents from two different species; some hybrids are also fertile and can produce further offspring

hybridise form double strands with nucleic acids derived from different sources

hybridoma a hybrid cell created by fusing a B cell clone with cells from a plasma cell tumour; produces monoclonal antibodies and divides repeatedly

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

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

hydrophilic hormone a hormone that is water soluble and binds to extracellular receptors to initiate a response in that cell; for example, peptide and some amine hormones

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

hydrophobic hormone a hormone that is water insoluble and binds to intracellular receptors; for example, steroid and thyroid hormones

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

immune having resistance to infection by a specific pathogen

immune system a complex network of cells, tissues and organs in the body that detects differences between self molecules and foreign organisms, and mounts an immune response that results in formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

immunisation the introduction of a vaccine to generate antibodies and memory lymphocytes that respond rapidly on encounter with the pathogen

immunodeficiency a state in which the immune system does not function properly, leaving a person susceptible to infections a healthy immune system could normally fight off

immunoglobulin (Ig) see **antibody**

immunohistochemistry a technique that can be used to detect the presence of molecules on cells or tissues on a microscope slide

immunosuppression reduction of the activation or efficacy of the immune system, for example by drugs that prevent transplant rejection or treat autoimmune diseases

immutable unchanging; the idea (now considered incorrect) that species did not change over time

independent variable a variable that is controlled by the experimenter, upon which another variable depends

induced-fit model a model to explain that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

inducer a signalling molecule that switches on expression of a gene

inflammation an innate response to infection or damage that causes swelling, pain, heat and redness

infraorder a taxonomic rank immediately above superfamily

inheritable capable of being passed on to the next generation

inhibitor a substance that slows down or prevents a particular chemical reaction; by binding to proteins, inhibitors change the protein conformation so it no longer performs its job

innate immune response a response to a pathogen that is not specific and does not generate antibodies or memory lymphocytes

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

insular dwarfism the trend for large mainland animals that colonise islands to evolve into smaller forms

intercellular occurring between cells

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

interleukin a subset of cytokines that assist with coordination of cells involved in immune responses

interpolation reading or construction of a new data point that has not been measured but is within the range of measured data

interstitial fluid a fluid that lies in the spaces between cells; also known as tissue fluid

intracellular occurring within a cell or cells

intrinsic pathway (of apoptosis) apoptosis activated by growth factor withdrawal or damage to DNA or organelles

intron a segment of DNA or RNA that does not code for a polypeptide and interrupts the sequence of a gene

ion atom or molecule with an overall positive or negative charge

ion channel a protein or protein complex that spans the plasma membrane, forming a channel to facilitate the movement of ions across the membrane

ion gradient the concentration gradient of ions across a membrane; also referred to as an electrochemical potential

isoenzymes enzymes that carry out the same biological reaction but are the products of different genes at different loci in the genome

isolating mechanism a mechanism that prevents organisms from mating or producing viable offspring

isotope atoms of an element that have the same number of protons but different numbers of neutrons and therefore different relative atomic masses

isotype a subtype of immunoglobulin; each isotype (IgG, IgM, IgA, IgE and IgD) performs a different function

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl coA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

lactic acid a product of anaerobic cellular respiration in animals

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

leukocyte the general term for white blood cell

ligand a molecule that binds to a receptor

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll; water molecules split to produce oxygen and hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions carbon dioxide, hydrogen ions and ATP produce carbohydrate

line of best fit the line that most accurately fits the data, usually calculated using linear regression

lineage in evolution, the line of descendant species that evolve from an ancestral species

linear regression a statistical tool used to model the dependence of one variable on another

lipopolysaccharide a complex bacterially derived molecule containing lipid and polysaccharide components; a PAMP that strongly activates inflammation

loaded describes coenzymes that are attached to the specific group of atoms they transfer

lock-and-key model a model suggesting that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

locus the specific location of a gene on a chromosome

logbook the record of an experiment or investigation kept by the scientist performing the experiment; it is a legal record of the experiments and their results

lymph a colourless fluid that originates from the extracellular (tissue) fluid

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and in removing foreign matter

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes for presentation to lymphocytes and initiation of an adaptive response

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting (verb: to lyse)

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

macroevolution the evolution of new groups of organisms comprising many related species through multiple speciation events; includes adaptive radiations

macrophage a large white blood cell in tissues that phagocytoses pathogens; originate as monocytes in circulation

major histocompatibility complex (MHC) protein markers found on the cell surface that are important in distinguishing self from non-self. There are two classes: MHC class I is found on all cells and MHC class II is found only on antigen presenting cells

MALT mucosal-associated lymphoid tissue; secondary lymphoid tissue in which adaptive immune responses occur

mandible the lower jawbone of the skull

mass extinction extinction of many species over a relatively short (geological) period of time

mast cell located in the tissues; when activated, releases granules containing histamine

master control gene a gene that coordinates expression of many other genes to control body patterning and cell differentiation during embryonic development; also referred to as 'master gene', 'master regulator', 'master switch' or 'toolkit gene'

maternally inherited describes a genotype and phenotype that are transmitted entirely from the female parent to the offspring

melting temperature in DNA–DNA hybridisation, the temperature at which 50% of double-stranded DNA in a sample has separated into single strands

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

metastasis when cancer cells leave the tissue of origin and invade neighbouring tissues or travel to new sites around the body

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

microevolution any change in the gene pool of a single population over a short time

microflora a community of micro-organisms, including fungi and bacteria, that live in or on another living organism

mirror neurons specific neurons that fire when an animal either performs an action or observes another animal performing the same action

missense mutation a gene mutation that results in one amino acid being replaced by another amino acid in the encoded protein

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

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

modern synthesis the theory of evolution incorporating our understanding of how traits are inherited

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

monoclonal antibody antibodies produced by a hybridoma with specificity against a single antigen; their specificity is identical to the antibodies produced by the original cell

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

monoploid (1*n*) describes a cell or organism that has a functional genome consisting of one copy of each chromosome, represented by 1*n*

morphological species concept to define a species using measurable anatomical criteria and characteristics

Mousterian a culture defined by stone tools from 300 000 to 30 000 years ago and associated with *Homo neanderthalensis*

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

multiregional origin a hypothesis that modern humans evolved from more ancient hominins simultaneously on all colonised continents

mutagen an agent capable of inducing mutations

mutation occurs when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

mya millions of years ago, sometimes expressed as millions of years before present (myBP), or simply millions of years (my); for example, a fossil dated as being 5 million years old lived 5 mya

myelin sheath the fatty layer surrounding and insulating the axons of many neurons; increases the speed at which electrical impulses travel along the nerve cell

NAD^+ unloaded form of the coenzyme nicotinamide adenine dinucleotide; has a role in cellular respiration

NADH loaded form of the coenzyme nicotinamide adenine dinucleotide; has a role in cellular respiration

$NADP^+$ unloaded form of the coenzyme nicotinamide adenine dinucleotide phosphate; has a role in photosynthesis

NADPH loaded form of the coenzyme nicotinamide adenine dinucleotide phosphate; has a role in photosynthesis

natural killer (NK) cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

natural selection the process whereby individuals with certain inheritable traits survive and reproduce more successfully than other individuals, leading to evolutionary change in the population

necrosis cell death that results from tissue damage or infection; results in inflammation

negative control a condition in which no substantial change is expected, providing a baseline to increase confidence that no unidentified independent variables are acting in the experiment

neuron nerve cell

neurotransmitter a chemical substance that carries the action potential across a synaptic cleft

neutralisation the process by which antibodies prevent toxins from acting; that is, by binding to them and blocking them from binding to their targets

neutral mutation a mutation that has no effect on an organism's chances of survival and reproduction

neutrophil a phagocytic leukocyte found in the blood and tissues

niche an organism's habitat; or way of life or function of an organism in its environment

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

NOD-like receptor (NLR) a type of pattern recognition receptor (PRR); intracellular sensors of PAMPs and DAMPs

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

non-disjunction the failure of sister chromatids in mitosis or homologues in meiosis to separate and go to opposite poles

non-self a molecule that is not recognised by the immune system as being part of the organism itself

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

non-specific describes a response that is the same regardless of the type of pathogen

nuclear envelope the double membrane that surrounds the nucleus in eukaryotic cells and separates DNA from the cytosol

nuclear pore an opening in the nuclear envelope

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

nucleolus a granular structure within the nucleus where ribosomal RNA is transcribed and ribosome subunits are assembled

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

nucleotide sequence the order of the four possible nucleotides in a segment of RNA or DNA. See also **DNA sequence** and **gene sequence**.

nucleus a membrane-bound compartment in eukaryotic cells that contains the chromosomal DNA

obligate restricted to a particular way of life

Oldowan a culture defined by stone tools from 2.5 to 1.2 mya and associated with australopithecines

oncogenes cancer-promoting genes

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

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

opsonisation a process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

optimum pH the pH at which an enzyme works at its fastest rate

optimum temperature the temperature at which an enzyme works at its fastest rate

orthograde a type of locomotion in which fore and hind limbs move in opposition to one another

orthologues similar genes in different species that evolved by speciation from a common ancestral gene

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

Out-of-Africa hypothesis See **recent single origin**

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

palaeoanthropology the field of study concerned with fossil hominins

pandemic a sudden outbreak of infectious disease that spreads rapidly across many countries

paracrine a type of signalling in which the signalling molecule acts to induce changes in nearby cells of a different type from the cell that released the signal

paralogues similar genes in the same genome that evolved by gene duplication and subsequent mutation

paraphyletic describes a taxonomic group that includes some, but not all, of the species that descended from a common ancestor

parasite an organism that lives in or on a host organism and derives nutrients from the host, at the host's expense

parthenogenesis a process by which the entire organism is regenerated from a single egg cell without the need for fertilisation

passive immunity immunity characterised by the transfer of antibodies from one individual to another; this type of immunity does not generate immunological memory

pathogen an organism foreign to the body that can cause disease

pathogen-associated molecular pattern (PAMP) a broad molecular pattern commonly shared by a number of pathogens and not normally present in the host

pattern recognition receptor (PRR) a receptor that recognises molecular patterns commonly shared by a number of pathogens; includes NOD-like receptors and toll-like receptors

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

peptide hormone a hydrophilic hormone composed of a chain of amino acids that can bind to extracellular receptors on target cells; for example, insulin and ADH

peptidoglycan a polymer in prokaryotic cell walls consisting of interlinked peptide chains and polysaccharides

period a division of geological time; periods and epochs together make up eras

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis the bulk transport of solids into a cell inside a vesicle

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

pheromones chemicals produced by animals that cause a change in the behaviour of another animal

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

phospholipid a lipid molecule that has a hydrophilic phosphate group 'head' and hydrophobic lipid 'tail'

phospholipid bilayer the two layers of phospholipids that form a plasma membrane

phosphorylation the addition of a phosphate group to a protein or other organic molecule

photoautotroph an organism that synthesises its own food from inorganic substances using light as its primary energy source

photorespiration an alternative pathway for Rubisco, the carbon-fixing enzyme in photosynthesis, in which oxygen is consumed and carbon dioxide is produced, decreasing the rate of photosynthesis; generally occurs when stomata close and the concentration of oxygen in the leaf exceeds that of carbon dioxide

photosynthesis anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose

phototropism directional growth of shoots towards a light source

phyletic evolution successive evolution of one species into another within a single evolutionary lineage

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny evolutionary relationships that exist between species, often expressed as a tree-like diagram

phylogram a type of rooted tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

phytoalexin a chemical produced by plants under attack

phytosterol a type of lipid found in plant cell membranes

pigment a molecule that absorbs certain wavelengths of light and reflects all others

pinocytosis the bulk transport of liquids into a cell inside a vesicle

plagiarism presenting someone else's work, including their words or ideas, as your own

plasma cell an effector B cell that has differentiated to become highly specialised for producing antibodies

plasma membrane the insoluble boundary of the living cell that maintains the contents of the cell and regulates the movement of substances into and out of the cell. All cells have a plasma membrane

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

platelet a cell fragment found in the blood involved in blood clotting

point mutation a mutation that affects a single base-pair position within a gene

polarity a term that refers to a molecule having distinct regions of opposite charge

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

polyadenylation the process of adding a chain of adenine nucleotides at the 3′ end of an mRNA

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

polypeptide a linear polymer built from amino acid monomers

polypeptide sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide

polyploidy condition of a cell or organism with a genome comprising three or more copies of each chromosome, represented by 3*n*, 4*n*, 5*n*, 6*n* etc.

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

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time in response to various evolutionary processes

positive control a condition in which an effect is expected, giving confidence that the experimental set-up can yield the expected results

postcranial all of the skeleton, except the skull

post-reproductive isolating mechanism a mechanism that prevents fertilisation occurring, or an embryo developing into viable offspring if fertilisation does occur

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manoeuvre an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of grasping

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

pre-reproductive isolating mechanism a mechanism that prevents organisms from being able to interact to reproduce

primary data data that you have measured or collected yourself

primary immunodeficiency an inherited immunodeficiency resulting from mutations in the genome

primary lymphoid organs the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

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

primer a single-stranded DNA molecule that acts as the start of the amplification process

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

pro-apoptopic signal a signal that results in apoptosis of the target cell

prognathism a condition in which the jaws protrude from the plane of the face

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

prostaglandins autocrine and paracrine hormones made from fatty acids

protein a polymer built from amino acid monomers; may comprise a single such polymer or multiple polymers bonded together into a functional molecule

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

proteomics the study of proteomes

pseudogene an obsolete gene for which there are no functional alleles, a consequence of inactivation by mutation during evolution

psychrophile an organism that lives in extremely cold conditions

punctuated equilibrium pattern of evolution in which changes in organisms occur in bursts separated by periods of stasis; the rapid changes occurring during the bursts may not be preserved in the fossil record

pyruvate the three-carbon molecule that is the end product of glycolysis

quadrupedalism a type of locomotion in which an organism walks on four limbs

qualitative measurement measurement with descriptive or non-numerical results

quantitative measurement measurement with numerical values

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

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

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents

receptor a molecule on the surface or interior of the cell that binds specifically to a substance (ligand) to detect or receive a stimulus

recognition protein a protein that acts as a marker on membranes

recombinant cytokines cytokines manufactured in bacteria using genetic engineering techniques

recombinant DNA technology transferring a gene from a cell of a member of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

reference the source of a specific piece of information or quotation

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

regulatory T (T_{reg}) lymphocyte/cell a class of lymphocytes that helps to negatively regulate the immune response

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

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

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

reproducible giving the same result, within uncertainty limits, when repeated measurements are made

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

resting potential the electrical potential difference between the two sides of an unstimulated nerve cell's plasma membrane; when this potential exists, the cell is ready for action

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

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

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

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

RNA polymerase the enzyme that catalyses the synthesis of RNA

robust of sturdy build

rooted tree a phylogenetic tree that depicts the ancestors and descendants through the course of evolution of a group of organisms

rough endoplasmic reticulum (rough ER) ER with ribosomes attached

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

scatter graph a graph or plot showing data points, without a line joining the points, and used to demonstrate or determine a mathematical relationship between variables; the axes are defined by the variables

secondary data data or information that has been collected by someone else

secondary immunodeficiency immunodeficiency acquired as the result of an environmental factor such as HIV infection

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and MALT

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

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

second messenger small molecules that relay a signal from receptors on the cell surface to target molecules inside a cell

secretory pathway the movement of proteins produced by ribosomes attached to the ER, through a series of compartments and vesicles, via the Golgi apparatus, to secretory vesicles for export from the cell by exocytosis

selection pressures factors that influence the survival of an individual within a population

selective breeding see **artificial selection**

selectively permeable see **differentially permeable**

self describes agents (e.g. cells, organisms, substances) that are recognised by the immune system of an organism as being part of that organism; the immune system tolerates all cells in the body without attacking them because cells carry marker molecules that identify them as self

self-antigen an antigen or molecule that is a normal body component

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self antigens to prevent an immune response to these antigens

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

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

sensitivity limit marks the portion of a curve that is non-linear; data that falls into these non-linear regions cannot be extrapolated

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

sexual dimorphism the situation where males and females of a species have different morphologies, often in shape or size

signal tranduction the process by which a cell converts one kind of signal into another; occurs when an extracellular signal binds to and activates a receptor, which, in turn, alters intracellular molecules to bring about a cell response

significant difference a difference between data values that is statistically significant; that is, the probability (p) of the difference being due to chance is so small (usually less than 5%) that the result is considered true

silent mutation see **synonymous mutation**

single nucleotide polymorphism (SNP) a nucleotide difference that occurs at a given position in the genomes of two or more individuals

short tandem repeat (STR) a short non-coding region of DNA consisting of a sequence of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be used in DNA profiling

smooth endoplasmic reticulum (smooth ER) ER with no ribosomes attached

sodium–potassium pump a membrane protein that moves potassium ions into, and sodium ions out of, a cell, using active transport

somatic describes a body cell that will not pass its genes on to the next generation

speciation the evolution of one or more new species from an ancestral species

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

spontaneous mutation a mutation occurring in the absence of exposure to mutagens

stabilising selection natural selection that tends to advantage organisms similar to their parents; this usually occurs when the environment is very stable and unchanging and selects against extremes of phenotype

standard deviation a measure of the dispersion of a set of data from its mean; expresses the variability of a population or set of data

stem cell an undifferentiated cell that can divide indefinitely to give rise to more cells of the same type or specialised cells through the process of differentiation

stereoscopic describes vision that has a sense of depth

sterile inflammation inflammation resulting from detection of DAMPs released during tissue injury in the absence of infection

steroid hormones hydrophobic signal molecules found in plants and animals; these are produced from cholesterol, giving them a common chemical structure; examples include oestrogen, testosterone and cortisone; these signalling molecules are lipophilic so they can pass through the plasma membrane and bind to intracellular receptors

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

stroma the jelly-like, semifluid interior of a chloroplast

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

subspecies distinct populations of a species, which can interbreed but usually do not due to geographical isolation

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

substrate a substance upon which an enzyme acts; a reactant for an enzyme-controlled reaction

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

superfamily a taxonomic rank immediately superior to the traditional rank of family; a superfamily may contain multiple taxonomic families

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

sympatric speciation speciation that occurs without physical or geographic isolation

synapse the point where an axon terminal meets another neuron, a muscle cell or a gland cell, separated by a synaptic cleft

synaptic cleft the space between the presynaptic cell and postsynaptic cell in a synapse, across which neurotransmitters diffuse to transmit a nerve impulse

synonymous mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a 'silent' mutation

synteny the conserved order of gene loci in a section of chromosome in two different species

systematics the branch of biology concerned with categorising organisms according to their evolutionary history

systemic acquired resistance a plant's reaction to invasion by a pathogen that leads to long-term resistance to a broad range of pathogens; 'systemic' refers to the whole body

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens presented on MHC proteins

target cell a cell that responds to a signalling molecule because it expresses specific receptors for that molecule

taxonomy a system of scientific conventions for naming and classifying organisms

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

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

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

thermophile an organism that lives in high-temperature environments

thylakoid membrane the interconnected, folded membrane within a chloroplast

thylakoid space the space inside a thylakoid membrane

toll-like receptor (TLR) a pattern recognition receptor in membranes that responds to PAMPs and DAMPs

transcribe copy DNA into RNA

transcription the process by which DNA is copied into RNA

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

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

transformation the process by which DNA is taken from one organism and inserted into another organism using a plasmid

transforming growth factor a secreted signalling protein with a role in stimulating cells to divide and differentiate

transgenic organism an organism that has been modified by incorporating a piece of foreign DNA into its genome

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

transmembrane protein a protein with one or more regions that span the membrane

transport protein a protein that carries molecules across membranes

tribe a taxonomic rank inserted between family and genus

tropism directional growth or turning movement of a plant in response to an environmental stimulus

t-test a statistical test commonly used to analyse differences between two sets of data

tumour an abnormal growth of tissue

tumour suppressor genes genes that prevent a cell from dividing to produce daughter cells that carry the mutation that enables the cell to escape apoptosis

uncharged tRNA tRNA without an attached amino acid

unloaded describes coenzymes that are not attached to the specific group of atoms they transfer

unrooted tree a phylogenetic tree that shows only the degree of relatedness between organisms and makes no reference to ancestry

up-regulate the process by which a cell increases the quantity of a cellular component, such as RNA or protein

vaccine an injected solution of dead or weakened antigens or pathogens, together with an adjuvant, that is used for the process of immunisation

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

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

variable traits traits that vary in the population due to differences in alleles carried by different individuals

vasodilation dilation (widening) of blood vessels, particularly arterioles

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

vesicle a small, membrane-bound sac in the cytoplasm that transports, stores or digests substances

vestigial structures structures found in organisms that have lost most, if not all, of their original function in the course of evolution; in ancestral organisms the structures served a purpose, but in their descendants the structures become atrophied or rudimentary

virus an obligate intracellular pathogen able to use the host cell's machinery to replicate itself: usually consists of a nucleic acid surrounded by a protein coat

wild-type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

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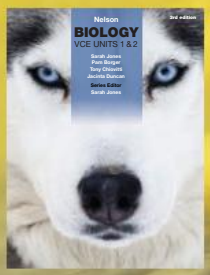

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