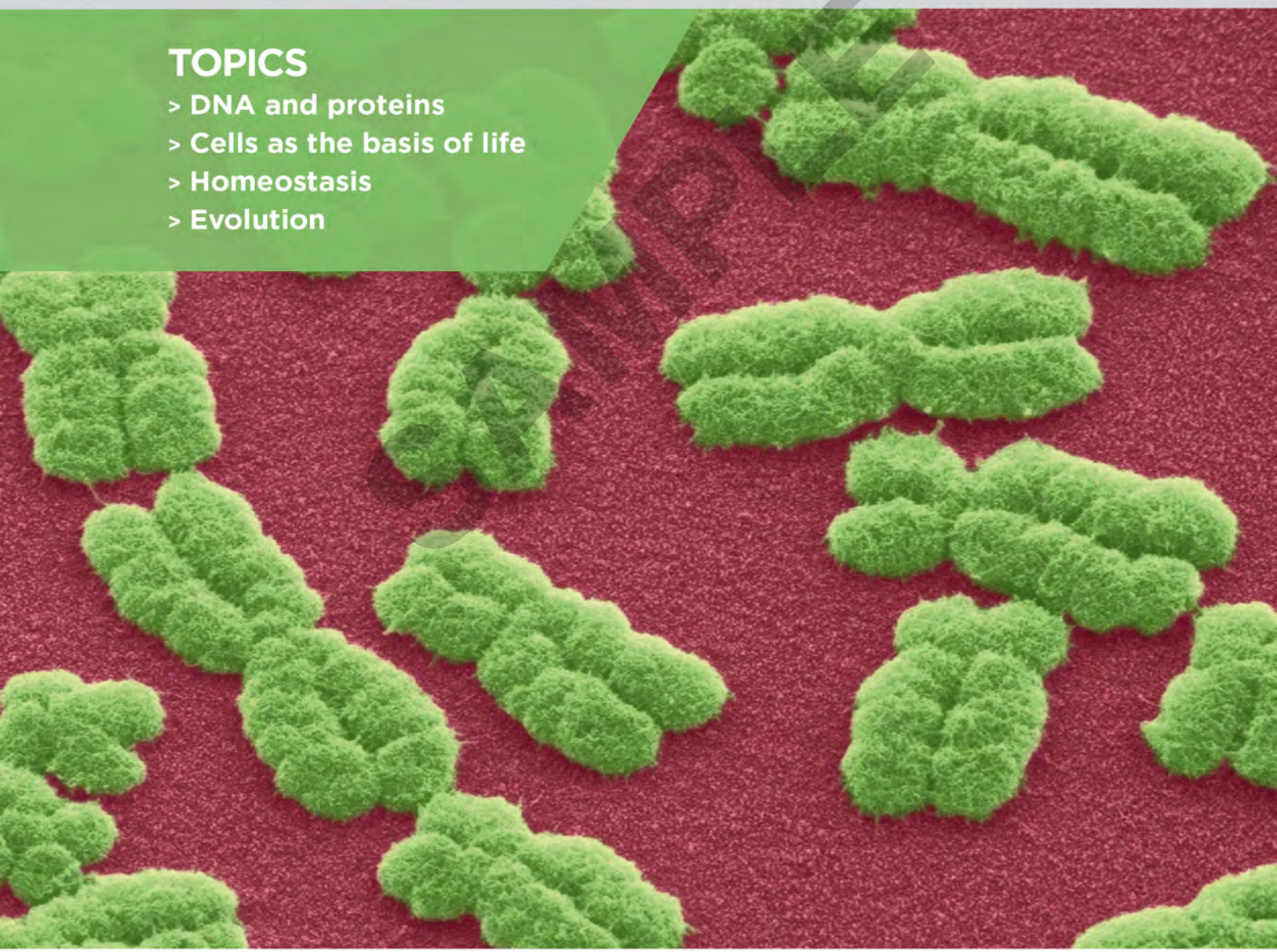
SACE STAGE 2
Australian Curriculum

WORKBOOK

Biology

TOPICS

- DNA and proteins
- Cells as the basis of life
- Homeostasis
- Evolution



SASTA
South Australian Science Teachers Association

Supporting Teachers of Science
Advancing Science Education

THIRD EDITION
Rhys Lewis

WORKBOOK

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SACE STAGE 2
Australian Curriculum

Biology

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Contents

CHAPTER 1

1.1: The Structure of DNA

DNA stores and transmits genetic information; it functions in the same way in all living things.

DNA is a helical double-stranded molecule.

In eukaryotes, DNA is bound to proteins in linear chromosomes, which are found in the nucleus.

DNA is unbound and circular in the cytosol of prokaryotes and the mitochondria and chloroplasts of eukaryotes.

- Compare chromosomes in prokaryotes and eukaryotes.

Deoxyribonucleic acid (DNA) is a large molecule in all living things that stores and transmits genetic information that coordinates all cellular activities. The information that programs a cell's activities is encoded in the structure of the DNA.

The Structure of DNA

DNA is a **nucleic acid**, a macromolecule that exists as polymers called **polynucleotides**. As shown in Figure 1.01, a polynucleotide consists of monomers called **nucleotides**, each containing a nitrogenous (nitrogen-containing) base, a sugar, and a phosphate group (Figure 1.01). There are four nitrogenous bases found in DNA, and each is represented by the first letter of its name: **thymine (T), cytosine (C), adenine (A) and guanine (G).** The nitrogenous bases are chemically bonded to a five-carbon sugar called **deoxyribose** which is bonded to a phosphate group. The phosphate group links the sugars of two nucleotides in a polynucleotide, resulting in a repeating sugar-phosphate pattern called the **sugar-phosphate backbone** (Figure 1.01).

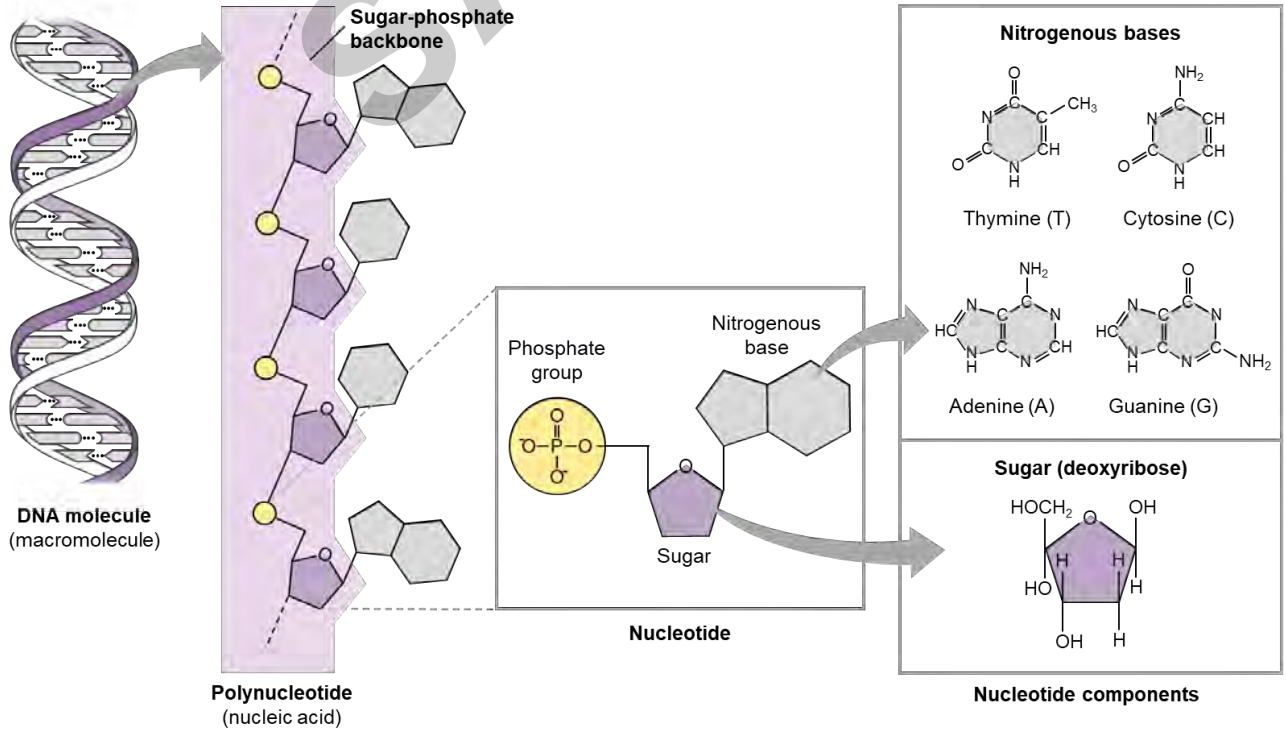


Figure 1.01: The structure of DNA

A DNA molecule has two polynucleotides, or "strands," that wind around an imaginary axis, forming a **double helix** (Figure 1.02). The two sugar-phosphate backbones run in opposite directions like a divided highway, an arrangement referred to as **antiparallel**. The sugar-phosphate backbones are on the outside of the helix, and the nitrogenous bases are paired in the interior of the helix. The two strands are held together by weak electrical forces of attraction called **hydrogen bonds** between the paired nitrogenous bases (Figure 1.02). Only certain bases in the double helix are compatible with each other. For example, Adenine (A) in one strand always pairs with thymine (T) in the other, and guanine (G) pairs with cytosine (C).

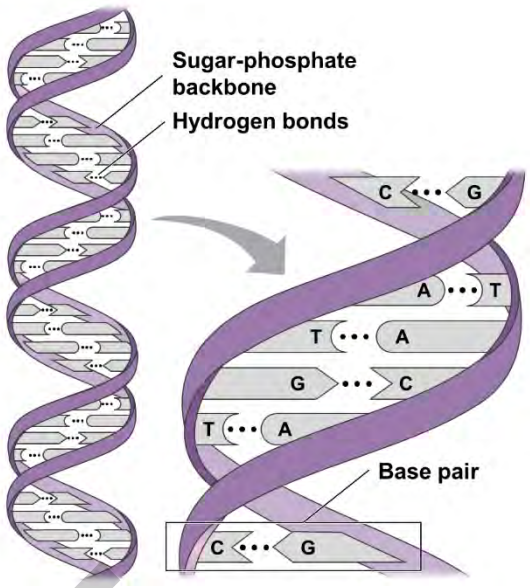


Figure 1.02: DNA double helix.

Chromosomes

Most DNA molecules are very long, containing thousands or even millions of base pairs. For this reason, cells arrange DNA molecules into one or more **chromosomes**, highly condensed structures composed of a single DNA molecule and associated proteins. In eukaryotes, each cell contains two or more linear chromosomes housed in the cell nucleus. Each eukaryotic chromosome contains **chromatin**, a mixture containing one long DNA molecule bound to proteins (Figure 1.03). Among the proteins bound to DNA in eukaryotes are **histones** (Figure 1.03), small round proteins that help coil the DNA molecule, reducing its length and allowing it to fit into the nucleus. When a eukaryotic cell is not dividing, the chromatin is relaxed, and the chromosomes are observed under an optical microscope as a spread-out mass, indistinguishable from one another. However, as a cell prepares to divide, the chromatin condenses, and the chromosomes become thick enough to be distinguished from one another under an optical microscope.

In addition, each eukaryotic species has a characteristic number of chromosomes. For example, a typical human body cell has 46 chromosomes in its nucleus, whereas a fruit fly body cell has only eight chromosomes.

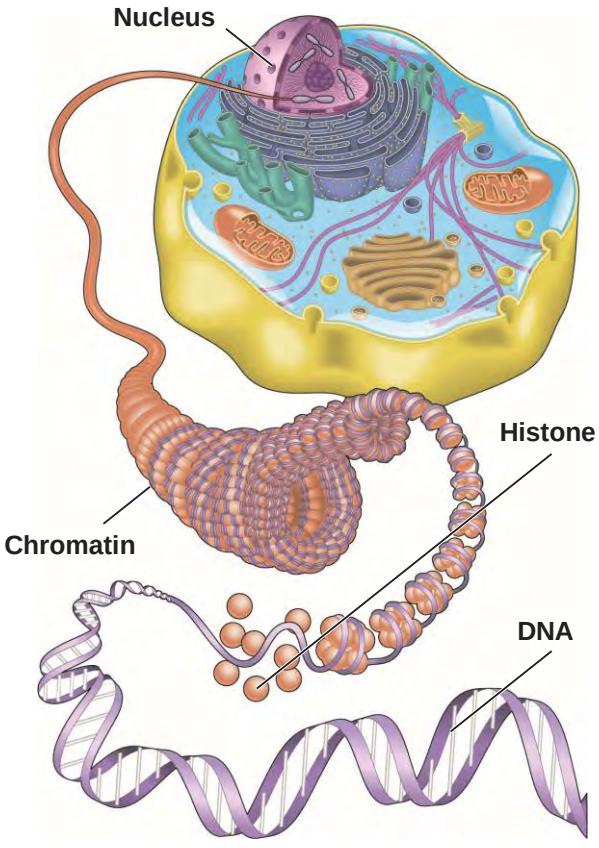


Figure 1.03: Chromosome structure eukaryotes

In contrast, prokaryotes each contain a single circular chromosome located in the **cytosol**, the fluid which fills the cell's cytoplasm. In prokaryotes, the cytosol region containing the chromosome is called the **nucleoid**. Prokaryotic chromosomes are composed of a single DNA molecule, but each is unbound and is not associated with proteins that regulate its length (Figure 1.04). In addition, the mitochondria and chloroplasts of eukaryotes also contain circular chromosomes, a reflection of their evolutionary past as free-living prokaryotes.

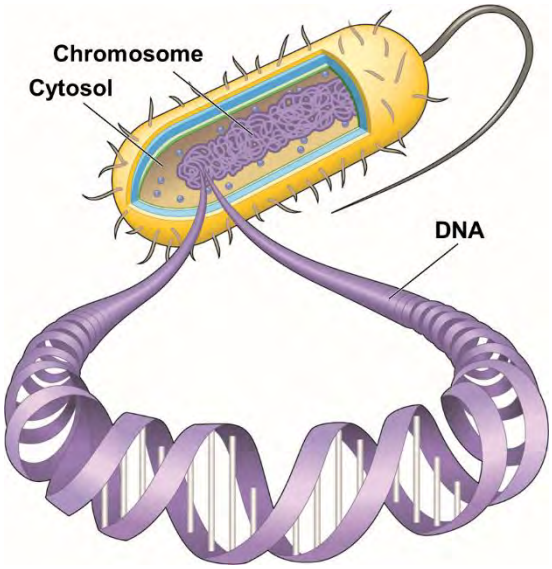
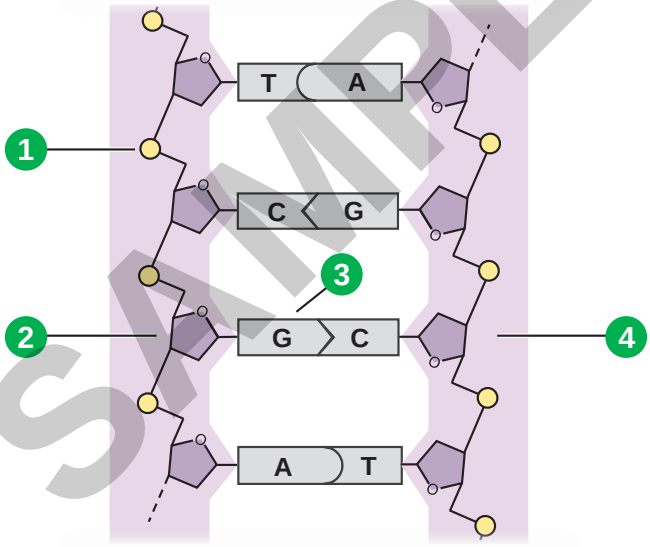


Figure 1.04: Chromosome structure prokaryotes

Question 1

The diagram below shows a short section of DNA.



(a) Name the components **1–4**.

1: _____ 2: _____

3: _____ 4: _____ (4 marks) **KA1**

(b) Describe the structure of a DNA molecule.

_____

_____

_____

_____

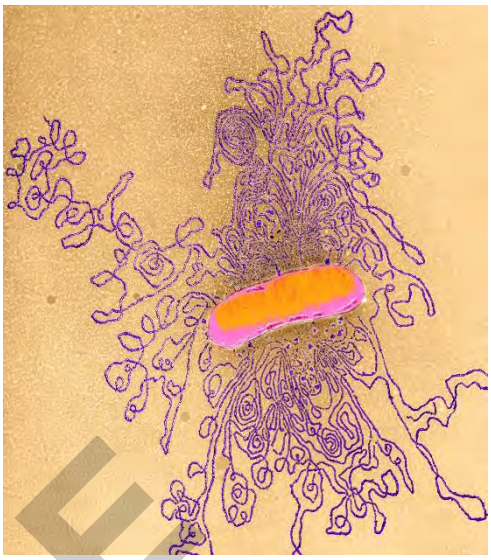
_____

_____ (3 marks) **KA2**

Question 4

The diagram opposite is a coloured transmission electron micrograph (TEM) of the bacterium *Escherichia coli*.

The cell has burst, and the chromosome has leaked out.



(a) Describe the structure and composition of the bacterial chromosome.

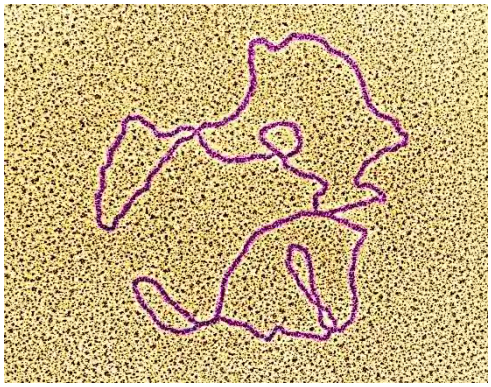
(2 marks) **KA2**

(b) State the location of the chromosome before it leaked out of the cell.

(1 mark) **KA1**

Question 5

The diagram opposite is a coloured TEM of mitochondrial DNA.



(a) Describe the structure of mitochondrial DNA using evidence from the diagram.

(2 marks) **KA2**

(b) Mitochondrial DNA has a similar structure to DNA in prokaryotes.

Give one reason for this.

(1 mark) **KA1**

(c) Mitochondria are one type of organelle containing DNA.

Name two other organelles that contain DNA.

(2 marks) **KA2**

1.2: The Replication of DNA

Replication of DNA allows for genetic information to be inherited.

Base-pairing rules and methods of DNA replication are universal.

- Describe the structural properties of the DNA molecule, including:
  - nucleotide composition and pairing
  - the weak bonds between strands of DNA that allow for replication.
- Explain the importance of complementary base pairing (A–T and C–G).
  - Describe and represent the process of semi-conservative replication of DNA.

DNA is the genetic material that organisms inherit from their parents. When a cell reproduces by dividing, its one or more DNA molecules are copied and passed along from one generation of cells, called **parent cells**, to the next, called **daughter cells**, making them genetically identical. This transmission of genetic information from parent to daughter cell depends on the structure and replication of DNA.

Base-Pairing Rules

A DNA molecule is composed of two polynucleotide strands that form a double helix. The strands run antiparallel and are held together by hydrogen bonds between base pairs. In base-pairing, only certain bases in the double helix are compatible with each other due to the positions of their atoms. For example, guanine (G) in one strand always pairs with cytosine (C) in the other, and adenine (A) pairs with thymine (T), as depicted in Figure 1.05. These base-pairing rules are universal and allow us to predict the sequence of bases along one strand of the double helix when we know the sequence of bases along the other strand. For example, if one strand has the base sequence –ACTAG–, the same stretch of the other strand must have the sequence –TGATC–. This is because the two strands of the double helix are **complementary**, meaning each is the predictable counterpart of the other. This feature of DNA makes it possible to generate two identical copies of each DNA molecule in a cell preparing to divide.

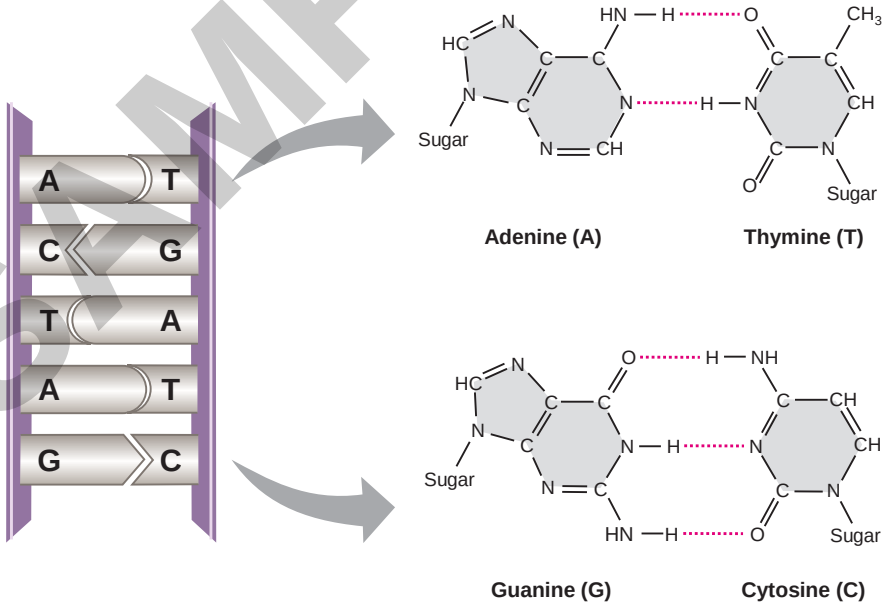


Figure 1.05: DNA base-pairing rules in DNA.

DNA Replication

Before a parent cell divides into daughter cells, its one or more chromosomes are copied, so the daughter cells each have a complete set of genetic information that programs a cell's activities. The process by which the chromosomes are copied is called **DNA replication**, a chemical reaction in which the two strands of a **parent DNA molecule** are separated, and free DNA nucleotides are used to construct complementary strands, forming two **daughter DNA molecules**, as in Figure 1.06. First, the two polynucleotide strands in the parent DNA molecule are separated, allowing each parent strand to serve as a template for synthesising one new strand. In living things, this separation is facilitated by the enzyme helicase, which breaks the weak hydrogen bonds between the base pairs in the parent strands. Next, the enzyme DNA polymerase binds free DNA nucleotides and attaches them to their complementary base pair on the exposed parent strands. Finally, DNA polymerase connects the nucleotides, forming the sugar-phosphate backbones of the daughter molecules.

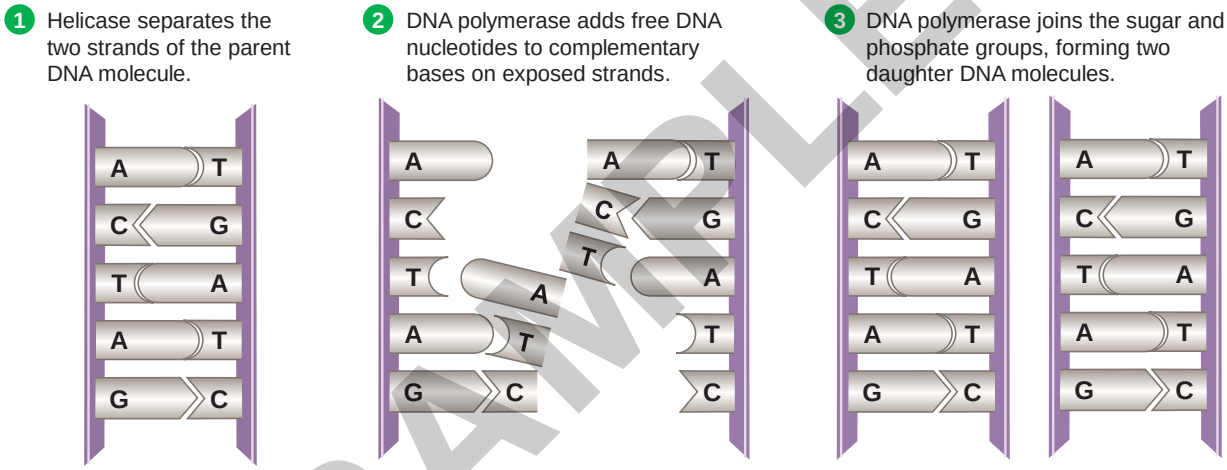


Figure 1.06: DNA replication process.

During DNA replication, the two strands of the parent molecule separate, and each functions as a template for synthesising a complementary strand. In this way, DNA replication is a **semi-conservative process** as when a double helix replicates, each of the daughter molecules has one old strand from the parent molecule and one new strand from DNA replication, as in Figure 1.07.

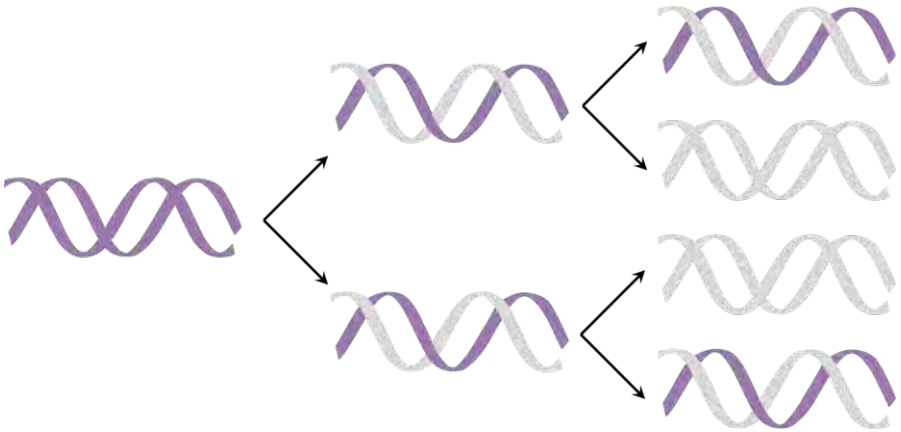


Figure 1.07: The semi-conservative model of DNA replication

DNA Replication and Inheritance

DNA replication allows genetic information to be inherited from a parent cell to daughter cells by binary fission in prokaryotes and mitosis and meiosis in eukaryotes. This inheritance process ensures that the daughter cells have a complete set of chromosomes containing the genetic information that programs a cell's activities, ensuring the daughter cells can survive and reproduce.

In eukaryotes, the process of inheritance begins with DNA replication. Before replication, the cell has two or more linear bodies called **unduplicated** or **unreplicated chromosomes.** During replication, each chromosome is replicated, and the two daughter DNA molecules are connected, forming X-shaped bodies called **duplicated** or **replicated chromosomes**. The two daughter DNA molecules are separated during cell division, and each is partitioned into a daughter cell, as in Figure 1.08. DNA replication occurs in the **synthesis 'S' phase** of the cell cycle in eukaryotes, and its length varies between species. In humans, S phase lasts 8 hours and replicates 46 chromosomes.

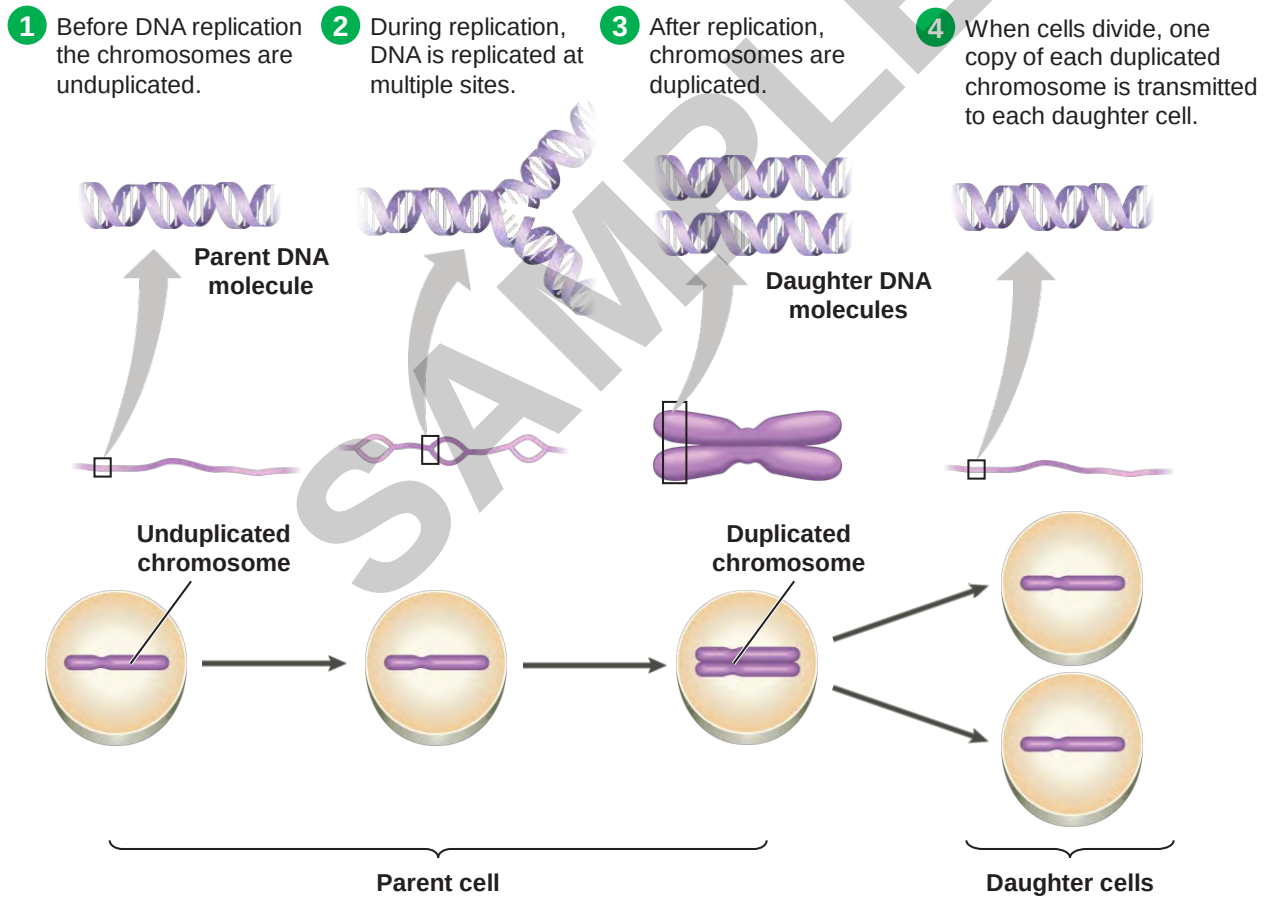


Figure 1.08: DNA replication and the transmission of genetic material.

In prokaryotes, DNA replication is more rapid as the reactant is a single circular chromosome much shorter in length than most eukaryotic chromosomes. In favourable environments, some bacteria can replicate their DNA in less than 15 minutes. Biologists have studied the processes of DNA replication extensively in prokaryotes and eukaryotes. They have concluded that most of the process is fundamentally similar, indicating that DNA replication methods are universal.

Genes

The DNA molecules in cells are arranged into one or more chromosomes, each containing hundreds to thousands of **genes**, nucleotide sequences that code for proteins and the RNA molecules that carry out protein synthesis. Proteins are macromolecules composed of long chains of amino acids folded into unique shapes that give cells their structure and functions. Genes are typically hundreds to thousands of nucleotides long, each having a unique nucleotide base sequence coding for the synthesis of part or all of a protein or RNA molecule. Genes are genetic information coding for inherited traits such as antibiotic resistance in bacteria, drought resistance in plants, and animal blood type. The DNA inherited by a living thing called its **genotype,** leads to specific physical and behavioural traits, called **phenotypes,** by dictating protein synthesis. For example, in the case of antibiotic resistance in bacteria, the resistance gene contains the information to synthesise an enzyme (protein) that breaks down antibiotics, protecting the cell from damage. The process by which DNA directs RNA and protein synthesis is called **gene expression** and includes two stages: **transcription** and **translation** (Figure 1.10). In transcription, the DNA nucleotide sequence of a gene is rewritten into a complementary RNA nucleotide sequence. The resulting RNA molecule is a reliable transcript of the gene's protein-synthesising instructions. The RNA molecule synthesised from a protein-coding gene is called **messenger RNA (mRNA),** as it carries the genetic message from genes to the cell's protein-synthesising machinery. In translation, the nucleotide sequence on the mRNA molecule is translated into an amino acid sequence, forming a polypeptide that is folded onto a functional protein. The sites of translation are **ribosomes**, macromolecular complexes that link the amino acids to form a polypeptide that folds into a functional protein.

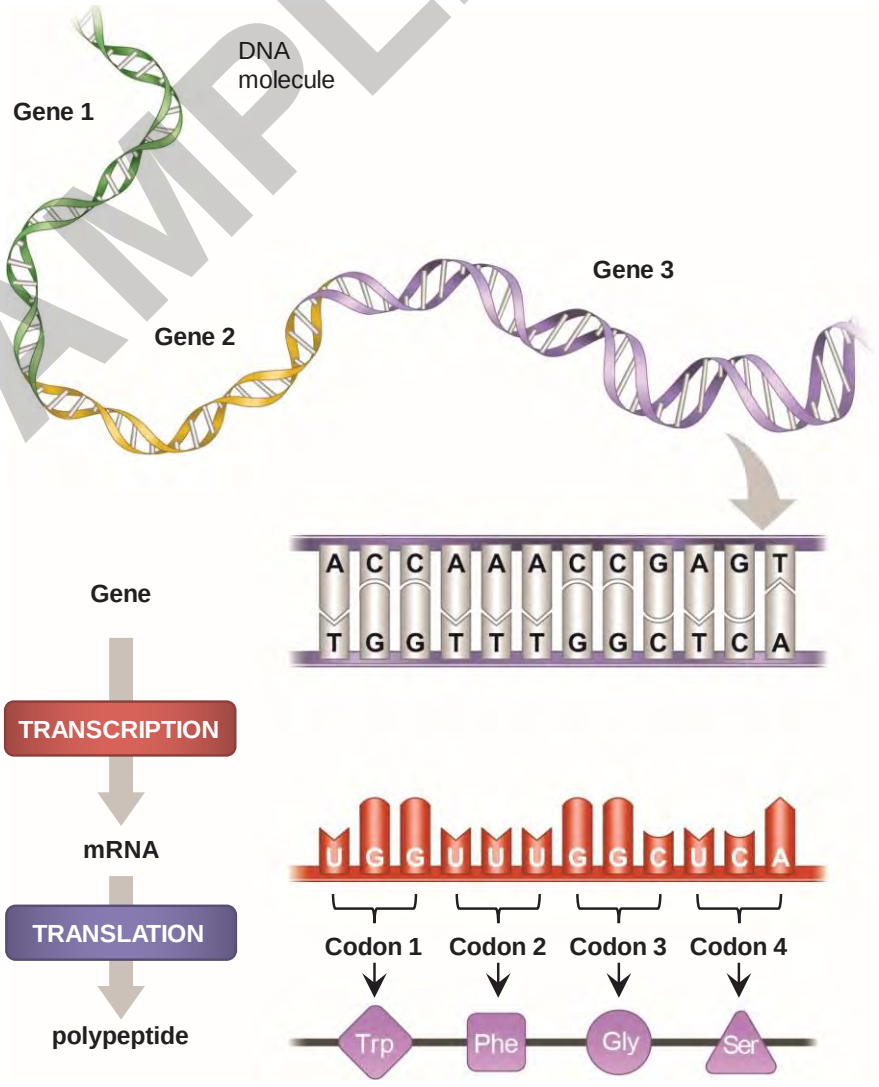
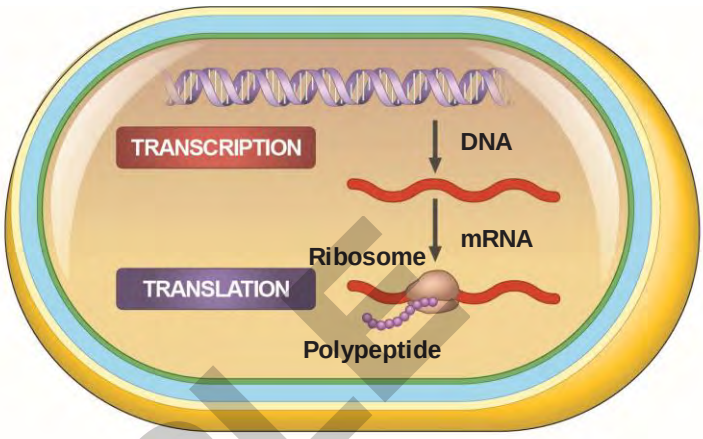
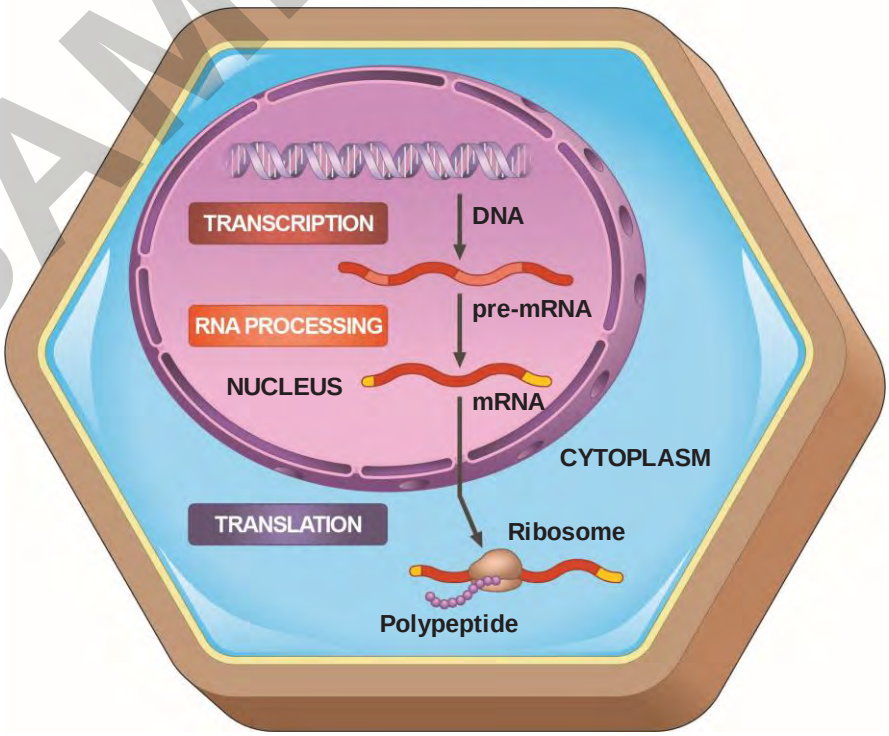


Figure 1.10: Gene expression.

Transcription and translation occur in all living things, with the basic mechanics being similar for bacteria and eukaryotes. Still, there is an essential difference in the flow of genetic information between the two cell types. Bacteria lack nuclei, so nuclear membranes do not separate bacterial DNA and mRNA from ribosomes and the other protein-synthesising machinery, as in Figure 1.11 1. This lack of compartmentalisation in bacterial cells allows translation to begin while transcription occurs, resulting in rapid protein synthesis. In contrast, eukaryotic cells have a nucleus with a membrane separating transcription from translation, as shown in Figure 1.11 2. Transcription occurs in the nucleus, but the mRNA must be transported to the cytoplasm for translation, preventing translation from starting until transcription has been completed. Furthermore, the transcription of a protein-coding gene in eukaryotes produces **pre-mRNA**, an RNA molecule with a complementary nucleotide sequence to its gene. In most cases, the pre-mRNA molecule is modified by enzymes in the nucleus to produce a mature mRNA molecule ready for translation. While processing RNA, both ends of the pre-mRNA molecule are modified, specific interior sections are cut out, and the remaining parts are spliced together. The mature mRNA molecule then leaves the nucleus and enters the cytoplasm, which is translated into a polypeptide by ribosomes.



1 Bacterial cell
Bacterial cells lack a nucleus, allowing translation to occur immediately after transcription.



2 Eukaryotic cell
Eukaryotic cells have a nucleus that provides a separate compartment for transcription. The original mRNA, called pre-mRNA is processed before translation.

Figure 1.11: Gene expression in a bacterial cell (top) and a eukaryotic cell (bottom).

RNA Processing

In eukaryotes, the transcription of genes produces pre-mRNA, a molecule with the same length as its gene and a nucleotide sequence complementary to its gene. Following transcription, enzymes in the eukaryotic nucleus modify pre-mRNA into a molecule ready for translation. Firstly, the ends of the pre-mRNA are modified by adding chemical groups that facilitate the export of the mature mRNA molecule from the nucleus to the cytoplasm while preventing degradation by enzymes and helping the molecule attach to ribosomes. Secondly, one or more portions of the pre-mRNA molecule are removed, and the remaining portions are reconnected, a process called **RNA splicing**. For example, a gene and the pre-mRNA molecule transcribed from it may be 28,000 nucleotides long. However, the mature mRNA molecule translated by ribosomes may only be 1,400 nucleotides long. This is because most eukaryotic genes and their RNA transcripts have long stretches of non-coding nucleotides that are not translated, many of which are interspersed between coding segments of the gene and pre-mRNA. The non-coding segments of the gene and pre-mRNA between coding regions are intervening sequences called **introns**. The coding segments are eventually expressed into a polypeptide and are called **exons**. In RNA splicing, the introns are cut out from the molecule, and the exons are joined together, forming an mRNA molecule with a continuous coding sequence (Figure 1.12).

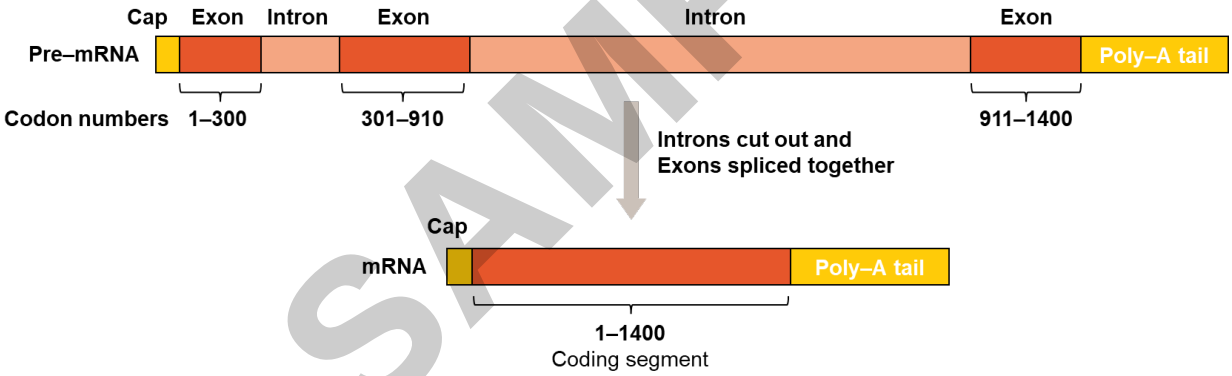


Figure 1.12: RNA processing

The removal of introns is accomplished by a **spliceosome**, a large complex made of protein and small RNA molecules. A spliceosome binds to several short nucleotide sequences along an intron, including critical sequences at each end and cleaves these regions to release the intron (Figure 1.13). The intron is rapidly degraded, and the spliceosome joins the two exons on either side of the intron.

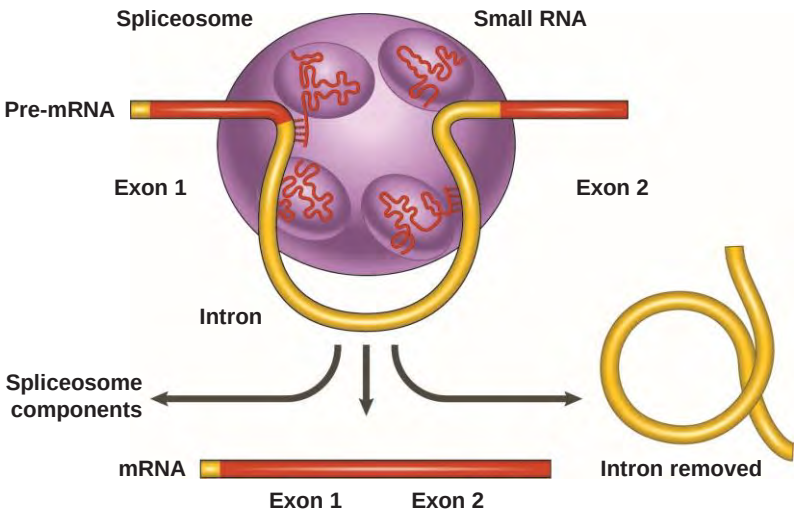


Figure 1.13: RNA splicing.

Transcription

Transcription is the synthesis of an RNA molecule, including messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). This text will focus on the synthesis of mRNA, the carrier of information from the template DNA strand of a gene to the cell's protein-synthesising machinery. First, an **RNA polymerase enzyme** binds to the target gene at a specific nucleotide sequence called the **promoter**. The enzyme then pries the two polynucleotide strands of DNA apart and joins together RNA nucleotides complementary to the DNA template strand, thus elongating the RNA polynucleotide strand (Figure 1.16). As the RNA polymerase moves along the gene, it unwinds the DNA double helix, exposing 10 and 20 nucleotides for pairing with complementary RNA nucleotides (Figure 1.17). As transcription proceeds, the newly synthesised RNA molecule behind the RNA polymerase peels away from the DNA template strand, and the DNA double helix re-forms. Transcription progresses at about 40 nucleotides per second in eukaryotes, and a single gene is often transcribed simultaneously by several RNA polymerase molecules following each other. The congregation of many polymerase molecules simultaneously transcribing a single gene increases the amount of mRNA transcribed from it, which helps the cell make the encoded protein in large amounts.

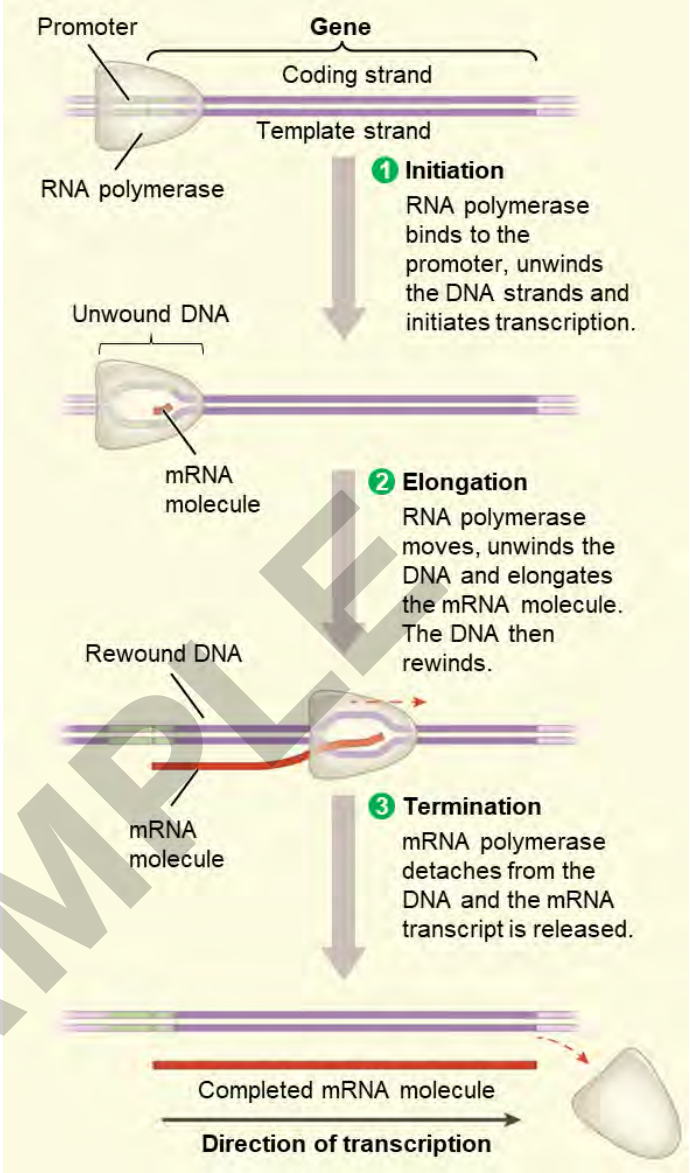


Figure 1.16: Transcription process

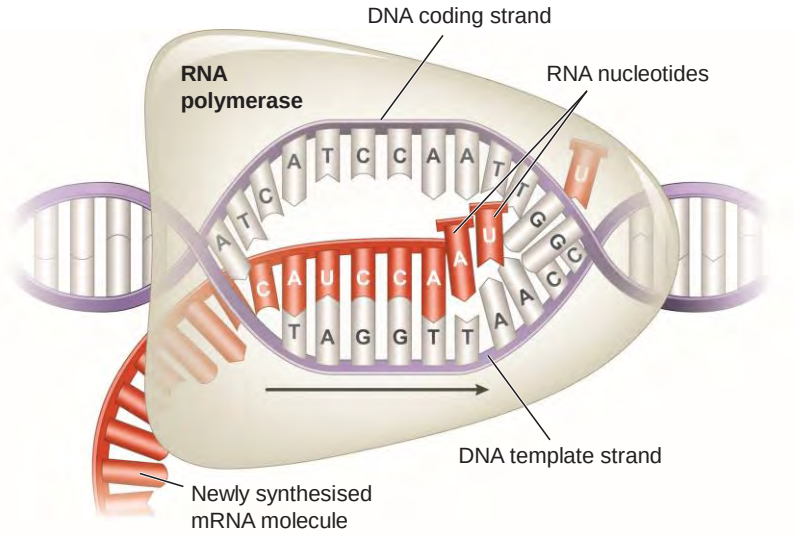


Figure 1.17: Transcription elongation.

Translation

In translation, the genetic message on mRNA is translated from an RNA nucleotide sequence to an amino acid sequence. The message is a series of codons along an mRNA molecule, and the translator is called a transfer RNA (tRNA). The function of a tRNA is to transfer an amino acid from the cytoplasm to a growing polypeptide in a ribosome. A cell keeps its cytoplasm stocked with all 20 amino acids by synthesising them from other compounds or taking them up from the surrounding solution. The ribosome, a structure made of proteins and ribosomal RNA, adds each amino acid brought to it by a tRNA to the growing end of a polypeptide chain (Figure 1.19).

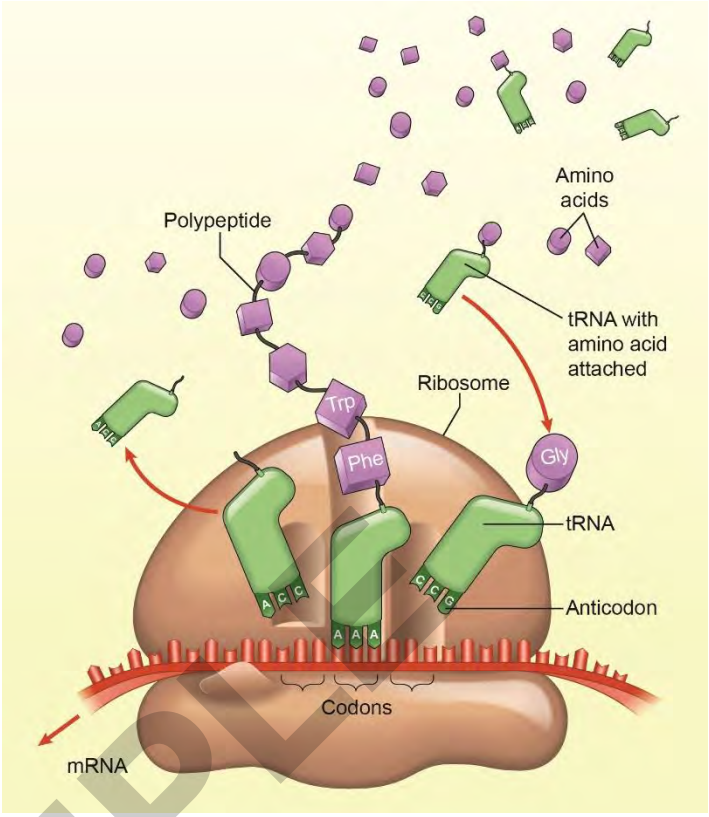


Figure 1.19: Translation

The key to translating a genetic message into a specific amino acid sequence is tRNA. A tRNA molecule has a specific amino acid binding site at one end of its structure, and a nucleotide triplet called an **anticodon** that base-pairs with the complementary codon on mRNA at the other (Figure 1.20). As an example of how tRNAs work, consider the mRNA codon UUC, which is translated as the amino acid phenylalanine. The tRNA has AAG as its anticodon and carries phenylalanine at its other end. As an mRNA molecule is moved through a ribosome, phenylalanine is added to the polypeptide whenever the codon UUC is presented for translation. Codon by codon, the genetic message is translated as tRNAs position each amino acid in the order prescribed, and the ribosome adds that amino acid onto the growing polypeptide.

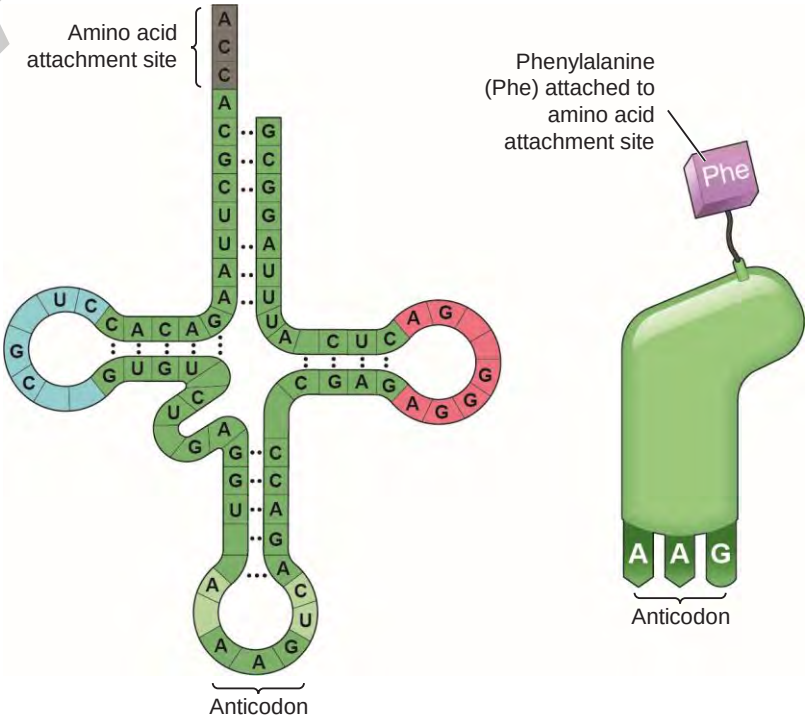


Figure 1.20: tRNA structure

The synthesis of a polypeptide occurs in three stages: initiation, elongation, and termination. The **initiation stage** of translation brings together an mRNA, a tRNA bearing the first amino acid of the polypeptide, and the two ribosomal subunits. First, a small ribosomal subunit binds to the mRNA, and a specific initiator tRNA carries the amino acid methionine (Met) to the start codon site. The large ribosomal subunit then attaches, completing the **translation initiation complex** (Figure 1.22). After the initiation process, the initiator tRNA sits in the central site of the ribosome, and the site vacated by this tRNA is ready to bind the next.

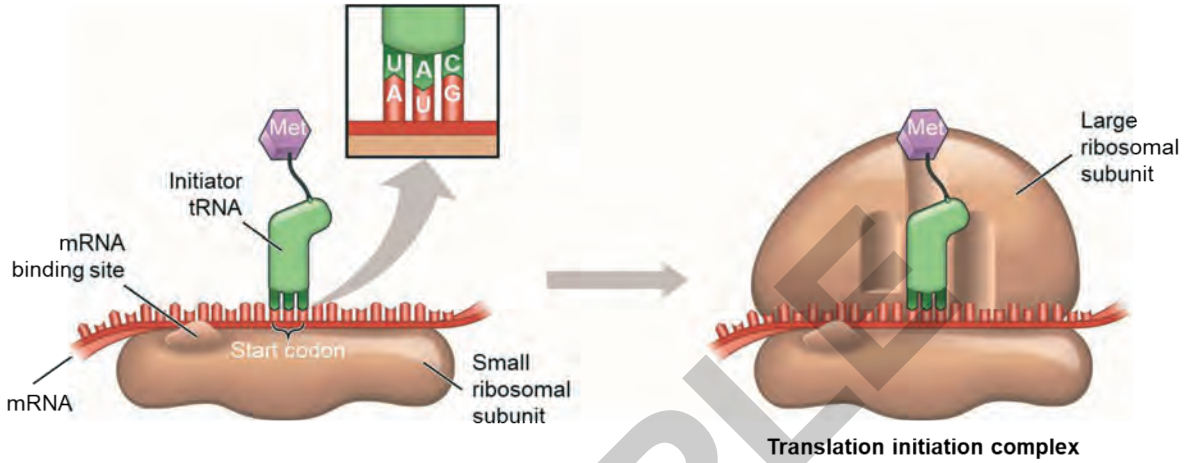


Figure 1.22: Translation Stage 1: Initiation

In the **elongation stage** of translation, amino acids are added to the growing polypeptide chain one at a time. The tRNAs released from the ribosome's exit site return to the cytoplasm, reloading them with the appropriate amino acid (Figure 1.23).

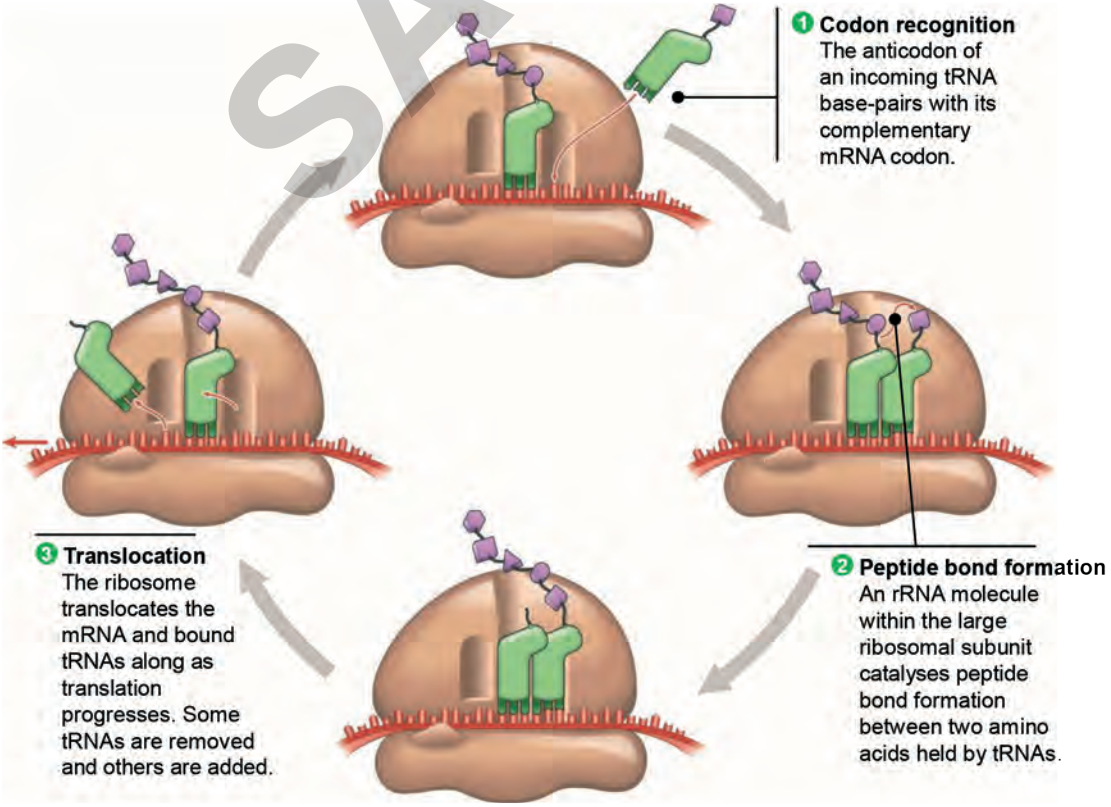


Figure 1.23: Translation Stage 2: Elongation

Question 21

The diagram shows the DNA nucleotide sequence coding seven amino acids in ribonuclease, a protein that breaks down mRNA after translation.

GTTTACTACTCTTCTTCTTTA

(a) Ribonuclease is translated from an mRNA 384 nucleotides long, including a stop codon.

State the number of amino acids in ribonuclease.

_____ (1 mark) **KA1**

(b) The number of each type of amino acid coded for by this sequence is shown below.

Amino acid	Number present
Arg	3
Met	2
Gln	1
Asn	1

Use the table to determine the amino acid sequence in this part of the protein.

Write your answer in the boxes below.

Gln						

(2 marks) **KA2**

Question 22

The table below shows part of the DNA sequence coding for a protein, the corresponding mRNA codons and the tRNA anticodons.

	Codon Position Number					
DNA template strand	GAC			CTC		
mRNA codon		CAC				AAG
tRNA anticodon			UCG		CGA	

(a) Complete the table. (3 marks) **KA1**

(b) Describe the role of hydrogen bonding in the interactions between DNA, mRNA and tRNAs.

_____

_____

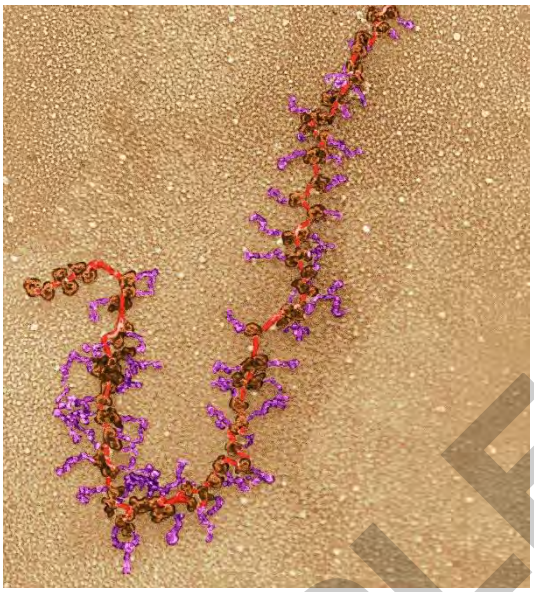
_____

_____ (2 marks) **KA2**

Question 24

The diagram below is a coloured TEM showing translation in a eukaryotic cell.

Several ribosomes (brown) are bound to the mRNA (red) and are synthesising proteins (purple).



(a) Describe the events in translation after the ribosome has attached to the mRNA.

(4 marks) **KA1**

(b) Explain the advantage to the cell of many ribosomes binding to the mRNA simultaneously during translation.

(2 marks) **KA2**

Question 25

The photograph below shows two species that are toxic to animals by ingestion.

1 **Death cap mushroom (*Amanita phalloides*)**

2 **Caster oil plant (*Ricinus communis*)**

(a) *Amanita phalloides* produce amatoxins that inhibit the function of RNA polymerase.

Describe how the ingestion of *Amanita phalloides* causes the death of liver and kidney cells.

(2 marks) **KA2**

(b) The seeds of *Ricinus communis* produce ricin, a highly toxic protein that alters the shapes of ribosomes in animal cells.

(1) Explain why ingestion of ricin causes serious illness in animals.

(2 marks) **KA2**

(2) Explain why the symptoms of ricin toxicity may take several days to appear in animals.

(2 marks) **KA2**

Substrate Specificity of Enzymes

Enzymes are particular in the reactions they catalyse. The reactant an enzyme acts on is called its **substrate**, and the binding of an enzyme to its substrate forms an **enzyme-substrate complex**. An enzyme can recognise its specific substrate even among closely related compounds. The specificity of the enzyme for its substrate results from its three-dimensional shape, which is a consequence of its amino acid sequence. The polypeptide chain folds into a tertiary or quaternary structure, creating a region that binds to the substrate. This region, called the **active site**, is typically a pocket or groove on the enzyme's surface where catalysis occurs (Figure 1.36). Usually, the active site is formed by only a few of the enzyme's amino acids, with the rest of the protein molecule providing a framework that determines the shape of the active site.

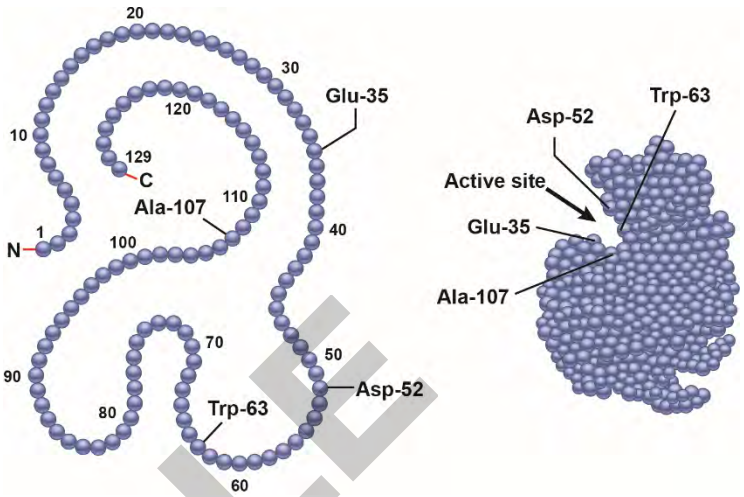


Figure 1.36: Active site of an enzyme

The specificity of an enzyme is attributed to a complementary fit between the shape of its active site and the shape of the substrate. However, the active site is not a rigid receptacle for the substrate. When the substrate enters the active site, the enzyme changes shape slightly due to interactions between the substrate's chemical groups and those on the side chains of the amino acid that form the active site. This shape change makes the active site fit more tightly around the substrate. The tightening of substrate binding after initial contact, called **induced fit**, is like a clasping handshake. Induced fit brings chemical groups of the active site into positions that enhance their ability to catalyse the chemical reaction. Following the conversion of substrate to product, the product departs from the active site, and the enzyme is free to bind to another substrate molecule. The entire catalytic cycle occurs so rapidly that a single enzyme molecule typically acts on thousands of substrate molecules per second. Figure 1.37 shows a catalytic cycle involving two substrates and two products.

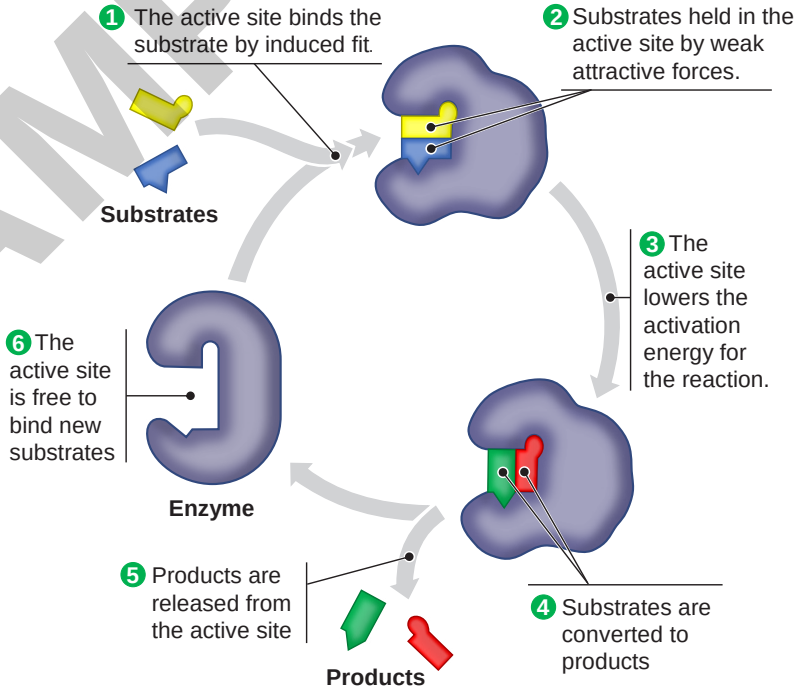
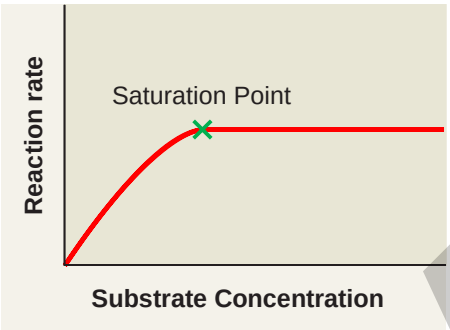


Figure 1.37: Catalytic cycle

Enzyme and Substrate Concentration

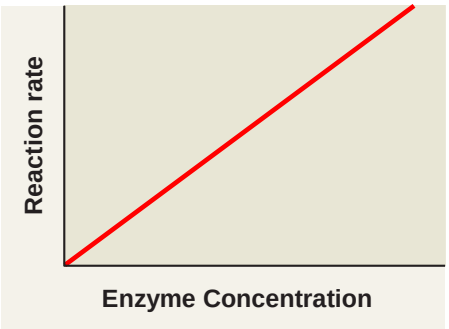
We have seen that the rate of an enzyme-controlled reaction depends on environmental factors such as temperature and pH and the presence of inhibitors. In addition, the rate of an enzyme-controlled reaction also depends on the concentration of the enzyme and its substrate. For example, the rate of lactase converting lactose to glucose and galactose depends on the number of available lactose molecules and the number of lactase enzymes to break them down. In general, the rate of an enzyme-controlled reaction increases with substrate concentration as more substrate molecules are available to access the vacant active sites of available enzymes. At low substrate concentrations, the reaction rate doubles with substrate concentration, but as substrate concentration is increased further, each additional increase results in a smaller increase in reaction rate, as shown in Figure 1.41. At some point, the substrate concentration becomes high enough that all enzyme molecules have their active sites occupied such that as soon as the product exits an active site, another substrate molecule enters. At this substrate concentration, the enzymes are said to be **saturated**, and the reaction rate becomes constant, as shown in Figure 1.41.



Example 1: Substrate concentration and reaction rate					
Lactase concentration (%w/v)	2	2	2	2	2
Lactose concentration (%w/v)	0	5	10	20	30
Reaction rate (μmol s^{-1})	0	25	50	65	65

Figure 1.41: Effect of substrate concentration on reaction rate.

When an enzyme population is saturated, the only way to increase the rate of product formation is to increase enzyme concentration. Cells often increase the rate of enzyme-controlled reactions by producing more enzyme molecules through protein synthesis. Increasing enzyme concentration will increase the reaction rate when the substrate concentration is high. As a general rule, the reaction rate doubles with each doubling of the enzyme concentration, as shown in Figure 1.42.



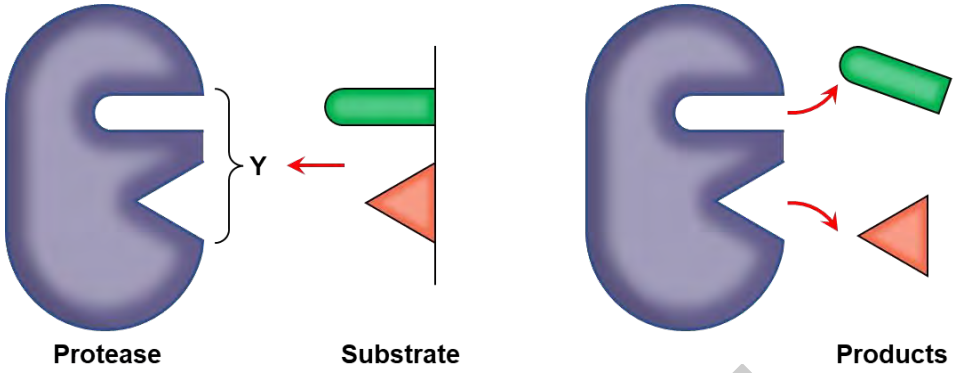
Example 2: Enzyme concentration and reaction rate					
Lactase concentration (%w/v)	0	1	2	4	8
Lactose concentration (%w/v)	10	10	10	10	10
Reaction rate (μmol s^{-1})	0	25	50	100	200

Figure 1.42: Effect of enzyme concentration on reaction rate.

Question 33

Proteases are enzymes that break down proteins.

The diagram below shows the stages of protease action.



(a) Name region Y on the diagram.

_____ (1 mark) KA2

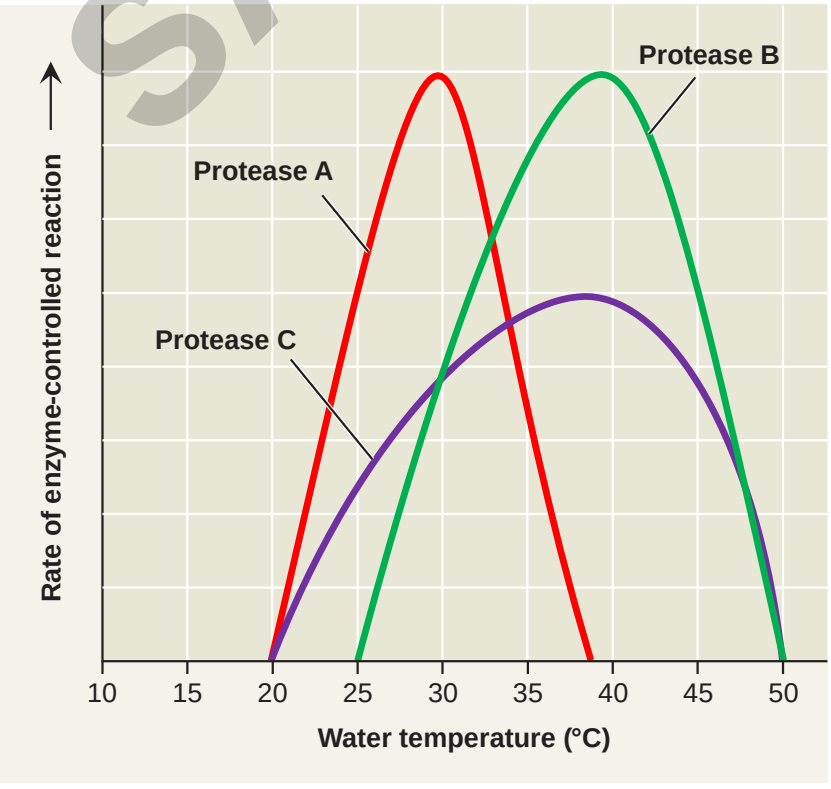
(b) Name the products of the protease-catalysed reaction.

_____ (1 mark) KA2

(c) Three proteases are used in a popular washing powder to remove protein-based stains.

The manufacturer claims its product works best at 35°C but offers superior stain removal at higher temperatures.

The graph below shows the rate of the enzyme-controlled reactions of the three proteases at different water temperatures.



The table below shows the different stains removed by each protease.

Protease	Source of protein-based stain		
	Egg	Meat	Blood
A	No	No	Yes
B	No	Yes	No
C	Yes	No	No

Use the table above and graph on the previous page to answer the following:

(1) Explain why the washing powder works best at 35°C.

(2 marks) KA2

(2) Identify the stain that is not removed when washing at 40°C.

(1 mark) KA2

(3) Suggest why three different proteases are needed to remove the different stains.

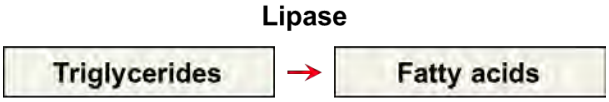
(2 marks) KA2

(4) Describe and explain why washing at 60°C would not be recommended for removing protein-based stains when using this washing powder.

(4 marks) KA2

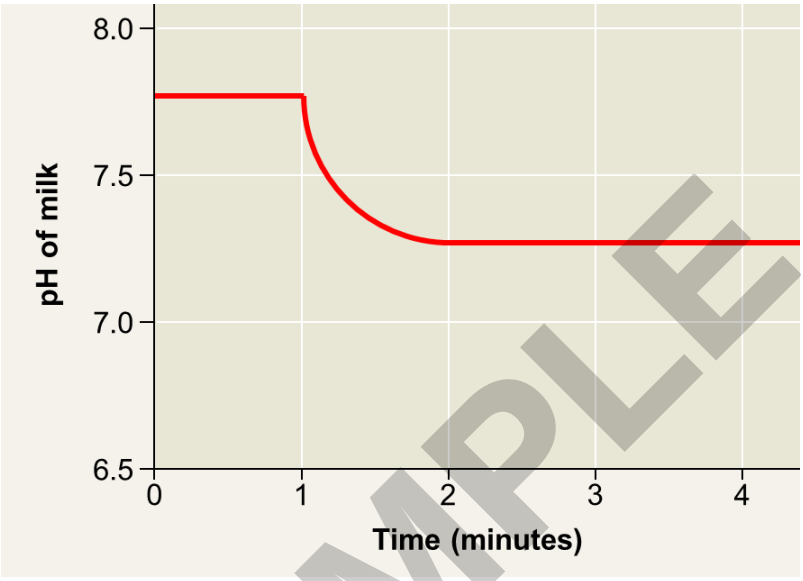
Question 38

Lipase is an enzyme that converts triglycerides in milk to fatty acids.



An experiment was conducted to investigate the conversion of triglycerides by lipase at 20°C.

The pH of a milk sample was measured with a pH meter before and after adding lipase.



(a) Suggest one advantage of using a pH meter rather than a pH indicator in the experiment.

(1 mark) IAE1

(b) State why pH decreases in the lipase-controlled reaction.

(1 mark) IAE1

(c) Suggest why the pH remained constant after two minutes.

(2 marks) IAE1

(d) The experiment was repeated at 10°C.

Draw a line on the graph to show the expected result.

(2 marks) IAE2

Question 39

Amylase is an enzyme in living things that converts starch to glucose.

An experiment was conducted to determine the effect of increasing temperature on the ability of amylase to catalyse the breakdown of starch, called amylase activity.

5 mL of 1% amylase solution and 5 mL of 5% starch solution were transferred to six test tubes.

The test tubes were transferred to baths containing water at different temperatures, and the amylase activity was recorded after five minutes.

(a) State one other variable held constant in the experiment.

_____ (1 mark) **IAE1**

(b) The results are shown in the table below.

Temperature (°C)	Amylase activity (a.u)
10	0.3
20	0.9
30	1.4
35	1.6
40	1.8
60	0.4

(1) Explain the change in amylase activity between 15°C and 35°C.

_____

_____

_____

_____ (2 marks) **IAE3**

(2) Explain why amylase activity decreased between 40°C and 60°C.

_____

_____

_____

_____ (2 marks) **IAE3**

(3) State why the optimum temperature cannot be accurately identified from these results and suggest how the investigation could be improved to measure this value accurately.

_____

_____

_____

_____ (2 marks) **IAE4**

1.7: Regulation of Gene Expression

The phenotypic expression of genes depends on factors controlling transcription and translation. These include the products of other genes and the environment.

Cellular differentiation associated with tissue growth and development is controlled by gene expression.

- Recognise that cytosine nucleotides in DNA can be methylated, altering gene expression.

Epigenetic changes can lead to phenotypic differences between identical siblings, phenotypic differences between clones and may cause human diseases.

- Explain how epigenetic modifications in genes that control cell division, such as changes in DNA methylation, can lead to cancer.

All living things, from single-celled bacteria to multicellular animals and plants, have inherited structural features and behaviours from their parents. These inherited traits, called **phenotypes**, are determined by the organism's **genotype**, its complete set of genes with information in the form of specific DNA nucleotide sequences. The genes inherited by an organism lead to specific phenotypes by dictating the synthesis of proteins and RNA molecules involved in protein synthesis. For example, the gene TYR contains the information to synthesise tyrosinase, an enzyme that produces the pigments associated with coat and skin colour in animals. A healthy copy of TYR results in the standard coat or skin colouring, while a faulty copy can result in albinism, a phenotype characterised by a lack of coat or skin colouring (Figure 1.43). The specific phenotypes of a cell or organism result from **controlled gene expression**, the process by which some genes are activated and expressed while others are deactivated and silenced. This chapter explores some factors affecting the phenotypic expression of genes.

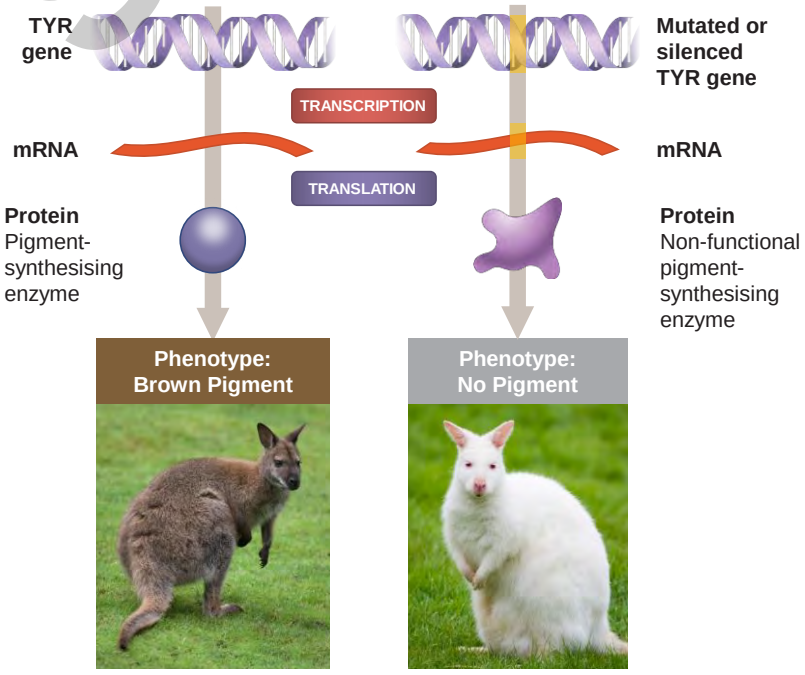
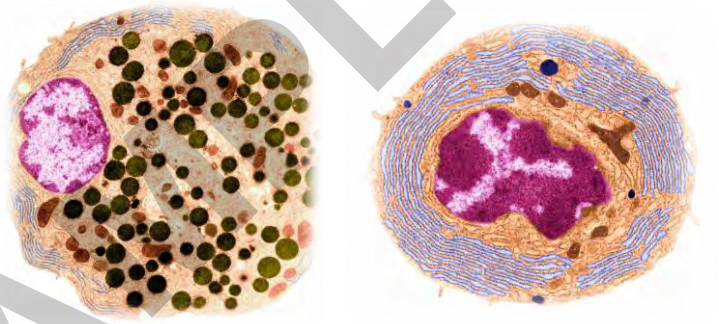


Figure 1.43: Link between genotype and phenotype in albinism.

Cellular Differentiation

Multicellular eukaryotes, including animals and plants, are composed of thousands of genetically identical cells. However, despite having identical genes, the cells in a multicellular eukaryote have vastly different structures and functions. This variation in cell structure and function results from **differential gene expression**, the expression of different genes by cells with the same genome. The unique gene expression pattern allows cells to synthesise some proteins and not others, resulting in a unique set of proteins that carry out specific functions not seen in other cell types. To perform its distinct role, each cell type must maintain a specific gene expression pattern in which certain genes are expressed, and others are not. For example, Figure 1.44 shows two human cell types containing the same genes. Some genes, such as those coding ribosomal proteins, DNA-repair proteins and cell cycle proteins, are expressed in both cells. In contrast, some genes, such as the insulin gene, are only expressed in pancreatic beta cells, whose primary function is synthesising and secreting insulin to regulate blood glucose. Similarly, genes coding antibody proteins are only expressed in B cells, whose primary function is synthesising and secreting antibodies to fight infection.



	Pancreatic beta cell	B cell
***RPL10A* gene (codes a ribosomal protein)**	✓	✓
TP53 gene (codes a DNA-repair protein)	✓	✓
***CCNE1* gene (codes a cell cycle protein)**	✓	✓
***INS* gene (codes insulin protein)**	✓	✗
***IGHA1* gene (codes an antibody protein)**	✗	✓

Figure 1.44: Differential gene expression in two human cell types.

The unique gene expression pattern in eukaryotic cells results from the expression of different sets of proteins called **transcription factors**. For a gene to be activated and its protein expressed, transcription factors must bind to the gene and facilitate the attachment of RNA polymerase. In their absence, RNA polymerase cannot bind, and the gene is not transcribed and is effectively **silenced**. In multicellular organisms, specific transcription factors activate some genes and silence others, resulting in differentiated cell types with specialised structures and functions.

Regulation of Gene Expression

All cells that conserve resources and energy have an advantage over cells that cannot. For this reason, cells regulate the genes they express at any given time, so they do not waste energy and resources such as nucleotides and amino acids by synthesising RNA and protein molecules when they are not required. For example, an individual *Escherichia coli* cell living in a large intestine depends on its animal host for nutrients. If the environment lacks tyrosine, an amino acid the bacterium needs to survive, the cell activates a metabolic pathway that makes tyrosine from another compound called prephenate. When the animal host later eats a tyrosine in a meal, the bacterial cell stops producing tyrosine, avoiding wasting resources to produce a substance readily available from the host. In this case, the bacterial cell can adjust the production level of certain enzymes in the metabolic pathway by regulating the expression of its genes. In this case, the transcription of genes coding the enzymes that synthesise tyrosine are switched off, inhibiting the transcription of mRNA that ribosomes would translate into enzymes facilitating tyrosine synthesis. Regulation of the tyrosine synthesis pathway is just one example of how cells switch genes on and off to tune their metabolism to changing environments. Both unicellular organisms and the cells of multicellular organisms continually turn genes on and off in response to signals from their external and internal environments. For example, a typical human cell might express about a third to a half of its protein-coding genes at any given time, while differentiated cells, such as muscle or nerve cells, express a smaller fraction. Figure 1.45 summarises the process of gene expression in a eukaryotic cell, highlighting critical stages in the expression of a protein-coding gene. Each stage is a potential control point at which gene expression can be turned on or off, accelerated, or slowed down. In prokaryotes, gene expression is commonly controlled at transcription and often occurs in response to signals from outside the cell, such as hormones or other signalling molecules. In eukaryotes, gene expression is also controlled by modifying DNA and chromatin and degrading mRNA and proteins (Figure 1.45).

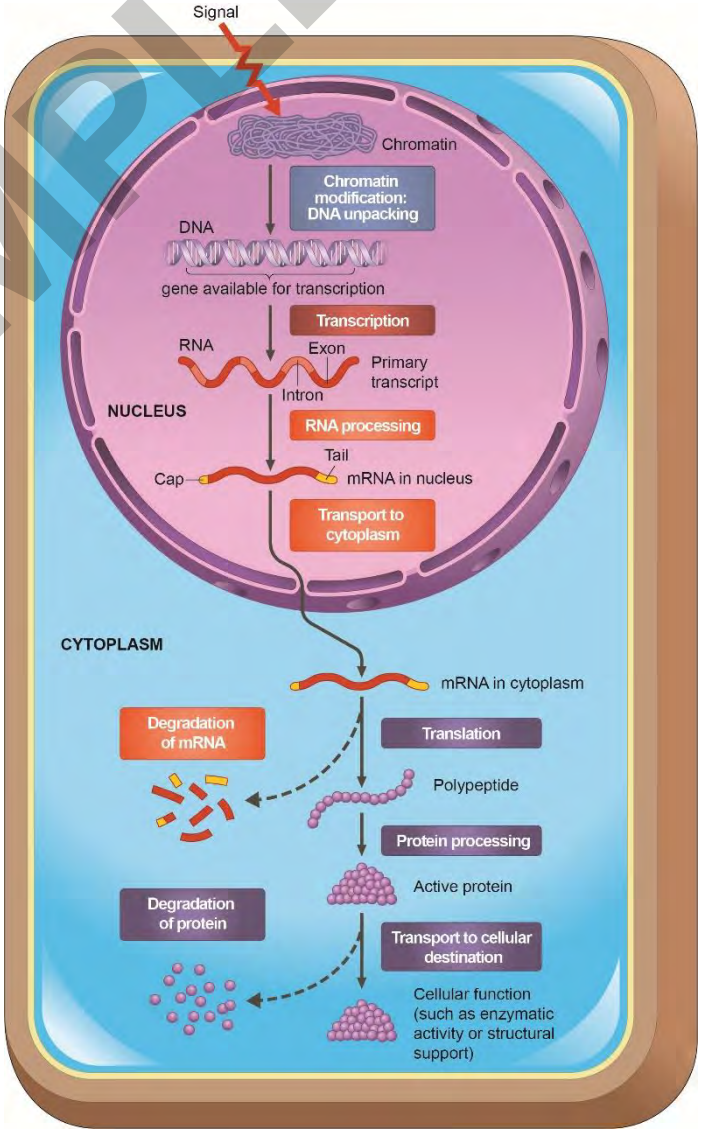


Figure 1.45: Gene expression in a eukaryotic cell

Mutagens

Mutations can arise in many ways. The most common is during DNA replication when errors pairing nucleotides lead to substitutions, insertions, or deletions. Mutations in DNA replication are passed on to daughter cells and occur at a rate of one nucleotide in every ten billion. This natural mutation rate is increased by exposure to **mutagens**, physical and chemical agents that interact with DNA in ways that cause mutations. For example, ultraviolet (UV) radiation induces disruptive thymine dimers that cause frameshift mutations further along the DNA molecule (Figure 1.54). Ionising radiation, including X-rays, gamma-rays, neutrons, protons, electrons and alpha particles, are mutagens with direct and indirect effects on DNA. For example, ionising radiation induces deletion mutations directly by causing single or double-stranded breaks in the sugar-phosphate backbone of DNA (Figure 1.54). In addition, ionising radiation induces mutations indirectly by initiating chemical reactions in cells with mutagenic products that damage DNA.

Chemical mutagens mutate DNA in different ways. The first type has chemical structures similar to DNA nucleotides and initiates mutations by pairing incorrectly during DNA replication. The second type induces mutations by reacting with nucleotides and altering the chemical structures of bases in a way that makes them pair incorrectly (Figure 1.54). The third type inserts itself into the double helix and distorts its shape, increasing the likelihood of mutation during DNA replication. The mutation rate is also increased by **viruses** that insert DNA into the host genome during infection.

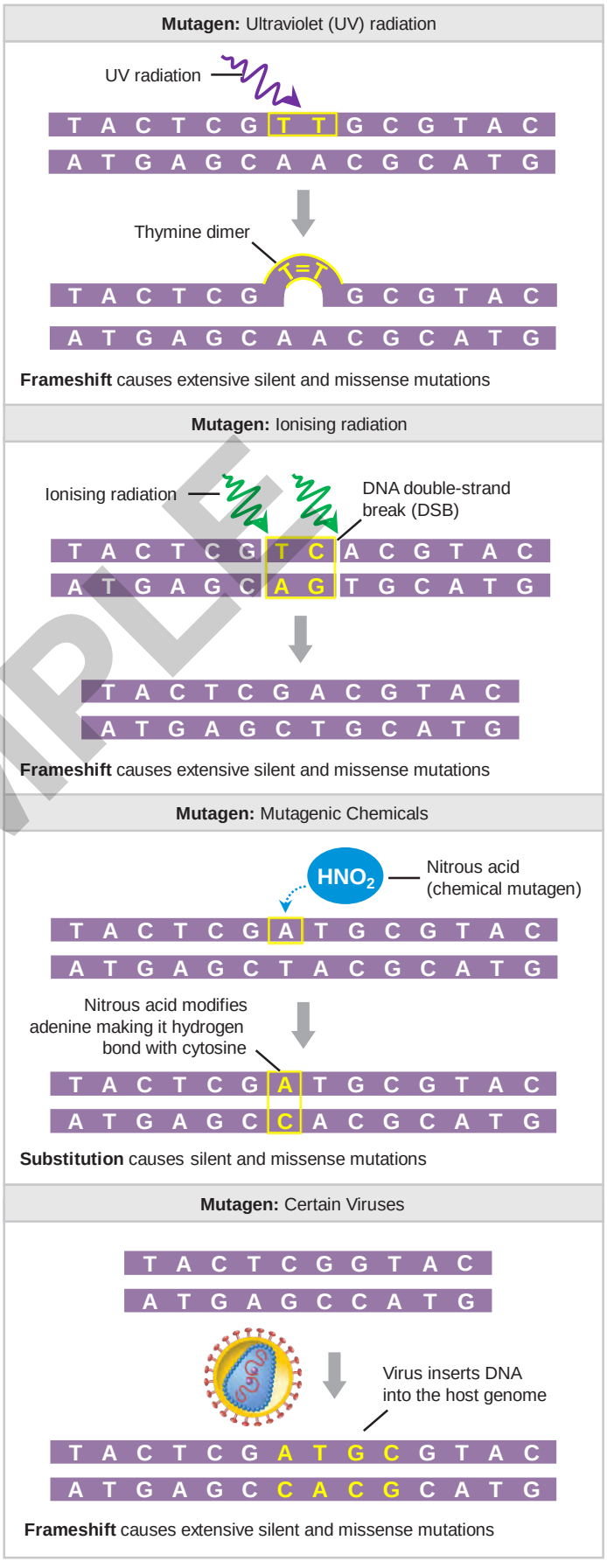


Figure 1.54: Effect of mutagens on DNA.

Mutations and Cancer

Cancer is a variety of diseases in which changes in gene expression cause cells to escape the control mechanisms that usually limit their growth and division. Most cancers are caused by mutations that modify the expression of proto-oncogenes that stimulate cell division and tumour suppressor genes that inhibit cell division. For example, mutations transform proto-oncogenes into **oncogenes**, cancer-causing genes encoding proteins that over-stimulate cell division (Figure 1.56). In other cases, a proto-oncogene is replicated many times, and its expression leads to an excess of proteins that stimulate cell division (Figure 1.56). In addition, certain viruses, including Epstein-Barr virus, Hepatitises B and C and Human papillomaviruses, can insert oncogenes into the host genome, initiating the onset of cancer. In contrast, mutations that decrease the activity of a tumour-suppressor protein may stimulate cell division and contribute to the onset of cancer (Figure 1.56).

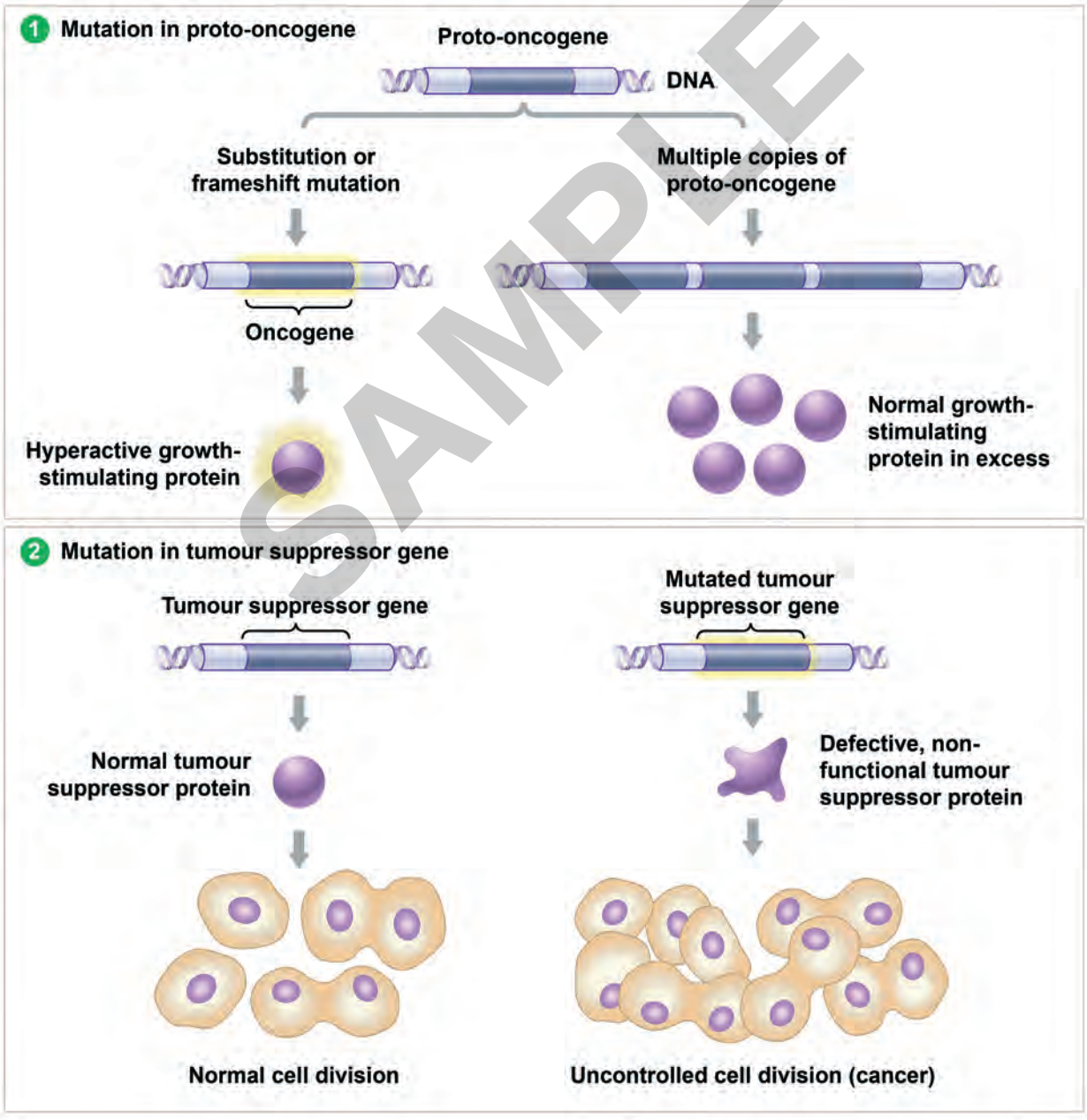
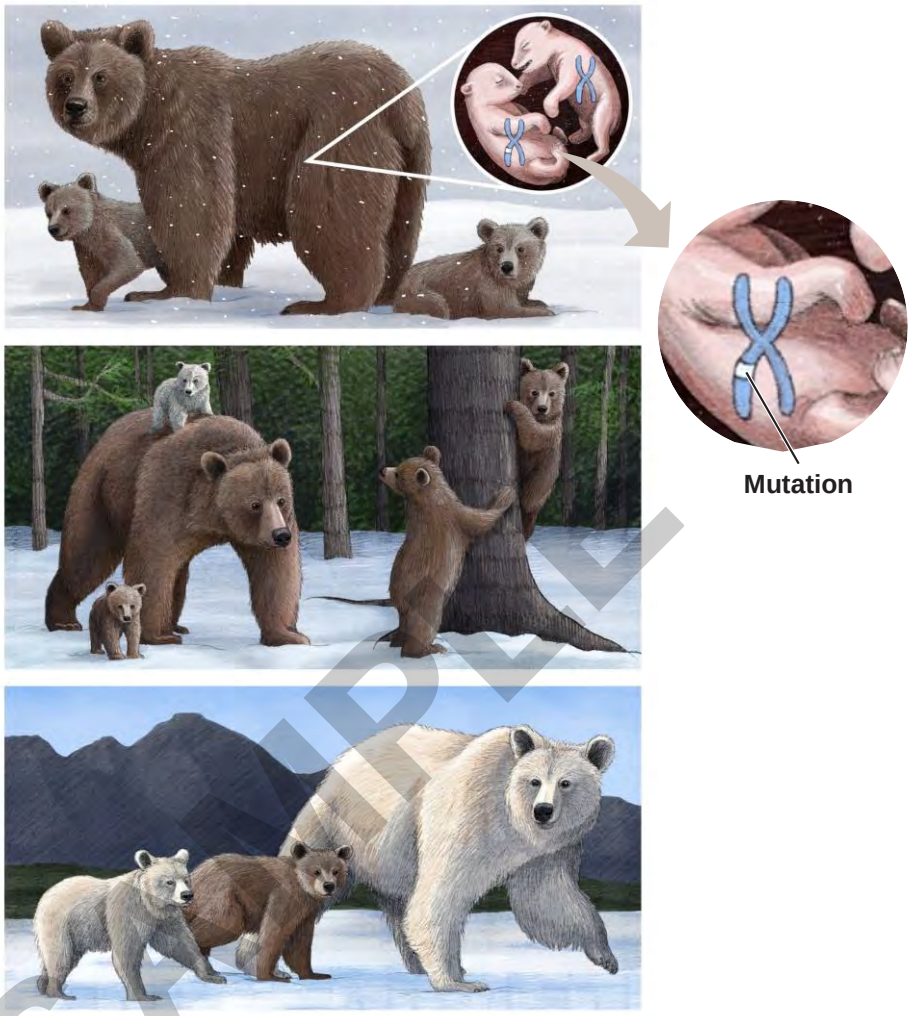


Figure 1.56: Mutations to proto-oncogenes and tumour suppressor genes.

Question 58

The diagram below describes how a mutation may lead to a new phenotype in brown bears.



(a) The white fur phenotype resulted from a mutation in a cell type in the mother.

State whether the mutation occurred in the mother's somatic or germ cell, giving evidence from the diagram to support your answer.

(2 marks) KA2

(b) State and explain whether the mutation is inheritable, giving evidence from the diagram to support your answer.

(2 marks) KA2

1.10: Electrophoresis and DNA Sequencing

The base sequence of DNA can be determined by electrophoresis.

- Describe electrophoresis.

The results of electrophoresis may be displayed in an electropherogram.

- Interpret electropherograms that illustrate DNA sequences.

DNA sequencing enables the mapping of species' genomes.

The discovery of the structure of DNA led to the development of **DNA technology**, a set of techniques used in biological research to copy, analyse, sequence and modify DNA. One of these techniques, the polymerase chain reaction, amplifies DNA to produce copies of a target sequence for analysis. A second technique, called **electrophoresis**, uses an electric field to separate DNA fragments of different lengths for analysis. The phosphate groups of DNA molecules have a negative charge, a property that causes them to accelerate in an electric field. In **gel electrophoresis**, the DNA fragments to be separated are transferred to a polymer gel (Figure 1.59) with microscopic holes of different sizes.

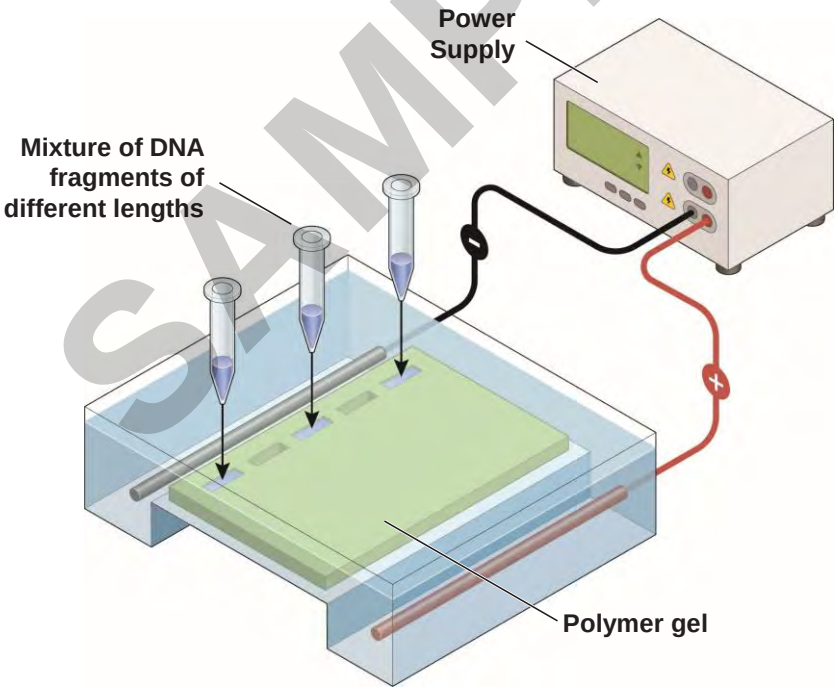


Figure 1.59: Gel electrophoresis apparatus.

The gel is less than 10 mm deep and contains 1 mm-deep-wells at one end. The DNA samples containing one or more fragments for analysis are loaded into separate wells with a micropipette, and the gel is submerged into an aqueous buffer solution. At each end of the gel is an electrode connected to either the positive or negative terminal of a power supply. When the power supply is switched on, an electric field is produced between the electrodes, forcing DNA molecules to move through the gel toward the positive electrode. Shorter fragments move faster through the gel than larger ones, causing the separation of fragments in the samples, as shown in Figure 1.60.

1.11: DNA Profiling

The results of electrophoresis can be used to construct DNA profiles. They may be displayed in an electropherogram or in a table of data.

DNA profiling identifies the unique genetic makeup of individuals.

- Interpret electropherograms and tables of data that illustrate DNA profiles.
- Explain how differences in DNA fragments identified by DNA profiling can be used in forensic science.
- Discuss the ethical, economic, and cultural issues related to the collection of genetic information.

The development of electrophoresis and DNA sequencing techniques during the 1970s and 80s led to the Human Genome Project (HGP), an international scientific research project to identify, map and sequence the human genome. The project was launched in 1990 and officially concluded in 2022 after the complete genome had been sequenced. Most of the sequencing was performed by members of the International Human Genome Sequencing Consortium (IHGSC), an international collaboration involving twenty universities and research facilities in the United States of America, the United Kingdom, Japan, China, Germany and France. The results of the HGP reveal the human genome contains nearly three billion base pairs encoding between 20,000 and 25,000 genes. Strangely, less than 1.5% of human DNA codes proteins, tRNAs or rRNAs, and the remaining 98.5% is **non-coding DNA**. The study of non-coding DNA has revealed the presence of **genetic markers**, nucleotide sequences that vary between individuals in the population and can be analysed to determine a person's unique **DNA profile**.

DNA Profiling

In violent crimes, the assailant or assailants may leave tissue samples at the scene or on the clothes or other possessions of the victim. Suppose enough blood, hair, semen, or skin is available. In that case, forensic laboratories can extract the DNA and analyse the non-coding regions of a person's DNA for genetic markers that vary between individuals in the human population. The most common genetic markers analysed by forensic scientists are **short tandem repeats (STRs),** repeated nucleotide sequences located in specific regions of the genome. STRs may be a short sequence repeated many times or tandemly repeated units of two-to-five different nucleotide sequences (Figure 1.71). To date, biologists have identified more than 20 STRs in non-coding regions of the human genome.

STR Locus	Chromosome number	Repeat Sequence
TPOX	2	AATG
D2S1338	2	$[TGCC]_n[TTCC]_n$
D3S1358	3	[AGAT], [TCTA]
CSF1PO	5	AGAT
TH01	11	AATG
vWA	12	AGAT
D16S539	16	GATA
Penta D	21	AAAGA
D21S11	21	[TCTA], [TCTG]

Figure 1.71: Some human STRs

The number of repeats present at an STR locus varies from person to person and may vary within an individual. Each person has two copies, or **alleles**, of each STR locus, one on the maternal copy of the chromosome inherited from their mother and the other on the paternal copy inherited from their father. Consequently, a person may have the same or a different number of repeats at each STR locus on their maternal and paternal chromosomes. For example, person A in Figure 1.72 has the sequence AATG repeated 6 times at the THO1 STR locus on the maternal copy of chromosome 11 and 9 times on the paternal copy. In contrast, person B has 12 repeats on the maternal and paternal copies of the chromosome. These two genotypes are expressed by the **allele values**: 6,9, and 12,12, where the number of repeats on the maternal chromosome is stated first in the sequence. People have two numbers in their genotype for an STR locus when the number of repeats differs between their maternal and paternal alleles and one number when they have the same number on both.

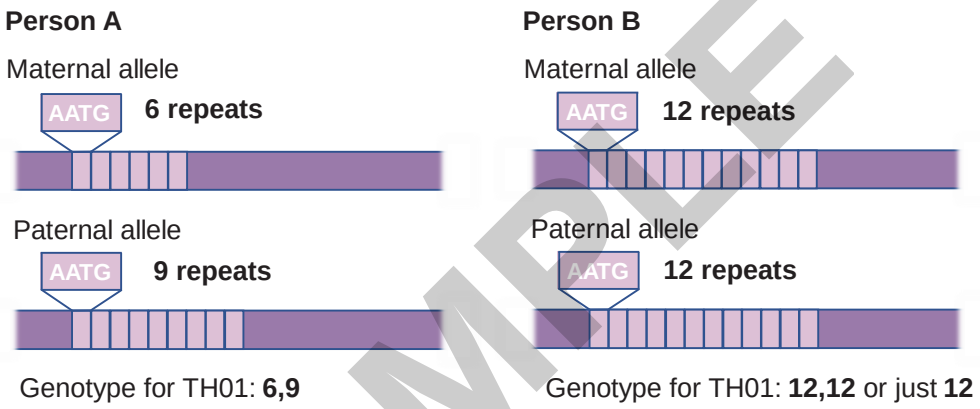


Figure 1.72: Comparing the number of repeats in TH01 in persons A and B.

Scientists produce a person's DNA profile by determining the number of repeats on the maternal and paternal alleles at some or all STR loci. In some cases, a DNA profile is displayed as a data table showing the number of repeats at various STR loci. For example, Figure 1.73 shows the DNA profiles of two people constructed using DNA from seven STR loci. The data shows significant differences in the number of repeats at each locus, a property distinguishing the two people.

Person A

STR Locus	Genotype
FGA	20,26
TH01	6,9
vWA	12,20
D3S1358	14
D8S1179	10,12
D18S51	16
D21S11	24,27

Person B

STR Locus	Genotype
FGA	18,21
TH01	12
vWA	20,21
D3S1358	13,16
D8S1179	9,11
D18S51	14,17
D21S11	25

Figure 1.73: DNA profiles of persons A and B in tables.

Using DNA Profiles

Scientists use DNA profiles to compare the genotypes of two or more people for applications, including forensic testing in criminal cases, paternity testing, and evolutionary research. In a criminal case, for example, forensic scientists produce DNA profiles of the suspect, victim, and tissue samples found at the crime scene to determine guilt or innocence of a violent crime. The forensic scientist extracts DNA from the tissue sample at the crime scene and amplifies selected STR loci using PCR. The PCR step enables the production of a suitable DNA profile even if the DNA is in poor condition or in tiny quantities. The DNA profiles from the crime scene and suspects are then compared to determine guilt or innocence. Figure 1.76 shows an electropherogram with data from three STR loci. The data shows the DNA from the crime scene matches Suspect B as the number of repeats on the paternal and maternal alleles are identical at all three STR loci.

Figure 1.76: Comparing DNA profiles in a criminal case.

Standard sets of STR loci are required to enter DNA genotype data into national or international databases to link serial offenders of violent crimes. For example, the United States of America currently requires 13 specific STRs to be analysed, while the United Kingdom requires 11. Genotype data from these STR loci provides a forensically valid DNA profile because the probability that two people who are not identical twins would have exactly the same genotype at each STR locus is incredibly low. For example, in forensic cases using 13 STR loci, the probability of two people having identical DNA profiles is between one in 10 billion and one in several trillion. Hence, despite problems arising from human error, flawed evidence or insufficient data, DNA profiles are accepted as evidence in criminal cases as they provide a high degree of discrimination between individuals.

DNA profiles are helpful for other purposes. For example, comparing the DNA of a child, their mother, and the purported father can conclusively settle a question of paternity. This is because a child's genotype at a given STR locus is a combination of their mother's and father's as they inherit one copy of each chromosome from their mother and the other from the father. Hence, paternity is determined by producing and comparing the DNA profiles of the child and the suspected parents. For example, Figure 1.77 shows the DNA profiles of a child, its mother and two suspected fathers. The evidence suggests Suspected Father 2 is the child's father as one of the child's two alleles is present in their DNA profile at each STR locus, with the other present in the mother's.

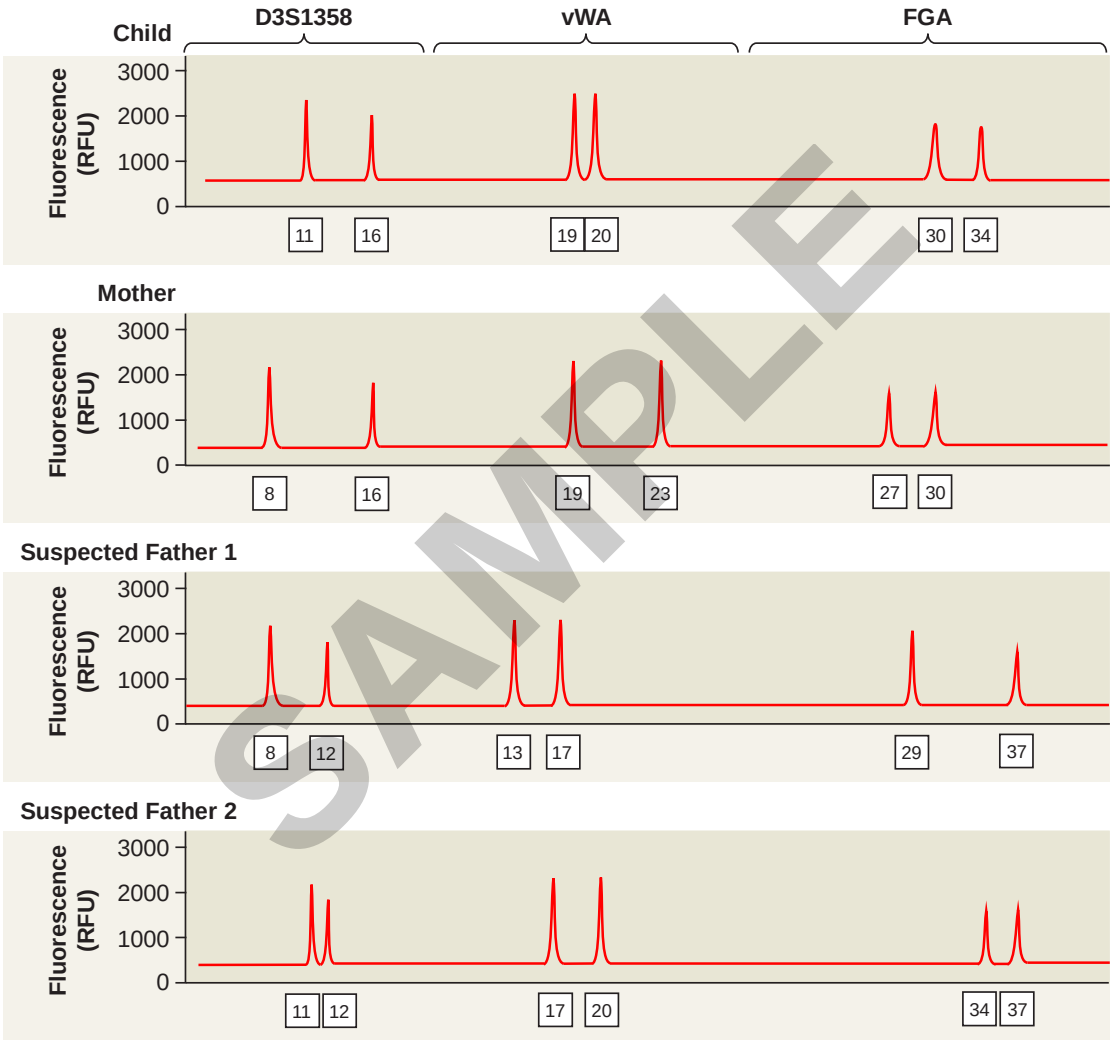


Figure 1.77: Comparing DNA profiles in a paternity case.

DNA profiles also identify victims of casualties, including mass causalities such as natural disasters and terrorist attacks. The largest such effort occurred after the attack on the World Trade Centre in September 2001, where thousands of samples of victims' remains were compared with DNA from personal items, such as toothbrushes provided by families. Ultimately, forensic scientists succeeded in identifying nearly 3,000 victims using DNA profiling. Similar techniques were used when identifying victims and survivors following the South East Asia Tsunami in 2004 and the Brazilian floods and mudslides in 2011.

Single Nucleotide Polymorphisms (SNPs)

Among the most valuable genetic markers used in the preparation of DNA profiles are single base-pair variations in the genomes of the human population. The human genome is approximately 3 billion base-pairs, and 99% of these nucleotides have the same position in the genomes of all humans. However, once in every 100-300 base-pairs of both coding and non-coding DNA sequences are **single nucleotide polymorphism (SNP)**, positions where the nucleotide sequence varies between individuals in at least 1% of the population. Every individual has a unique SNP pattern that scientists use as genetic markers to build DNA profiles. For example, Figure 1.78 shows electropherograms for DNA profiles of three modern human populations using seven SNPs. The data shows persons A and C are the most closely-related individuals as they share more SNPs.

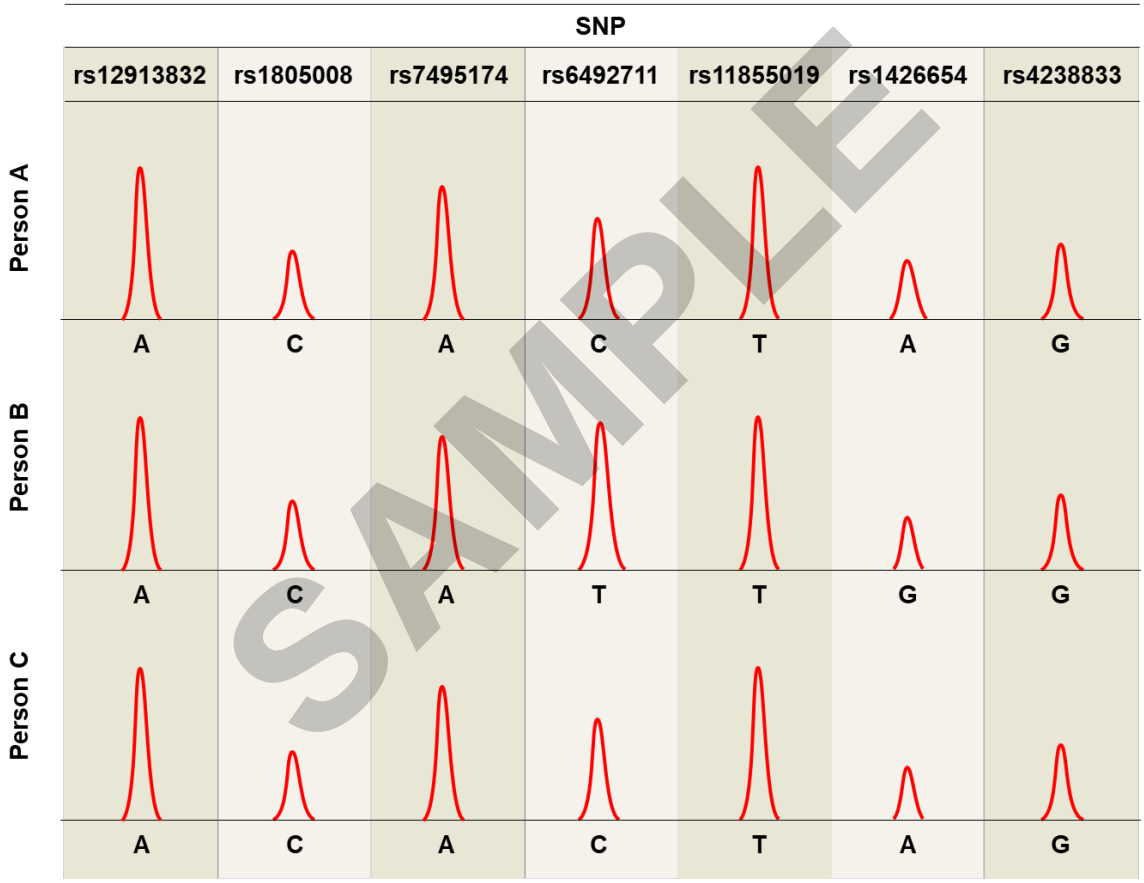


Figure 1.78: DNA profiles produced using seven SNP loci.

Since SNPs occur at a much higher frequency in the human genome than STRs, researchers can build a more comprehensive DNA profile using between 4 and 5 million SNPs, dramatically improving the accuracy and validity of conclusions from genetic testing. Furthermore, SNPs serve as genetic markers of disease-causing alleles, a property that allows scientists to determine the probability of a person developing a particular genetic disease. SNPs are currently used by scientists studying human evolution and genetic disease, by for-profit DNA testing companies offering customers insight into their family history and genetic health, and by pharmaceutical companies producing drugs and vaccines that provide therapeutic benefits to people with genetic diseases.

Question 66

In 1984, Earl Washington was convicted for the assault and murder of Rebecca Williams.

In 2000, forensic scientists working in collaboration with the Innocence Project compared Washington's DNA profile with one of the DNA evidence at the crime scene.

Source	STR locus 1	STR locus 2	STR locus 3
Crime scene DNA	17,19	13,16	12,12
Earl Washington	16,18	14,15	11,12
Kenneth Tinsley			

(a) Describe and explain how the DNA profiles above were produced.

(2 marks) **KA2**

(b) Earl Washington was released from prison in 2001 based on the evidence in the table.

(1) State the evidence that Earl Washington was innocent of this crime.

(1 mark) **KA2**

(2) Another man, Kenneth Tinsley, was found guilty of the murder using DNA evidence.

Complete the table above by writing Tinsley's genotype into each box.

(1 mark) **KA1**

(c) The Innocence Project is an organisation that uses DNA testing of archived samples from crime scenes to revisit old cases and overturn wrongful convictions,

As of 2022, their forensic and legal work has overturned 375 wrongful convictions.

Give one example of how the work done by the Innocence Project demonstrates the interaction between science and society.

(1 mark) **KA3**

Question 68

The table below shows DNA profiles for samples taken from a crime scene, victim and two suspects.

STR locus	Crime scene	Victim	Suspect A	Suspect B
D3S1358	15,17,19	15,19	15,17	16,17
TH01	9,11,12	9,11	9,12	7,9
D21S11	29,30,31,32.2	31,32.2	29,30	28,32
D18S51	12,16,18	12,16	12,18	10,12
D5S818	11,12	11,12	11,11	10,13
D13S317	11,13	11,11	11,13	9,11
vWA	15,17,18	17,18	15,17	13,16.1
FGA	21,23	21,23	23,23	20,21

(a) State what the two numbers represent in the DNA profiles of the victim and suspects.

(1 mark) **KA2**

(b) Identify the suspect whose DNA was found at the crime scene using evidence from the table.

(2 marks) **KA2**

(c) Eight STR loci were analysed in this crime scene investigation.

Explain the benefit of analysing many STR loci in crime scene investigations.

(2 marks) **KA2**

(d) Police often retain people's DNA profiles after they have been used in investigations.

Suggest one reason people may object to having their DNA retained by police.

(1 mark) **KA2**

1.12: Biotechnology

Biotechnology can involve the use of plasmids and viruses as vectors, bacterial enzymes, and yeasts. Techniques include bacterial transformations, electroporation, and microinjection.

- Describe how particular genes can be selected using probes and removed using restriction enzymes.
- Describe how selected genes can be transferred between species.
- Describe how CRISPR can be used to edit and/or transfer genes.
- Discuss the design of new proteins and their uses.

In the last section of this chapter, we explore the practical applications of DNA-based **biotechnology**, the manipulation of living things or their components to make useful products. Today, significant applications of biotechnology include research, medicine, agriculture, and environmental cleanup.

Genetic Engineering

The structure of DNA and the genetic code is the same in all living things. For this reason, DNA from the cells of one living thing can be transcribed and translated in the cells of another. This property of DNA is the basis of **genetic engineering,** the manipulation of genes for practical purposes. One technique, called **DNA cloning**, isolates a DNA segment carrying a gene of interest from one cell and makes multiple identical copies using a host cell, usually a bacterium. In addition to their large circular chromosome, many bacteria also have **plasmids**; small, circular DNA molecules replicated separately from the chromosome. A plasmid has only a small number of genes the bacterium expresses in a particular environment but is not required for its survival or reproduction under most conditions. Scientists remove the gene of interest from its source and insert it into a plasmid, forming **recombinant DNA,** a molecule containing DNA from two different sources. The recombinant plasmid is returned to a bacterium, producing a **recombinant cell** that reproduces through repeated cell divisions to create a large population of genetically identical cells called **clones**. Because the dividing bacteria replicate the recombinant plasmid and pass it on to their descendants, the foreign DNA and any genes it carries are cloned at the same time. The production of multiple copies of a single gene is a type of DNA cloning called **gene cloning** (Figure 1.81).

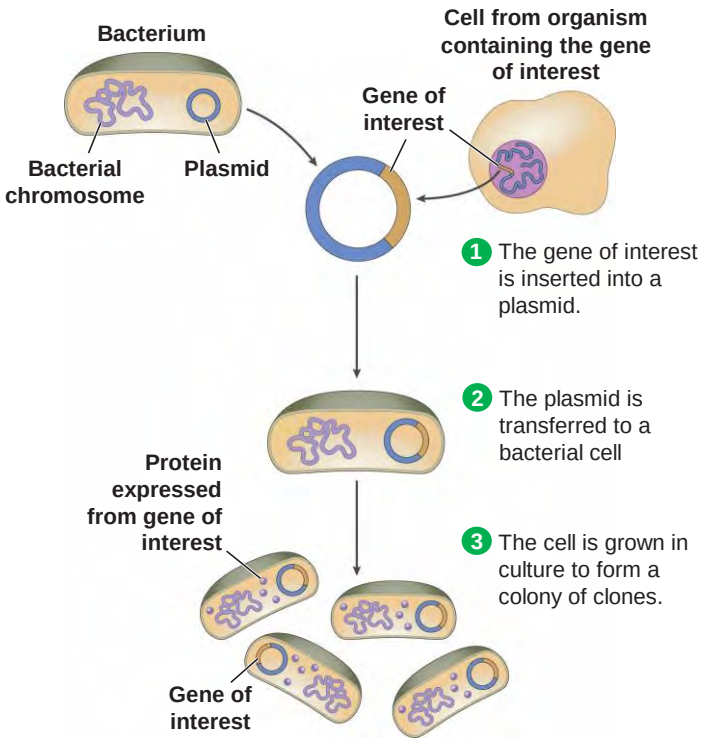


Figure 1.81: Gene cloning

In Figure 1.81, the plasmid acts as a **vector**, a DNA molecule that carries foreign DNA into a host cell, where it is replicated and expressed. Bacterial plasmids are widely used as vectors as they are easily manipulated by inserting foreign DNA and readily introduced into bacterial cells. Gene cloning has two primary functions: to make many copies of a particular gene and express its protein product. Recombinant bacteria, yeast and mammalian cells carrying cloned genes are harvested from cultures to produce large quantities of proteins with a therapeutic or medical use, such as insulin to treat diabetes, taxol to treat certain cancers, and human growth hormone to treat stunted growth (Figure 1.82). Alternatively, researchers can isolate copies of a cloned gene to study gene function or insert them into another living thing to produce a genetically modified organism (GMO) with a desirable phenotype. For example, Figure 1.83 describes how scientists extracted the insect resistance "*cry*" gene from the bacterium *Bacillus thuringiensis* and transferred it to different plant crops. The resulting GMOs synthesise proteins that deter insects, protecting themselves from tissue damage.

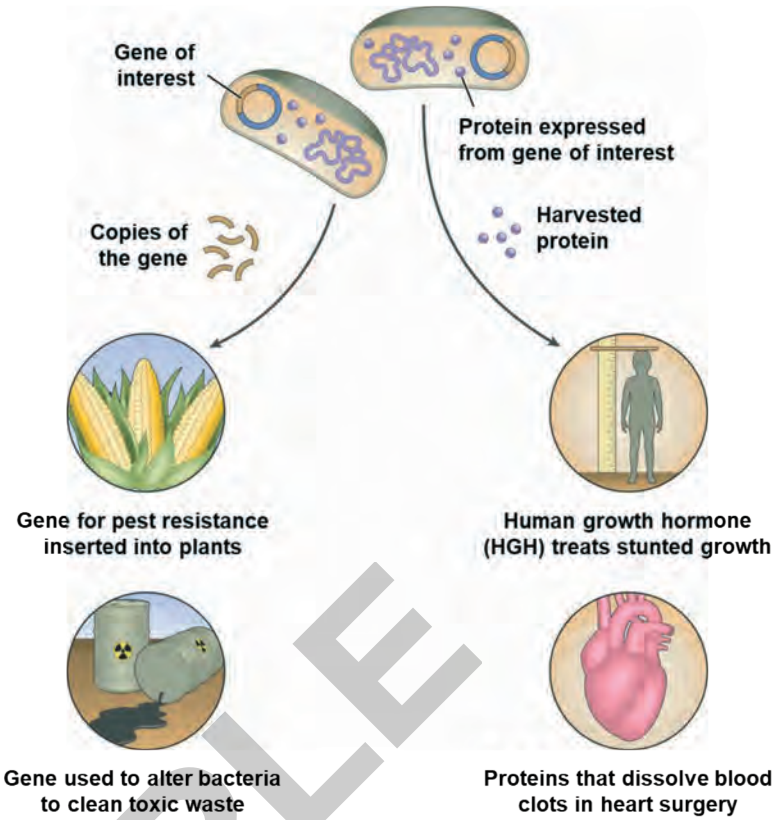


Figure 1.82: Some uses of gene cloning

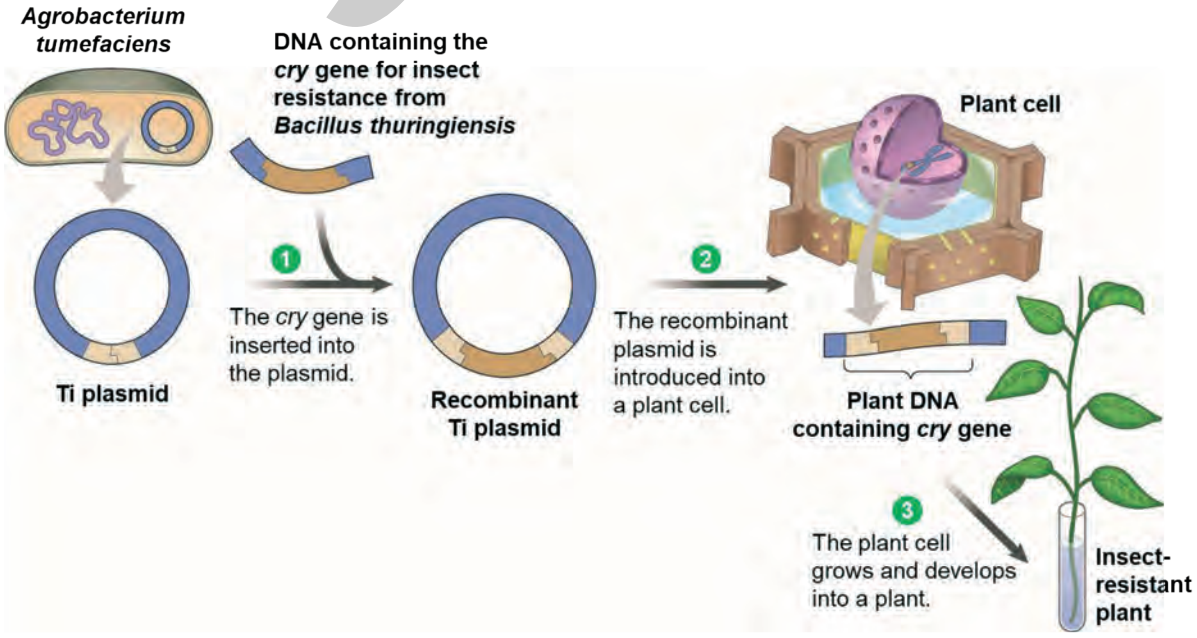


Figure 1.83: Transfer of insect resistance genes from bacteria to plants.

Restriction Enzymes

Gene cloning uses **restriction enzymes,** bacterial enzymes that cut DNA molecules at a limited number of specific locations. Hundreds of restriction enzymes have been identified and isolated, each recognising a particular short DNA sequence, called a **restriction site**, and cutting both DNA strands at precise points within this site. Figure 1.84 shows how restriction enzymes are used to clone a foreign DNA fragment into a bacterial plasmid. At the top of the figure is a bacterial plasmid with a single restriction site recognised by a particular restriction enzyme. As shown in this example, most restriction sites are symmetrical, as the nucleotide sequence is the same on both strands when read in the opposite direction. The most commonly used restriction enzymes recognise sequences containing four to eight base-pairs. Because these short sequences usually occur by chance many times in a long DNA molecule, a restriction enzyme will make many cuts, yielding a set of **restriction fragments** of different lengths. Since restriction enzymes always cut at the same DNA sequence, copies of any given DNA molecule exposed to the same restriction enzyme always yield the same set of restriction fragments. The most useful restriction enzymes cut the sugar-phosphate backbones of the two strands in a staggered manner, as shown in Figure 1.82 1. The resulting double-stranded restriction fragments have at least one single-stranded end, called a **sticky end**. These short extensions can hydrogen-bond with complementary bases on sticky ends on other DNA molecules cut with the same restriction enzyme, such as the inserted DNA fragment shown in Figure 1.82 2. The temporary associations formed in this way are made permanent by DNA ligase, an enzyme that closes the sugar-phosphate backbones of DNA strands, as in Figure 1.82 3. The end product is a stable recombinant plasmid containing the gene of interest.

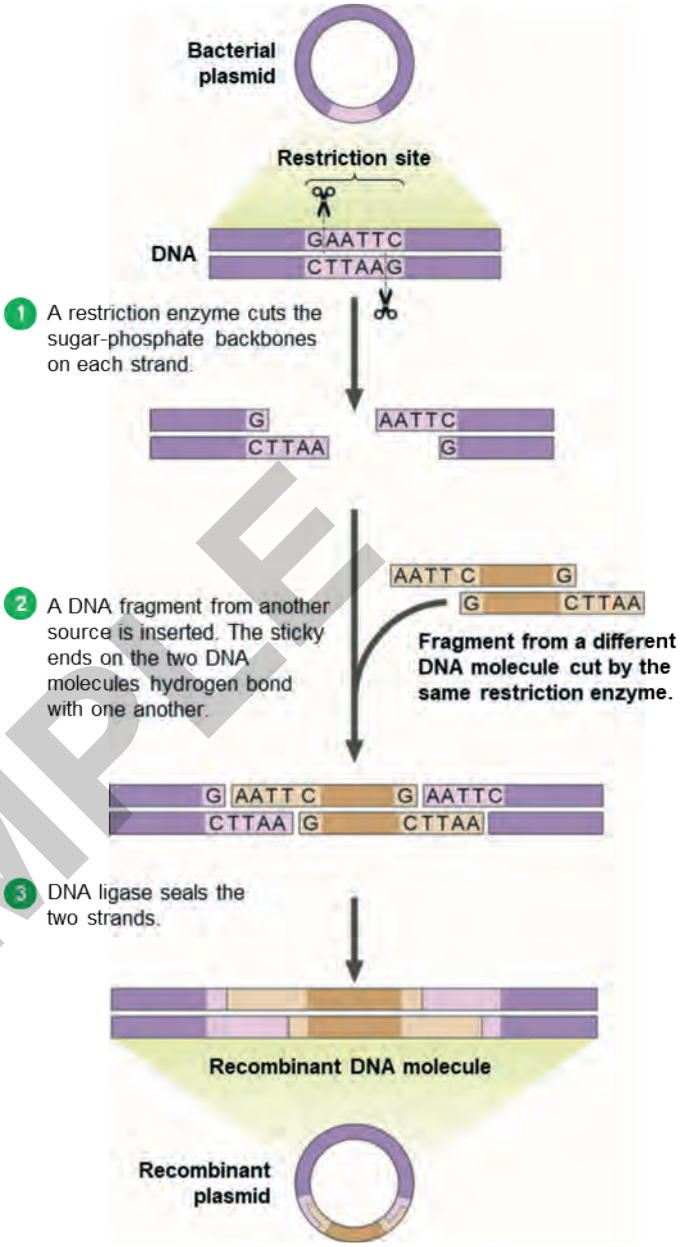


Figure 1.84: Restriction enzymes in DNA cloning.

Probes

The gene cloning process involves isolating the gene of interest from its host's genome and inserting it into a plasmid. In many cases, the gene is isolated by firstly breaking down the hosts' cells and separating one or more DNA molecules into fragments with specific restriction enzymes. Next, the fragments are separated by gel electrophoresis into thousands of bands, some of which contain the gene of interest. The DNA is then denatured, transferred to a filter paper and soaked with fluorescently or radioactively-labelled **probes**, small, single-stranded DNA or RNA molecules with a nucleotide sequence complementary to part of the gene of interest. When a solution containing probes is washed over the filter paper, it binds to the gene of interest and emits visible light or ionising radiation (Figure 1.85), enabling its detection. Once isolated, the gene of interest is typically amplified with PCR before being mixed with plasmids to form recombinant DNA. Finally, the recombinant plasmids produced during gene cloning are transferred to and expressed in bacterial cells or the nucleus of a recombinant eukaryotic cell such as yeast, plants or mammalian cells. DNA molecules are too large to diffuse across the cell membrane, so biologists use different techniques to transport recombinant plasmids into the cytoplasm or nucleus of a recombinant cell. One technique, **electroporation,** uses a brief electrical pulse to create temporary pores in the cell membrane through which plasmids can enter. A second technique, called **microinjection**, uses a microscopically-thin glass needle to inject recombinant plasmids into the cytoplasm or nucleus of a recombinant cell. A third technique, called **transformation**, involves bacteria's uptake of foreign DNA. In one transformation process, called **conjugation**, recombinant bacteria in a population share their recombinant plasmids with neighbouring cells, increasing the proportion of recombinant cells in a population (Figure 1.86).

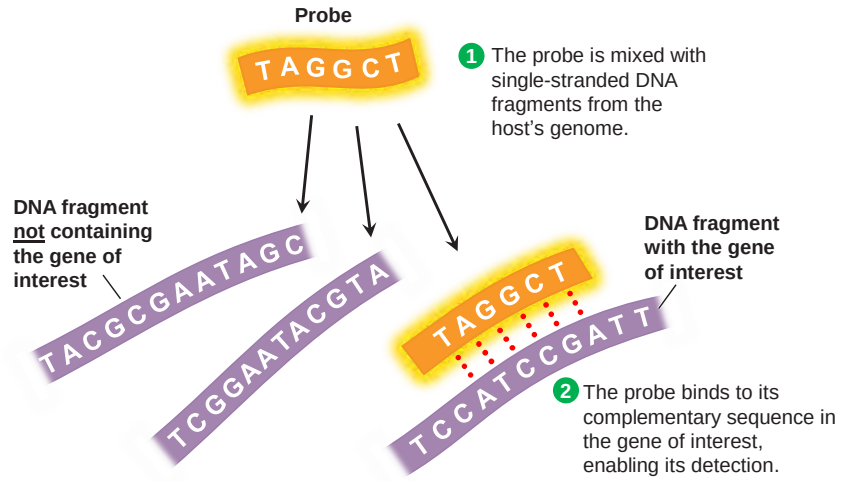


Figure 1.85: Probe binding to gene of interest

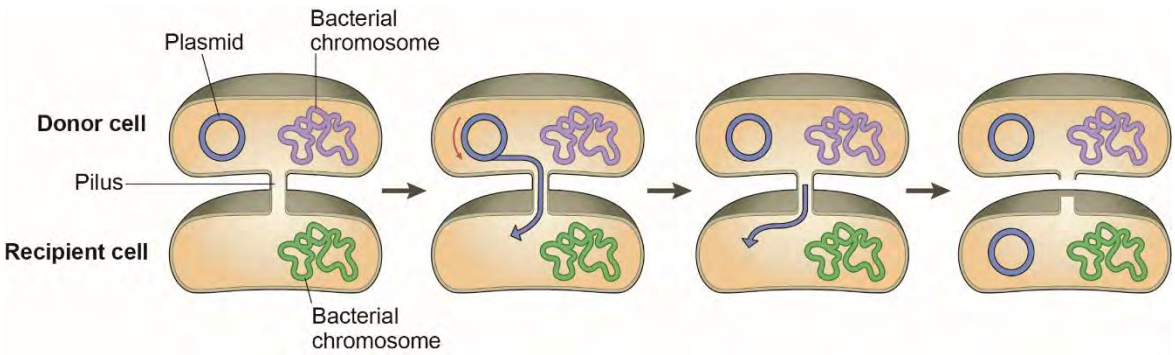


Figure 1.86: Bacterial transformation shares a recombinant plasmid.

CRISPR

Since biologists have understood gene mutation's role in the development of certain diseases, they have sought methods of **gene editing**, altering genes in a specific, predictable way. Over the past 20 years, biologists have developed a powerful new technique for gene editing called the **CRISPR-Cas9 system**. Cas9 is an enzyme that defends bacteria against infection by bacteriophage. During infection, Cas9 binds a **guide RNA (gRNA)** coded by the CRISPR region of the genome and uses it to locate phage DNA before cutting both strands of any complementary DNA sequence. Scientists have exploited the function of Cas9 by introducing a Cas9-guide RNA complex into cells to disable a gene or repair a mutation (Figure 1.90). In either case, the guide RNA is engineered to have a nucleotide sequence complementary to the target gene, and Cas9 cuts both strands, effectively disabling the gene. Scientists can then introduce a segment from the normal (functional) gene along with the CRISPR-Cas9 system if the target gene has a mutation. After Cas9 cuts the target DNA, repair enzymes use the normal DNA as a template to repair the target DNA at the breakpoint. This way, the CRISPR-Cas9 system edits the defective gene to correct it (Figure 1.90). Researchers, physicians, and patients are excited about the potential of CRISPR technology to treat or even cure human genetic diseases such as Sickle cell, Alzheimer's, and Parkinson's diseases, as well as some types of cancer. However, there are still concerns about using CRISPR-treated cells in humans because of the possible editing of genes that are not being targeted.

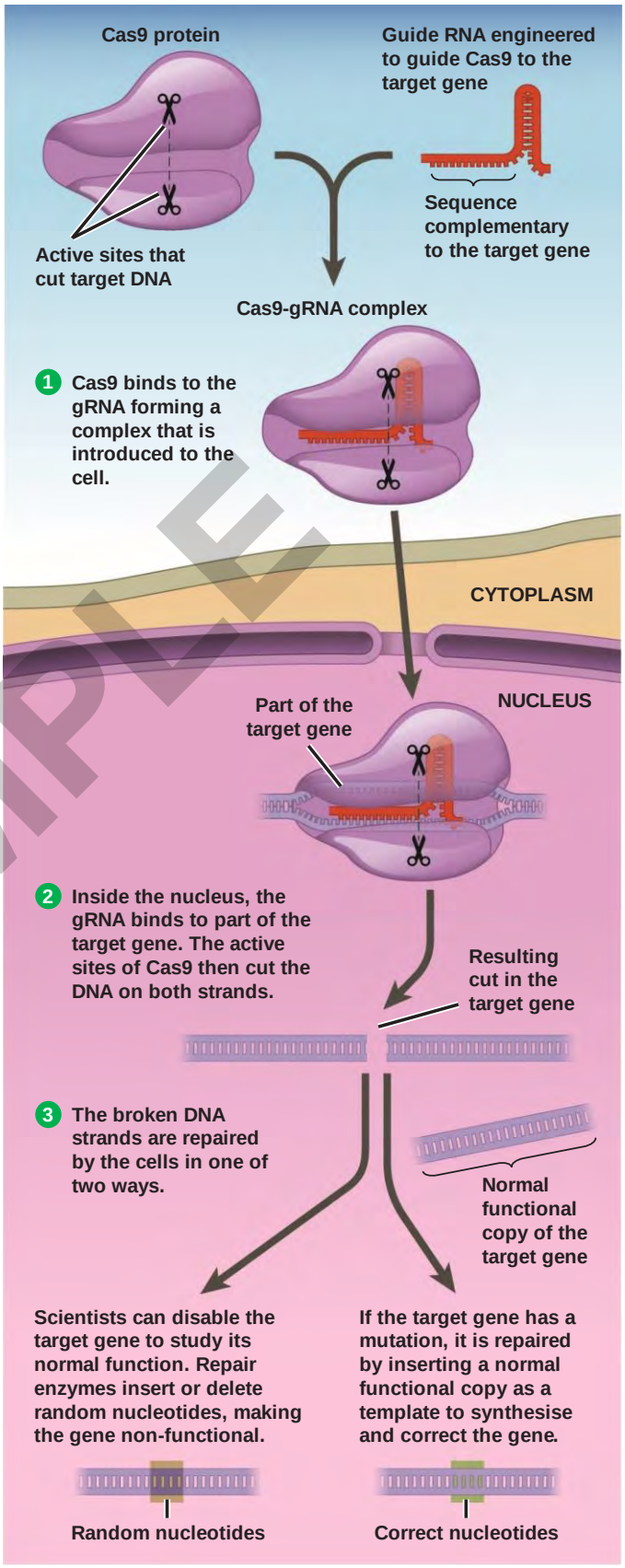


Figure 1.90: Gene editing with CRISPR

Designer Proteins

Developing our scientific knowledge and understanding of DNA and biotechnology has enabled scientists to design and manufacture proteins for scientific and medicinal use. One example is **monoclonal antibodies**, defensive proteins prepared from cloned B cells grown in culture. The monoclonal antibodies produced by such a culture are identical and specific for the same antigen. As a result, monoclonal antibodies have provided the basis for many recent medical diagnoses and treatment advances. For example, home pregnancy tests use monoclonal antibodies to detect human chorionic gonadotropin (hCG) in a woman's urine, a reliable indicator for a very early stage of pregnancy. Monoclonal antibodies are also used as therapies for many human diseases, including **targeted chemotherapy** to treat cancer. Figure 1.91 shows some monoclonal antibodies used in targeted chemotherapy.

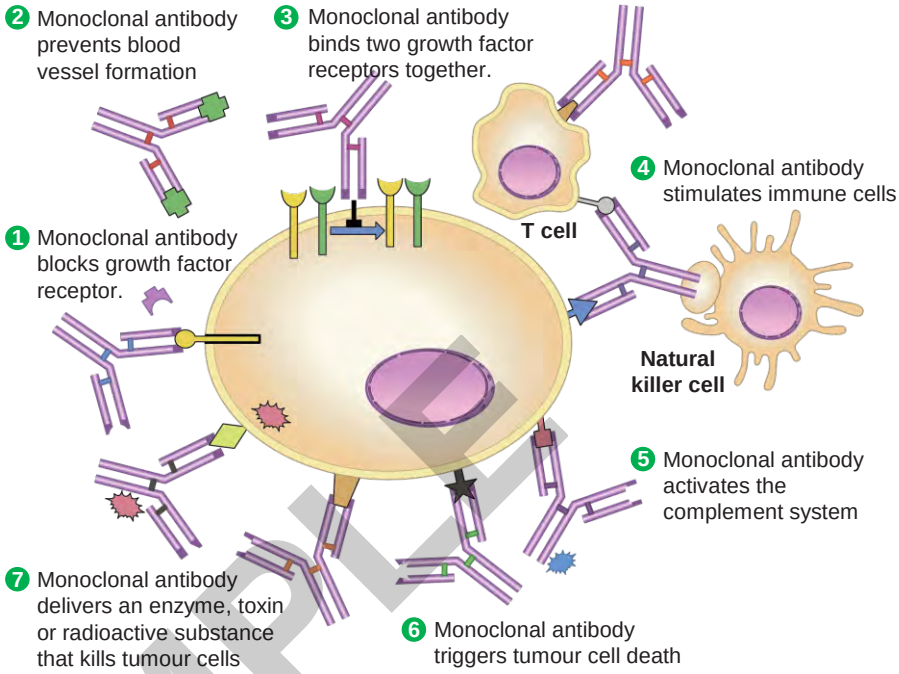


Figure 1.91: Monoclonal antibodies used in chemotherapy.

Another example is **biosensors**, devices that use designer proteins for near-immediate detection and identification of specific molecules, including drugs and microbial antigens. Biosensors are typically integrated with a computer microchip for information management, forming a **biochip**. The designer proteins, such as monoclonal antibodies and enzymes, recognise specific substrates, and their binding produces a response amplified by a transducer. Some of the applications of biochips include disease diagnosis and the detection of drugs and pathogens.

Other types of biosensors are used in medical **diagnostics**. For example, continuous glucose monitors use designer proteins to measure glucose levels continuously to gain insight into patterns and trends in glucose levels. The designer protein is an enzyme that converts glucose to other substances, producing an electronic current in proportion to glucose concentration.

Designer proteins are also used as **biomaterials**, substances engineered to interact with the body for medical purposes. Protein-based biomaterials like collagen, keratin, elastin, silk protein and, more recently, recombinant proteins have emerged as attractive alternatives to synthetic polymers due to their desirable properties like cell-to-cell interaction, structural support and cellular communications. Several protein-based biomaterials are being utilised in the biomedical field with applications including tissue engineering, medical science, regenerative medicine, and drug delivery.

Review Test 1

Questions 1 to 10

Questions 1 to 10 are **multiple-choice questions.** For each multiple-choice question, indicate the best answer to the question by shading in the bubble [O] beside it.

1. Plant cells have DNA in their nucleus, mitochondria, and chloroplasts.

   Which of the following describes chloroplast DNA?

○ **J** multiple linear chromosomes
○ **K** a single linear chromosome
○ **L** multiple circular chromosomes
○ **M** a single circular chromosome

(1 mark) **KA1**

2. In a DNA molecule, 17% of the nucleotide bases were cytosine.

   What percentage of the nucleotide bases were adenine?

○ **J** 17%
○ **K** 33%
○ **L** 66%
○ **M** 83%

(1 mark) **KA1**

3. The table shows mRNA codons and their amino acids.

mRNA codon	Amino acid
ACC	Threonine
AUU	Isoleucine
CUU	Leucine
UCC	Serine

   A tRNA has the anticodon AGG.

   Which one of the following amino acids does this tRNA bring to the ribosome?

○ **J** Leucine
○ **K** Isoleucine
○ **L** Serine
○ **M** Threonine

(1 mark) **KA1**

4. A segment of one DNA strand in a target gene contains the nucleotide sequence below.

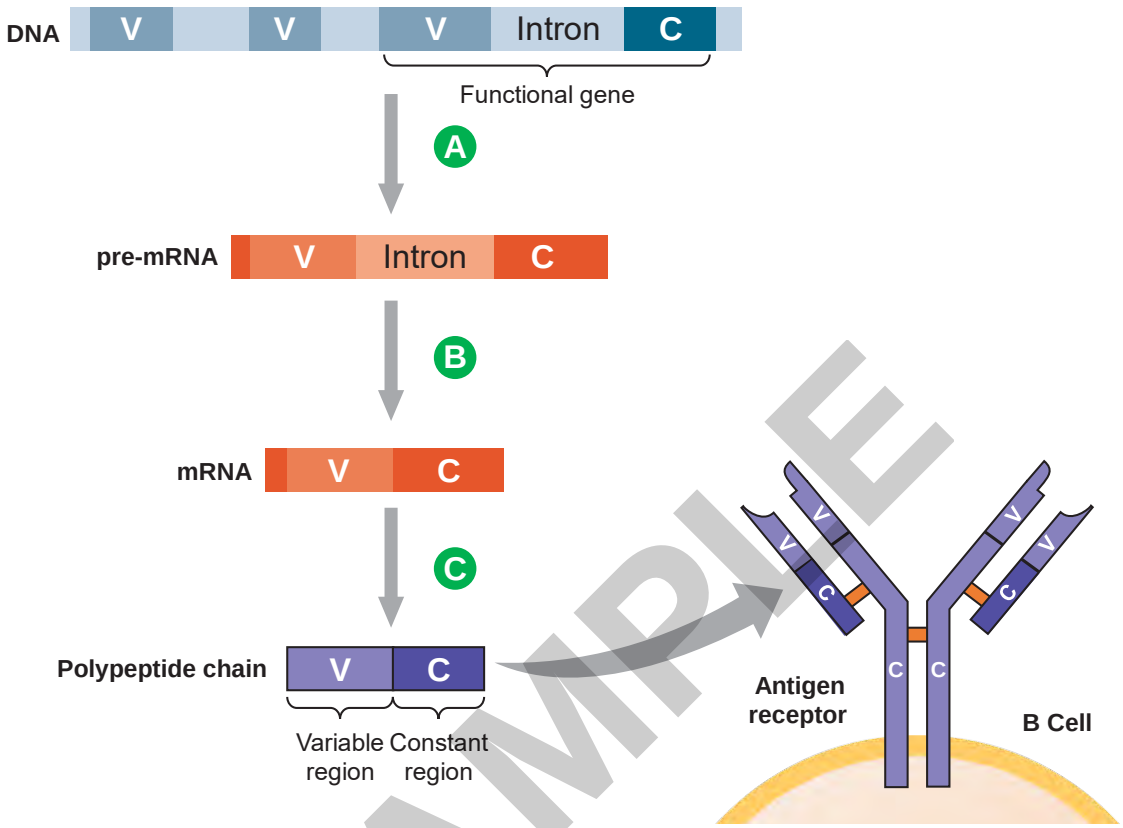
   AACGAGGACCTGGCG

   Which one of the following sequences could be used as an RNA probe to locate this segment?

○ **J** UCCUGGAC
○ **K** TTGCTCCT
○ **L** UUGCTCCT
○ **M** GCUGGUCC

(1 mark) **KA1**

5. B cells are immune cells in humans that synthesise defensive proteins called antibodies and antigen receptors. B cells have genes that code for the polypeptides that comprise an antigen receptor. The diagram below shows the gene expression stages synthesising a polypeptide chain in the B cell antigen receptor.



Which of the following combinations correctly identifies processes A, B and C?

		A	B	C
○	J	Translation	Transcription	RNA processing
○	K	Transcription	RNA processing	Translation
○	L	Translation	RNA processing	Transcription
○	M	Transcription	Translation	RNA processing

(1 mark) **KA2**

6. DNA polymerase is an enzyme that joins DNA nucleotides during DNA replication.

Which one of the following statements about DNA polymerase is ***not*** correct?

○ J DNA polymerase lowers the activation energy for DNA replication.

○ K DNA polymerase has an active site complementary to DNA nucleotides.

○ L DNA nucleotides bind to the active site of DNA polymerase by induced fit.

○ M DNA polymerase joins nucleotides more rapidly at higher temperatures.

(1 mark) **KA1**

In addition to lipids, the cell membrane contains hundreds of different proteins, forming the majority component of most membranes, averaging 50% of their total composition. Proteins are composed of amino acids with hydrophilic or hydrophobic side chains (Chapter 1.5), a property that enables their interaction with the phospholipid bilayer. Proteins with hydrophilic side chains typically bind to the hydrophilic surfaces of the membrane, and those with hydrophobic side chains penetrate the hydrophobic core, as depicted in Figure 2.08. Proteins attached to the surface of the membrane are called **peripheral proteins**, and those penetrating the lipid bilayer are called **integral proteins**. In 1972, American biologists S.J. Singer and G.L. Nicolson proposed the widely accepted **fluid mosaic model**, which states that a biological membrane is a mosaic of different protein molecules attached to or embedded within the fluid matrix of the lipid bilayer. The fluid mosaic structure of the cell membrane is illustrated in Figure 2.09.

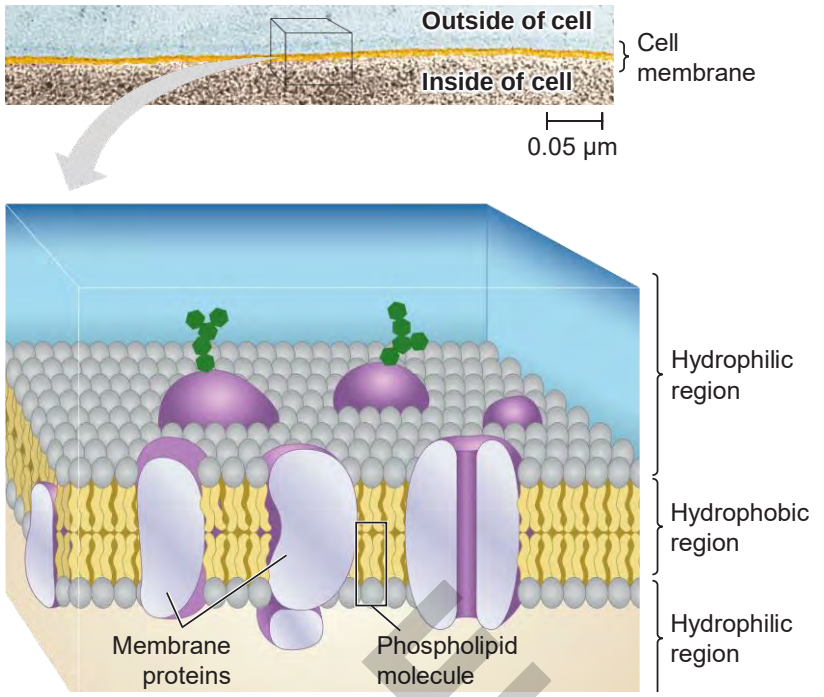


Figure 2.08: Arrangement of proteins in the cell membrane

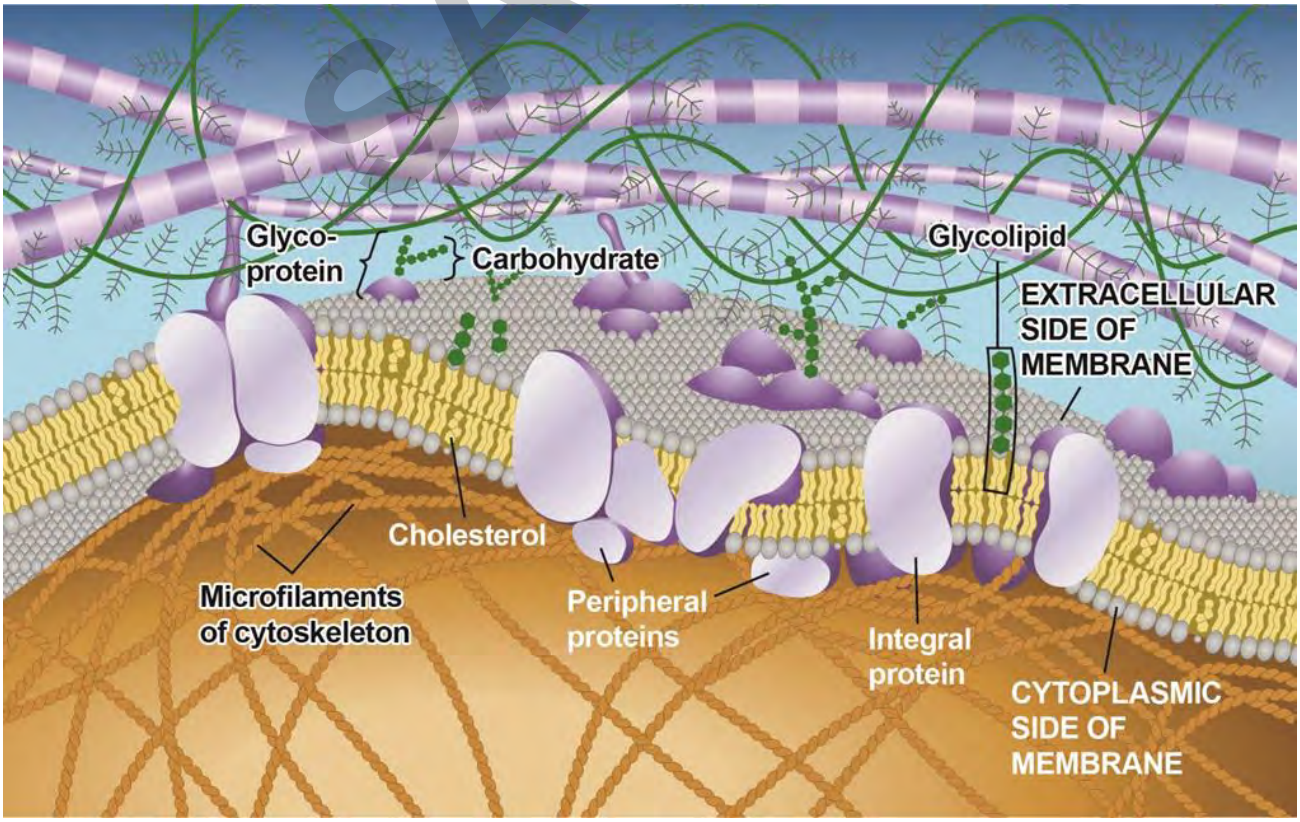


Figure 2.09: The fluid mosaic structure of the cell membrane.

The peripheral and integral proteins of the cell membrane are not randomly distributed. Instead, most proteins occupy a specific membrane region where they carry out various functions depicted in Figure 2.10. The most common function of membrane proteins is transporting materials between the cell and its surroundings. In addition, many essential enzymes are embedded in the cell membrane, catalysing specific metabolic reactions. Furthermore, some membrane proteins act as receptors for signal molecules that cause changes in cellular activities. In contrast, others help cells recognise one another, which is essential in normal development and immunity. Finally, membrane proteins anchor cells to each other and the extracellular matrix (ECM) in animal cells.

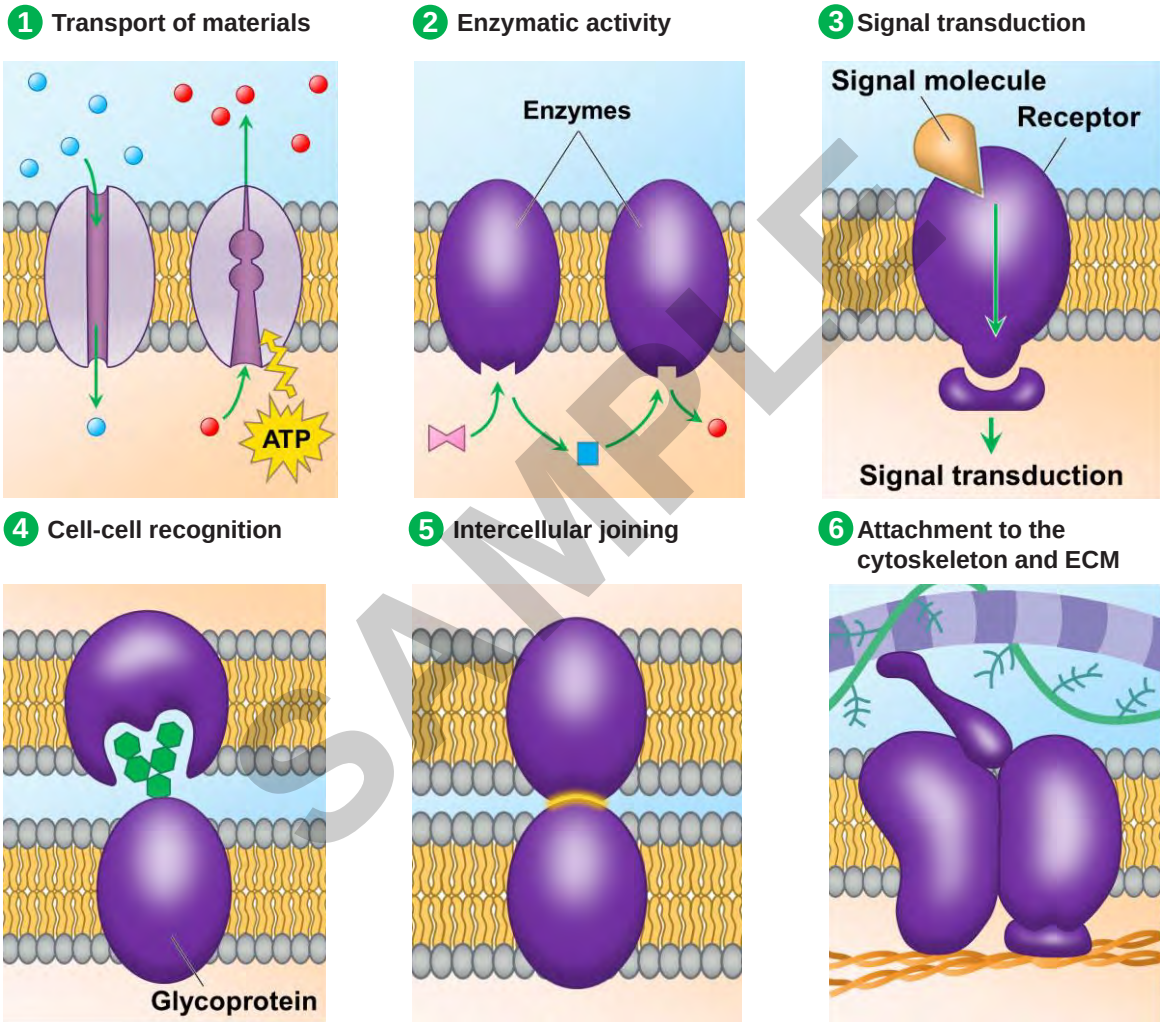


Figure 2.10: The functions of different membrane proteins.

In addition to lipids and proteins, the cell membrane contains a small percentage of carbohydrates. Carbohydrates may be chemically bonded to lipids (forming **glycolipids**) or, more commonly, to proteins (forming **glycoproteins**) on the external side of the cell membrane. The type of carbohydrate molecules that form glycoproteins and glycolipids vary among species, individuals, and the different cell types in an individual. Both glycolipids and glycoproteins play an essential role in cell-cell recognition, a process in which different cells bind to each other using molecules, often carbohydrates, on the extracellular surface of the cell membrane. Cell-cell recognition typically triggers a response such as communication, cooperation, transport, defence, or growth.

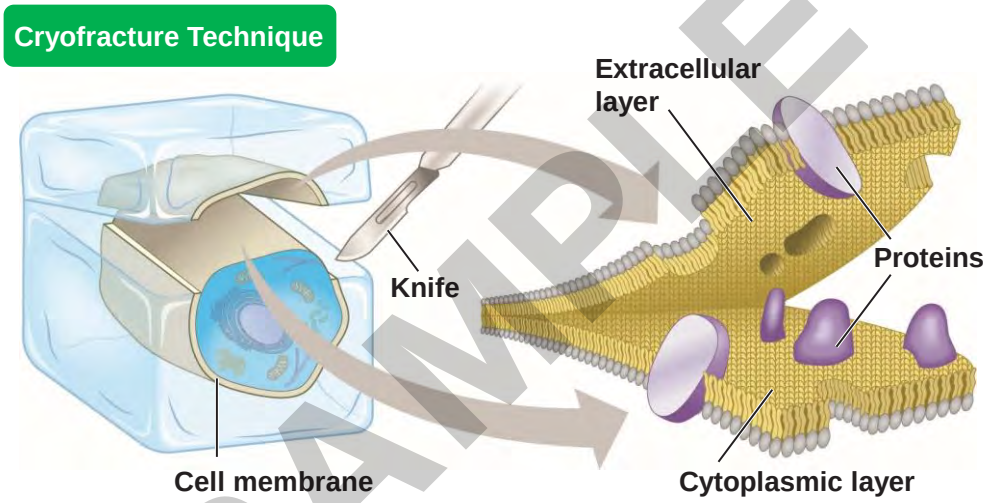
Question 89

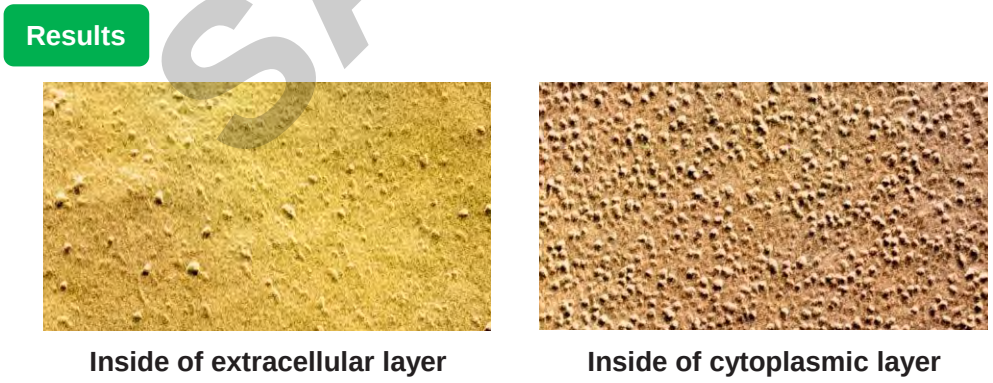
In 1972, Singer and Nicolson proposed the fluid mosaic model of cell membranes.

(a) Describe the structure of the cell membrane according to the fluid mosaic model.

(2 marks) KA2

(b) Cryofracture is a technique that splits the cell membrane along the middle of the bilayer.



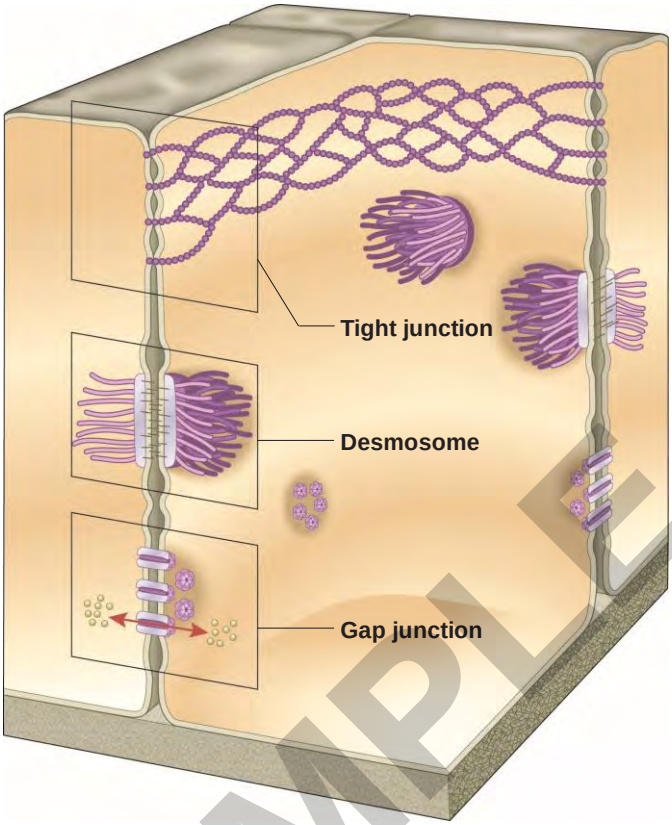


Describe the evidence from the diagram supporting the fluid mosaic model.

(2 marks) KA2

Question 91

The diagram below shows some different proteins linking the membranes of epithelial cells.



(a) At tight junctions, the membranes of neighbouring cells are pressed together.

Describe how the cell membranes of neighbouring cells are connected.

_____

_____

_____ (1 mark) **KA1**

(b) At desmosomes, neighbouring cells are fastened together into strong sheets.

Predict how desmosomes anchor to the cytoplasm.

_____

_____

_____ (1 mark) **KA1**

(c) At gap junctions, membrane proteins form hollow channels passing through the membranes of two neighbouring cells.

Suggest a likely function of gap junctions.

_____

_____

_____ (1 mark) **KA2**

Eukaryotic Cells

Eukaryotes are more complex living things that likely evolved from prokaryotes in endosymbiotic events between 1.5 and 2.5 billion years ago (See Chapter 4.2). Eukaryotes comprise one of the three domains of living things, called **Eukarya** which includes four kingdoms: protists, fungi, animals, and plants (Figure 2.13). Eukaryotic cells are much larger than prokaryotic cells, ranging from 10 to 100 micrometres in length. In addition, the basic shape of the eukaryotic cell is spherical, but this varies and is ultimately determined by its function. Thus, the cell's shape may be variable (i.e., frequently changing) or fixed. For example, variable or irregular shape occurs in Amoeba and white blood cells (leucocytes), and fixed shape occurs in almost all protists (e.g., *Euglena*, *Paramecium*), plants and animals. Furthermore, the number of cells varies from one in a unicellular eukaryote such as yeast, protozoa and protophyta to many cells in multicellular eukaryotes such as most fungi, plants, and animals.

Figure 2.13: Eukaryotes

Eukaryotic cells vary in size and structure, but each shares some common features. The defining feature of eukaryotic cells is a **nucleus** (Figure 2.14) that stores the genetic material. In fact, the term eukaryote is derived from the Greek word meaning "nucleated." In eukaryotes, the hereditary material is on two or more linear chromosomes, each composed of a single DNA molecule wrapped tightly around histones. The chromosome number ranges in eukaryotes from two to more than a thousand. In addition to the nucleus, the cytoplasm of a eukaryotic cell contains many membrane-enclosed **organelles**, literally meaning "tiny organ". Each organelle in a eukaryotic cell is isolated from the cytosol by a lipid bilayer membrane similar to the cell membrane. Within an organelle is a specialised set of proteins and other molecules that facilitate one or more essential processes in the cell, including respiration, protein synthesis and cell division. These organelles and the structures of eukaryotic cells will be explored in detail in Chapter 2.3.

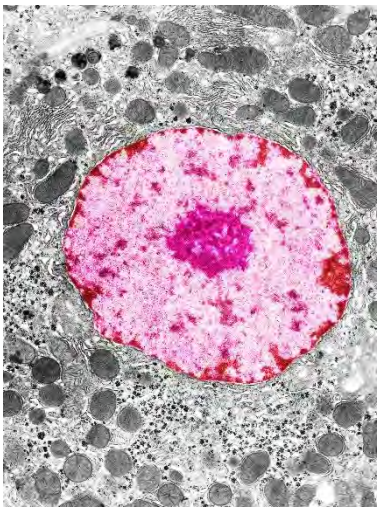


Figure 2.14: Nucleus

Comparing Prokaryotic and Eukaryotic Cells

Prokaryotic and eukaryotic cells have similarities and differences (Figure 2.15). Each contains a cytoplasm filled with cytosol enveloped by a cell membrane. In addition, both cells contain DNA that codes enzymes and other proteins that carry out the activities necessary for a cell to sustain itself and reproduce. These proteins are assembled by ribosomes using the information stored on DNA molecules. Finally, both cells are powered using energy stored in adenosine triphosphate (ATP) molecules. These similarities between prokaryotic and eukaryotic cells reflect their common evolutionary past, a concept discussed later in Chapter 4.2. However, there are many significant differences between prokaryotic and eukaryotic cells. First, prokaryotic cells are much smaller than eukaryotic cells. Second, most of the DNA is in a membrane-bound organelle called the nucleus in a eukaryotic cell and is concentrated in a region of the cytoplasm called the nucleoid in a prokaryotic cell. Third, prokaryotes contain a single, circular chromosome, whereas eukaryotic cells contain multiple linear chromosomes (Figure 2.16). Finally, eukaryotes contain membrane-bound organelles that facilitate specialised functions that typically occur in the cytoplasm in prokaryotic cells.

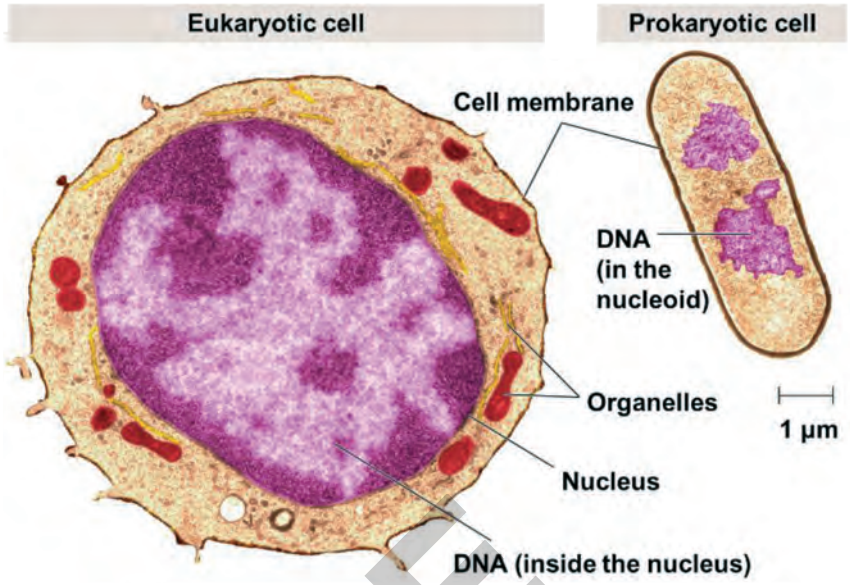


Figure 2.15: TEM showing the two cell types.

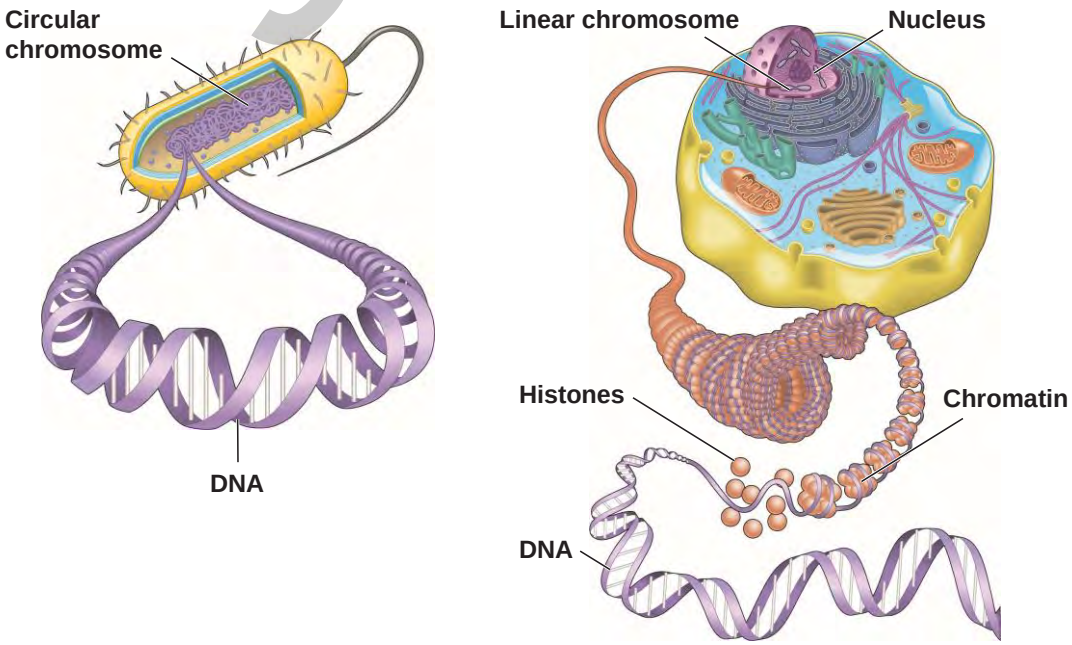


Figure 2.16: Chromosome structure in prokaryotes and eukaryotes.

2.3: The Structure of Eukaryotic Cells

Eukaryotic cells have specialised organelles which facilitate biochemical processes.

- Represent the structure and describe the function of:
  - nucleus
  - nucleolus
  - mitochondrion
  - chloroplast
  - vacuole/vesicle
  - Golgi body
  - endoplasmic reticulum (rough and smooth)
  - ribosome
  - lysosome
  - cytoskeleton.
- Compare the structures of plant and animal cells.

In addition to the cell membrane at its outer surface, a eukaryotic cell has extensive, elaborately arranged internal membranes that divide the cell into compartments—the organelles mentioned in Chapter 2.2. The cell's compartments provide different local environments that support specific metabolic functions so that incompatible processes can occur simultaneously in a single cell. The cell and organelle membranes also participate directly in the cell's metabolism because many enzymes and transport proteins are built into the membranes. Before continuing with this chapter, examine the eukaryotic cells in Figure 2.17. The generalised diagrams of an animal and a plant cell introduce the various organelles and show the key structural differences between animal and plant cells. Note that animal and plant cells share many features, but each has unique ones.

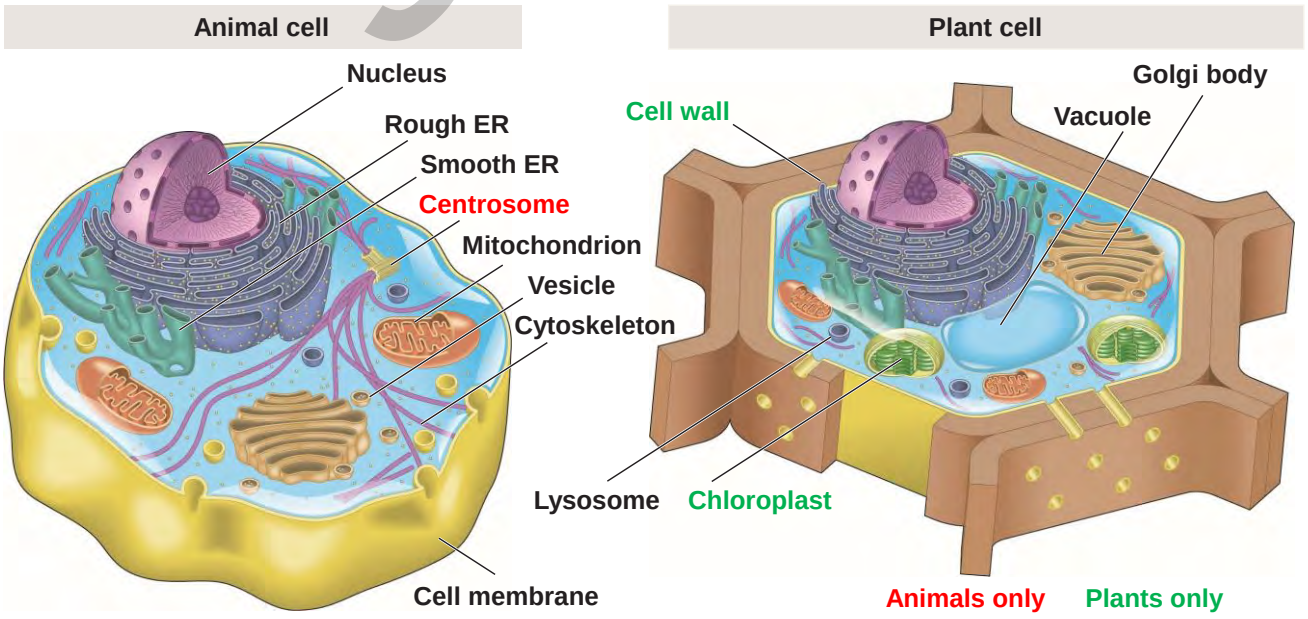


Figure 2.17: Generalised diagrams of an animal cell and a plant cell

The Nucleus

The nucleus contains most of the genes in the eukaryotic cell. It is usually the largest and most visible organelle, averaging about 5 μm in diameter, roughly half the length of the cell (Figure 2.18). The nucleus is enclosed by the **nuclear envelope**, a double lipid bilayer membrane containing various proteins that separate its contents from the cytoplasm (Figure 2.18). The envelope is perforated by **nuclear pores** that regulate the entry and exit of macromolecules, including RNAs and proteins that diffuse between the nucleus and cytoplasm. The DNA is organised within the nucleus into chromosomes, which carry genetic information. Each chromosome is made from **chromatin**, a mixture containing one long DNA molecule and its associated proteins that help coil the DNA, reducing its length and allowing it to fit into the nucleus. When a cell is not dividing, stained chromatin appears as a diffuse mass, and the chromosomes cannot be distinguished from one another. However, as a cell prepares to divide, the chromosomes condense and become thick enough to be distinguished as separate structures when viewed through a microscope.

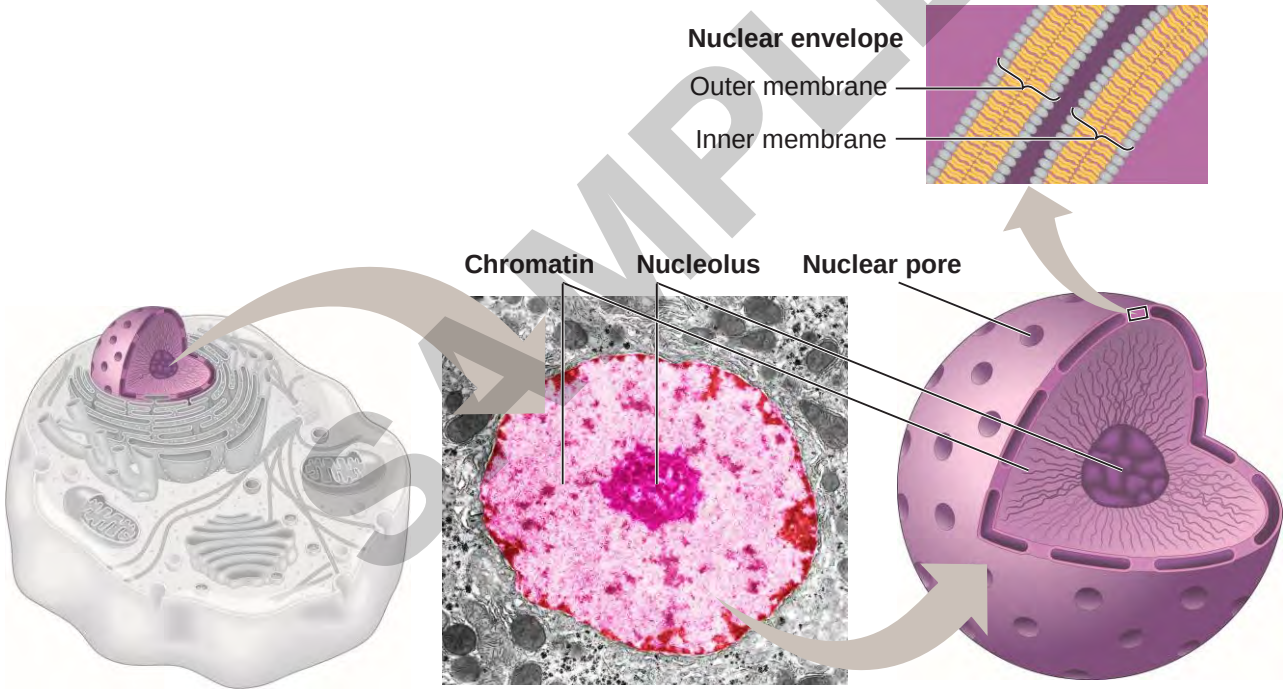


Figure 2.18: The nucleus and its features.

A prominent structure within the nucleus is the **nucleolus**, where ribosomal RNA (rRNA) is synthesised from genes in the DNA. Also, in the nucleolus, proteins imported from the cytoplasm are assembled with rRNA into large and small subunits of ribosomes (Figure 2.19). These subunits exit the nucleus through the nuclear pores to the cytoplasm and assemble into a ribosome. As we saw in Chapters 1.3 and 1.4, the nucleus directs protein synthesis by synthesising messenger RNA (mRNA) that carries information from the DNA. The mRNA is then transported to the cytoplasm via nuclear pores. Once an mRNA molecule reaches the cytoplasm, ribosomes translate the mRNA's genetic message into the primary structure of a specific polypeptide.

Ribosomes

Ribosomes are macromolecular complexes made of ribosomal RNAs and proteins that carry out protein synthesis (Chapter 1.4). Although ribosomes carry out a specific function in cells, they are not membrane-bound and are not considered organelles. Cells with high protein synthesis rates have vast numbers of ribosomes and prominent nucleoli to facilitate rapid ribosome assembly. For example, a pancreatic beta cell, which synthesises the hormonal protein insulin, has a few million ribosomes and a plasma cell, which makes antibodies, has up to ten million. Ribosomes build proteins in two cytoplasmic regions. At any given time, **free ribosomes** are suspended in the cytosol, while **bound ribosomes** attach to the outside of the rough endoplasmic reticulum (Figure 2.19).

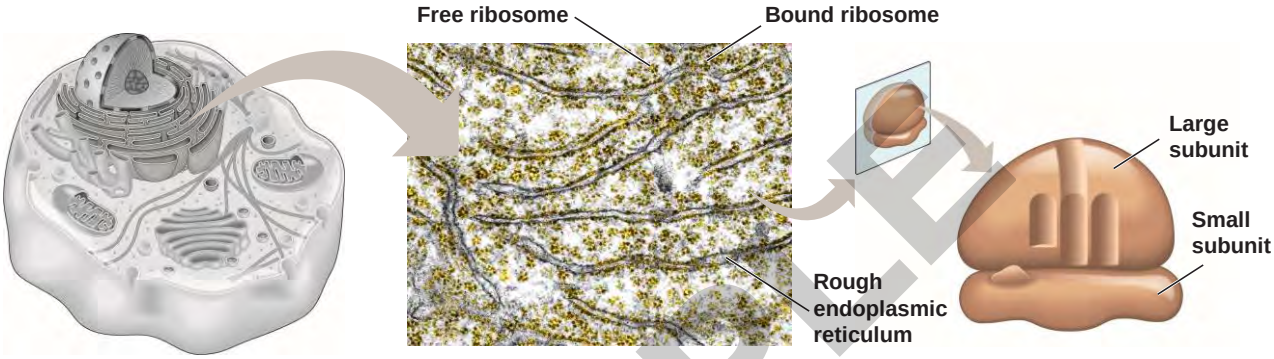


Figure 2.19: Ribosomes and their features.

Bound and free ribosomes are structurally identical, and ribosomes can play either role at different times. Most of the proteins made on free ribosomes function within the cytosol such as the enzymes that catalyse the first steps of respiration. Bound ribosomes make proteins destined for insertion into membranes, packaging within specific organelles or secretion from the cell. Such polypeptides have a **signal peptide**, a short amino acid sequence recognised by a protein-RNA complex called a **signal-recognition particle** (SRP) that escorts the polypeptide and ribosome to the endoplasmic reticulum as in Figure 2.20.

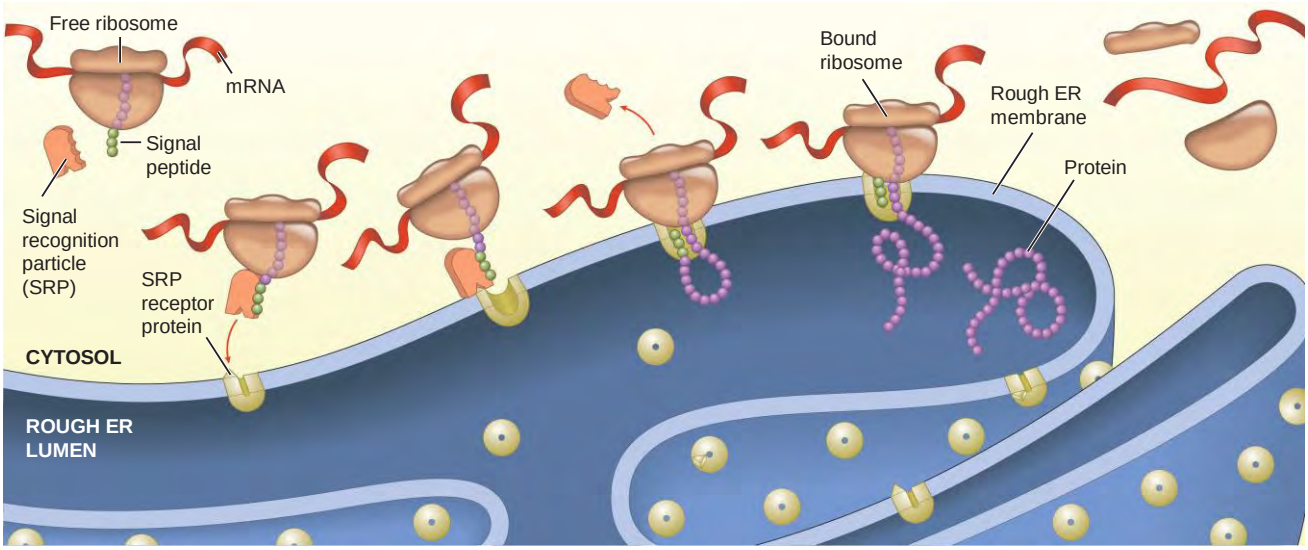


Figure 2.20: Bound ribosomes

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is an extensive network of membranes that accounts for more than half the total membrane content in many eukaryotic cells. The ER consists of a network of fluid-filled, membrane-bound sacs called **cisternae**. A single lipid bilayer, the ER membrane, separates the internal compartment of the ER, the **ER lumen,** from the cytosol. The ER lumen is continuous with the space between the two membranes of the nuclear envelope. The ER has two distinct, though connected, regions called the **smooth ER** and **rough ER** that differ in structure and function. The rough ER is studded with ribosomes on its membrane's outer surface and appears rough through the electron microscope (Figure 2.21). The smooth ER is so named because its surface lacks ribosomes.

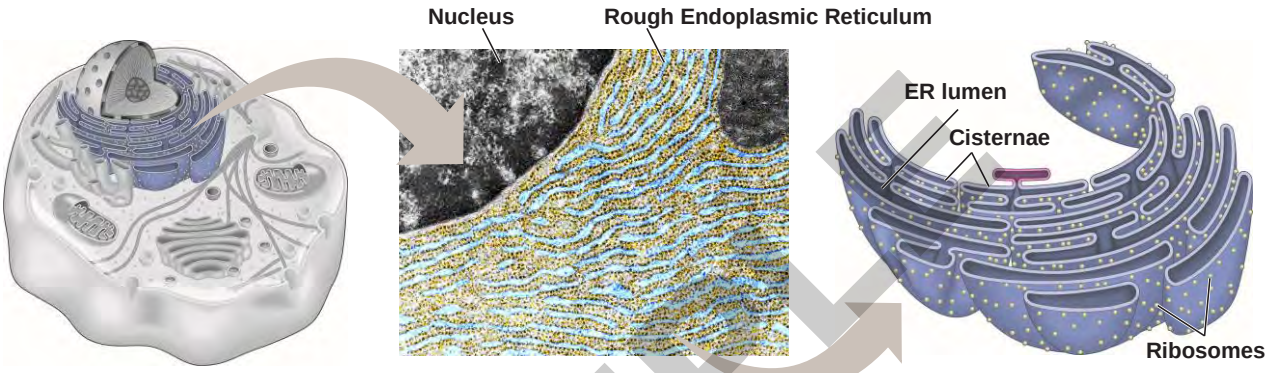


Figure 2.21: The rough endoplasmic reticulum

The rough ER specialises in synthesising and secreting proteins. As a polypeptide chain grows from a bound ribosome, the chain is threaded into the ER lumen through a pore formed by a protein complex in the ER membrane. The new polypeptide folds into its functional shape as it enters the ER lumen. Folded proteins depart from the ER wrapped in membrane-bound **vesicles** that bud like bubbles from the ER membrane (Figure 2.22). Vesicles in transit from one part of the cell to another are called **transport vesicles**.

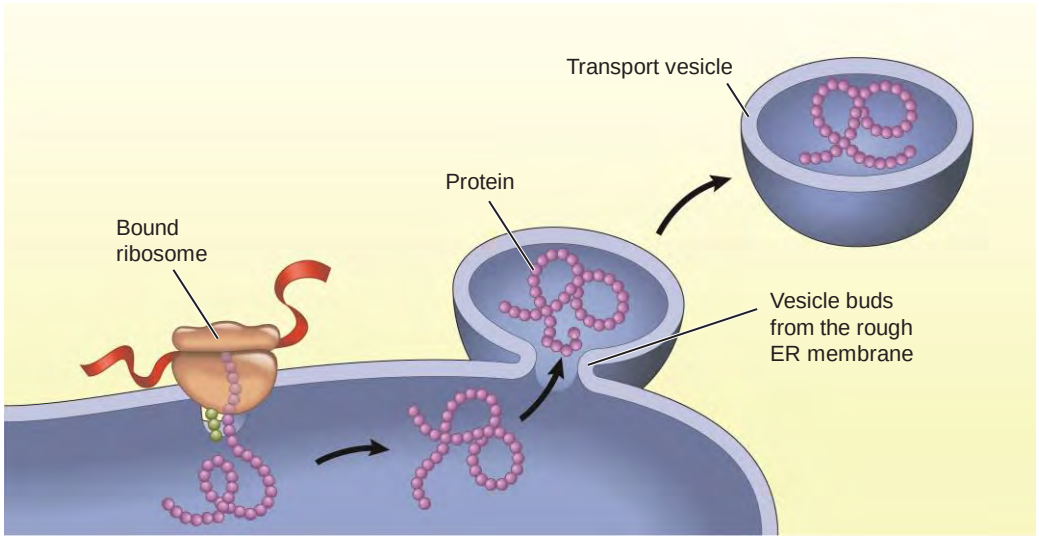


Figure 2.22: The rough ER folds polypeptides and exports proteins.

The smooth ER (Figure 2.23) has various functions in different cell types, including synthesising lipids, detoxifying drugs and poisons, and storing calcium ions. Enzymes of the smooth ER are important in synthesising lipids, including membrane phospholipids, cholesterol, and steroid hormones. Among the steroids produced by the smooth ER in animal cells are the sex hormones of vertebrates and the various steroid hormones secreted by the adrenal glands (see Chapter 3). Other enzymes of the smooth ER help detoxify drugs and poisons, especially in liver cells. Detoxification usually involves adding hydroxyl groups to drug molecules, making them more water-soluble and easier to flush from the body. The smooth ER also stores calcium ions crucial to muscle movement and the propagation of nerve impulses.

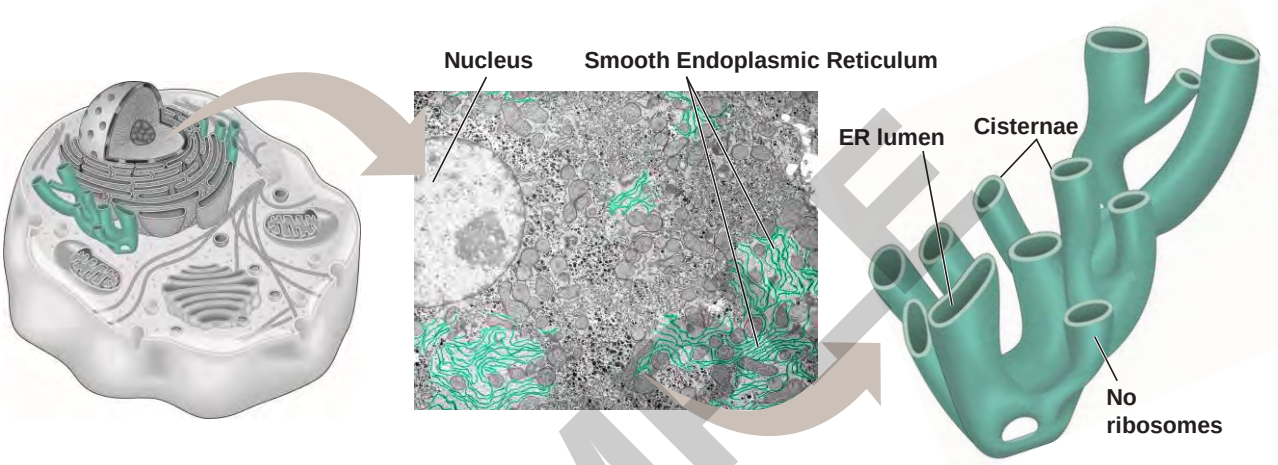


Figure 2.23: Smooth endoplasmic reticulum

Like the smooth ER, the rough ER also synthesises membrane phospholipids; enzymes built into the ER membrane assemble phospholipids from substrates in the cytosol. The rough ER is a membrane factory for the cell; it grows in place by adding membrane proteins and phospholipids to its membrane. As polypeptides destined to be membrane proteins grow from the ribosomes, they are inserted into the ER membrane and anchored there by their hydrophobic portions. As the ER membrane expands, portions of it are transported via vesicles to other cell components. A vesicle has a fluid-filled core where macromolecules like polypeptides or many ions or smaller molecules like neurotransmitters or hormones are stored and enclosed by a lipid bilayer (Figure 2.24).

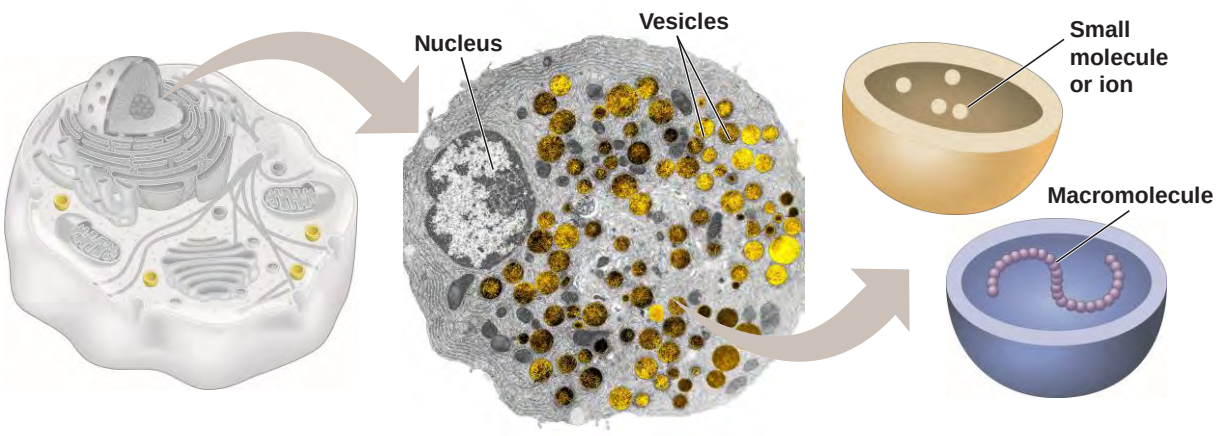


Figure 2.24: Vesicles

Cytoskeleton

In the early days of microscopy, biologists thought the organelles of a eukaryotic cell floated freely in the cytoplasm. However, the development of higher-resolution light and electron microscopes has revealed the **cytoskeleton**, a network of fibres extending throughout the cytoplasm (Figure 2.34). Bacterial cells also have fibres that form a type of cytoskeleton constructed of proteins similar to eukaryotic cells, but we will focus on eukaryotes.

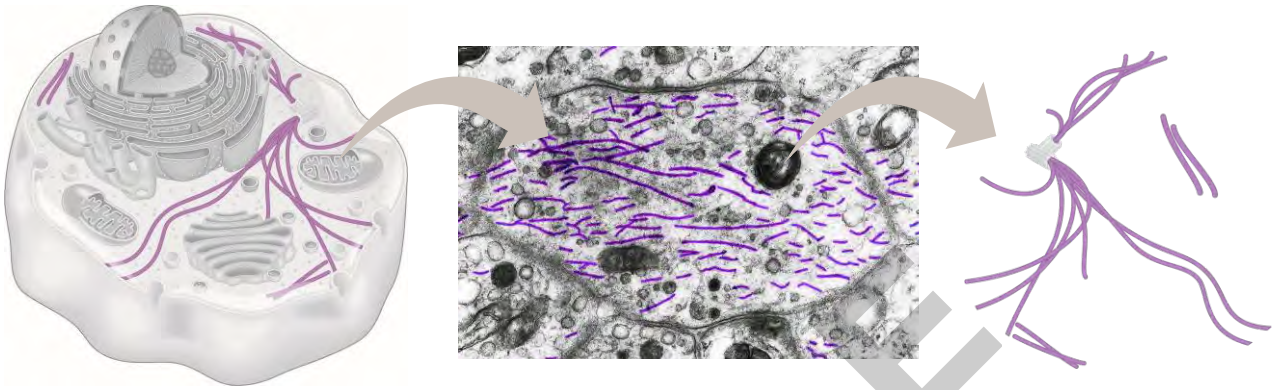


Figure 2.34: Cytoskeleton in eukaryotes.

The eukaryotic cytoskeleton plays a significant role in organising the structures and activities of the cell. The most apparent function of the cytoskeleton is to give mechanical support to the cell and maintain its shape. This is especially important for animal cells, which lack cell walls. The remarkable strength and resilience of the cytoskeleton are based on its architecture. Like a dome tent, the cytoskeleton is stabilised by a balance between opposing forces exerted by its components. And just as the skeleton of an animal helps fix the positions of other body parts, the cytoskeleton provides anchorage for many organelles and enzymes. The cytoskeleton is more dynamic than an animal skeleton, however. It can be quickly dismantled in one part of the cell and reassembled in a new location, changing the cell's shape. The cytoskeleton also manipulates the cell membrane, bending it inward to form food vacuoles. Finally, the cytoskeleton facilitates **cell motility**, including changes in cell location and movement of cell parts. Cell motility generally requires the interaction of the cytoskeleton with motor proteins. Inside the cell, motor proteins "walk" vesicles and other organelles to their destinations along tracks provided by the cytoskeleton (Figure 2.35).

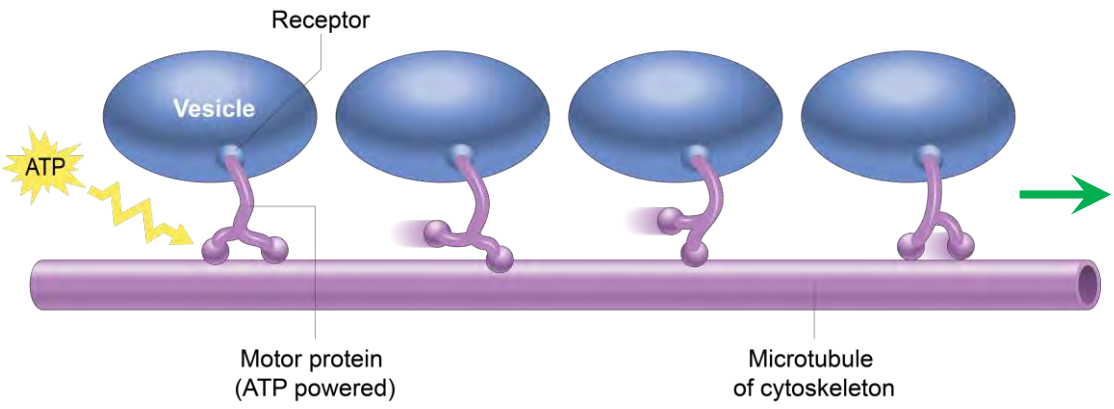


Figure 2.35: Motor proteins transporting vesicles along the cytoskeleton.

The cytoskeleton comprises three main types of fibres, each a complex of one or more proteins. **Microtubules** are the thickest of the three types; **microfilaments** are the thinnest, and **intermediate filaments** are fibres with diameters in the middle range. The structures and functions of these three types of fibres are summarised in Figure 2.36.

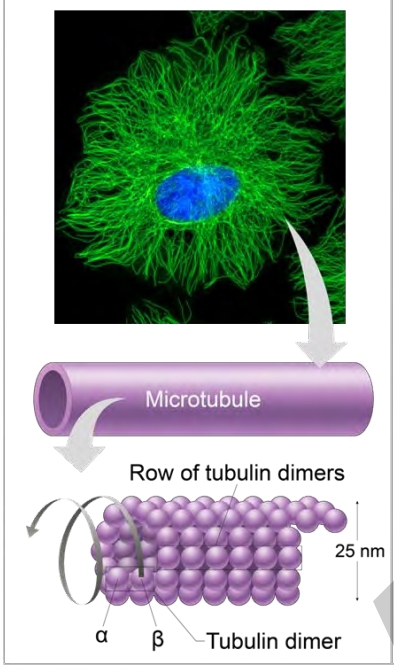
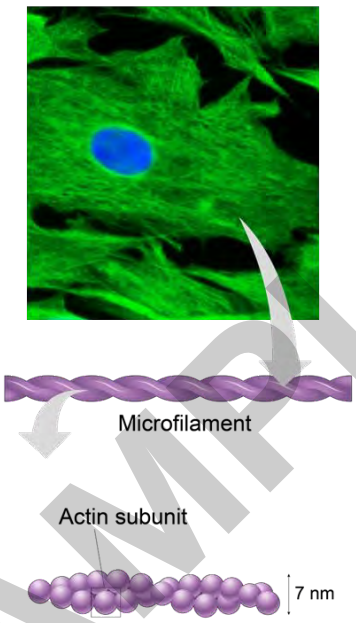
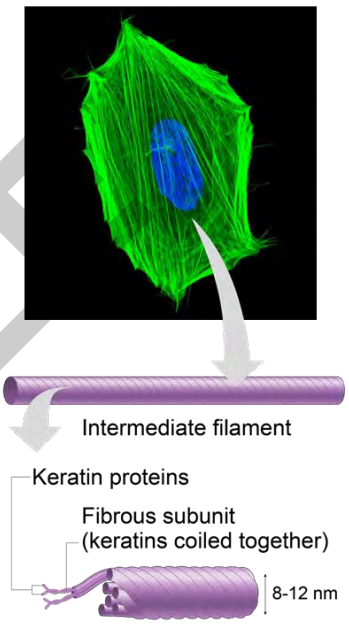
Microtubules	Microfilaments	Intermediate filaments
Hollow tubes around 25 nm in diameter composed of the proteins α–tubulin and β–tubulin. Microtubules maintain cell shape, move chromosomes during cell division and provide a framework for transporting vesicles and organelles.	Thin fibres 7 nm in diameter composed of two intertwined strands of the protein actin. Microfilaments maintain and change cell shape, facilitate muscle cell contraction and are essential to cell motility and division.	Fibrous protein coiled into cables between 8 and 12 nm in diameter composed of the protein keratin. Intermediate filaments maintain cell shape as well as anchoring the organelles including the nucleus.
		

Figure 2.36: The structure and function of cytoskeletal fibres.

In animal cells, microtubules grow out from a **centrosome**, a region often located near the nucleus. Within the centrosome is a pair of **centrioles**, each about 0.25 picometres in diameter. The two centrioles are arranged at right angles, each composed of nine sets of triplet microtubules arranged in a ring (Figure 2.37). Although centrosomes with centrioles may help organise microtubule assembly in animal cells, many other eukaryotic cells lack centrosomes with centrioles and instead organise microtubules using **microtubule-organising centres (MTOC)**.

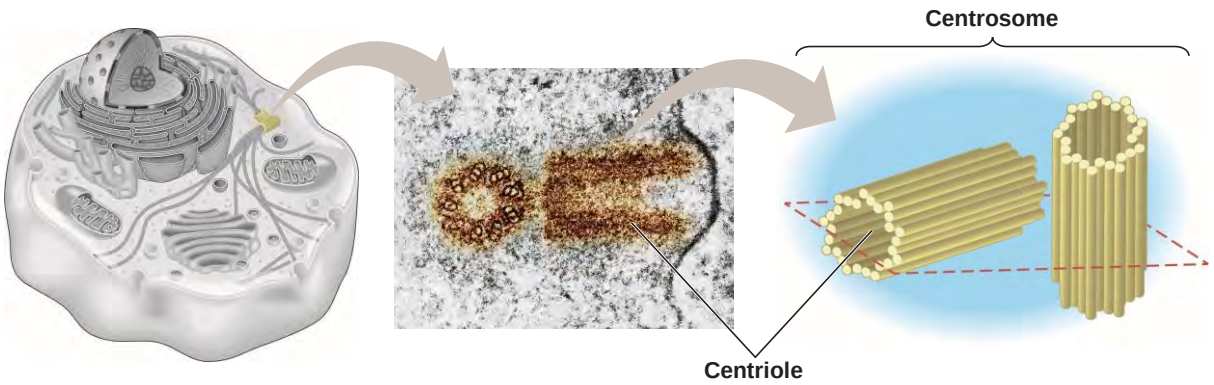


Figure 2.37: Centrosome structure.

Comparing Plant and Animal Cells

Plants and animals are two kingdoms of the eukaryotes, each a multicellular organism composed of specialised eukaryotic cells. Plant and animal cells share many features common to eukaryotes, including a nucleus, mitochondria, cytoskeleton, and an endomembrane system which includes the smooth and rough ER, Golgi body, vesicles, vacuoles, lysosomes, and cell membrane. The features common to both cell types are illustrated in Figure 2.38. However, there are differences in some organelles and structures common to plant and animal cells. For example, the cytoskeleton in animals is initiated and organised from a centrosome, a structure that is absent in plant cells. Another example is the vacuole. Plant cell vacuoles store significant quantities of water and are much larger and more prominent than vacuoles in animal cells that typically store macromolecules.

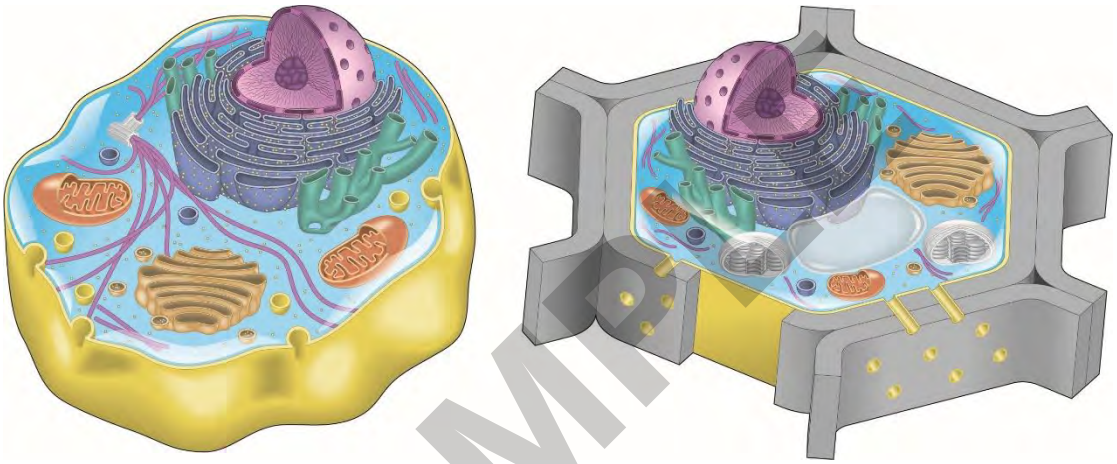


Figure 2.38: Similar structures in plant and animal cells.

Despite their similarities, plant and animal cells show distinct differences in structure (Figure 2.39). Firstly, plant cells have chloroplasts, organelles required for photosynthesis. Second, plant cells are enveloped in a **cell wall**, a rigid structure composed of cellulose that protects the cell, maintains its shape and prevents excessive uptake of water. The plant cell wall is perforated with **plasmodesmata**, channels that connect cells and facilitate the exchange of materials. In contrast, animal cells have an extracellular matrix, an elaborate network of fibrous proteins like collagen and fibronectin that connect animal cells to form tissues.

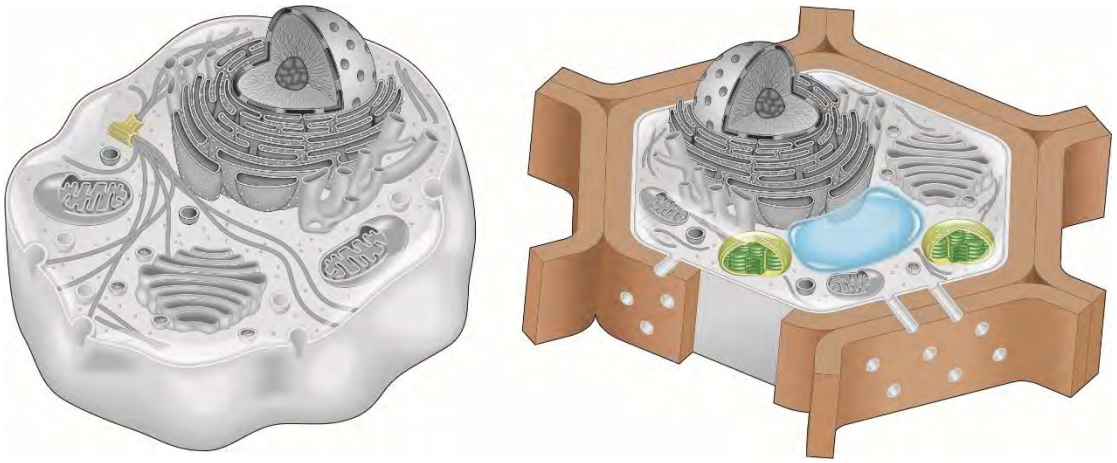


Figure 2.39: Different structures in plant and animal cells.

Autotrophs and Heterotrophs

Most living things derive energy from the breakdown of energy-rich nutrients or **food sources** such as carbohydrates and fats. Living things synthesising their food source using energy and inorganic molecules in the environment are called **autotrophs**. For example, plants, phytoplankton, and green algae are **photoautotrophs** synthesising carbohydrates and other energy-rich molecules using light, carbon dioxide, and water in photosynthesis. In contrast, some prokaryotes are **chemoautotrophs** that synthesise energy-rich molecules using the energy released in the chemical reactions of inorganic molecules. Autotrophs play an essential role in ecosystems as **primary producers,** transforming sunlight into other energy forms that **consumers** may utilise. Examples of photoautotrophs and chemoautotrophs are shown in Figure 2.40.

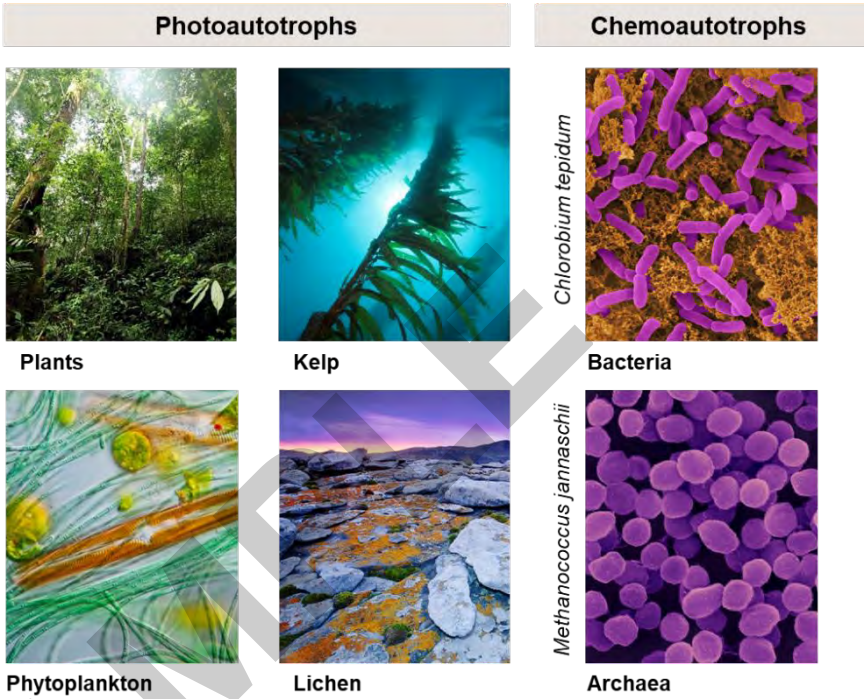


Figure 2.40: Autotrophs

In contrast, living things that derive their food from living or formerly living things are called **heterotrophs**. A heterotroph typically obtains energy and nutrients by eating and digesting other living things, usually plants and animals, for energy and nutrients. Thus, heterotrophs are consumers in ecosystems as they consume producers or other consumers. Heterotrophs that eat plants exclusively are called **herbivores**, those that eat animals exclusively are called **carnivores**, and those eating plants and animals are called **omnivores**. Heterotrophs that consume dead and decaying matter are called **detritivores**. These organisms obtain food by feeding on the remains of plants and animals, and faecal matter. Examples of heterotrophs are shown in Figure 2.41.

Figure 2.41: Heterotrophs

Photosynthesis

The sun is the principal energy source in most ecosystems on Earth. The sun's light contains energy that photoautotrophs transform into chemical energy in **photosynthesis**. Photosynthesis is a complex chemical process that uses light energy to transform carbon dioxide and water into glucose ($C_6H_{12}O_6$) and other organic compounds, including lipids and amino acids. The remarkable ability of photoautotrophs to harness light energy and use it to drive the synthesis of organic compounds emerges from the structural organisation in the cell. Photoautotrophs contain light-absorbing pigment molecules like **chlorophyll** and photosynthetic enzymes grouped as specialised molecular complexes in the **thylakoid membrane**, a biological membrane enabling the necessary series of chemical reactions to be carried out efficiently. In existing photosynthetic bacteria, infolded thylakoid membranes function similarly to those in the chloroplasts of photosynthetic eukaryotes (Figure 2.42). During photosynthesis, light energy is absorbed by pigments like chlorophyll and the energy is subsequently transformed into chemical energy. Thus, photosynthesis is an essential chemical process that supplies photoautotrophs with the glucose needed for respiration. The process of photosynthesis is summarised by the symbol and word equations below.

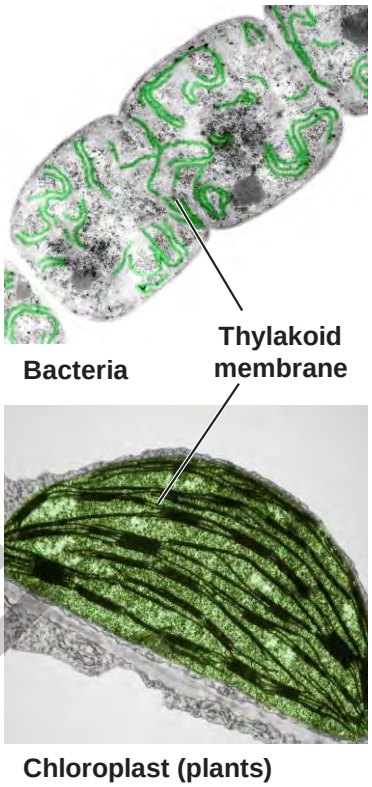


Figure 2.42: Thylakoid membranes in prokaryotes (top) and eukaryotes (bottom)

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2$$

$$\text{carbon dioxide + water} \xrightarrow[\text{chlorophyll}]{\text{light}} \text{glucose + oxygen}$$

All green parts of a photoautotroph have thylakoids with membranes packed with chlorophyll. The chlorophyll molecules of chloroplasts selectively absorb violet-blue and red light wavelengths and reflect or transmit green light (see Figure 2.43). This is why photosynthetic tissues in autotrophs appear green. However, the energy transformation efficiency in photosynthesis is limited as more than half of the incident sunlight is composed of wavelengths too long to be absorbed, and some of the remainder is reflected or lost to surrounding tissues. Other pigments improve energy efficiency, but more than half is lost.

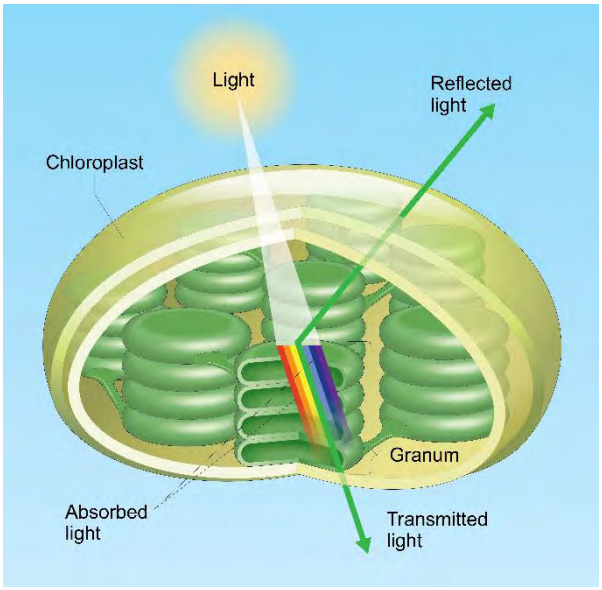


Figure 2.43: Interaction of light with chloroplast

2.6: Metabolic Pathways

Cell metabolism is critical to the survival of cells.

Biochemical processes in the cell are influenced by the nature and arrangement of internal membranes and the presence of specific enzymes.

- Explain how the structure of internal membranes of mitochondria and chloroplasts facilitates some biochemical processes.
- Explain that in a metabolic pathway:
  - there are many regulated steps;
  - each step loses some energy as heat;
  - some steps produce intermediate compounds;
  - specific enzymes are required at each step.

Biochemical processes in the cell are influenced by environmental factors.

Chemicals can interfere with cell metabolism.

- Discuss possible benefits and/or harmful effects of chemicals that human beings use.

The cells of living things are an aqueous solution filled with colliding molecules. When two molecules collide with the activation energy, a chemical reaction occurs, and their atoms are rearranged into new substances. Each cell is a highly chemically active environment, with billions of **metabolic reactions** occurring each second. Most reactions in cells are part of a **metabolic pathway**, a highly organised sequence in which one or more reactants are altered in a series of defined steps that produce one or more products. Some metabolic pathways, called **catabolic pathways**, break down complex molecules into simpler compounds, releasing energy for cellular work. For example, one major catabolic pathway is aerobic respiration, which breaks down glucose in the presence of oxygen to carbon dioxide and water, releasing energy cells use to synthesise ATP. Other metabolic pathways, called **anabolic pathways**, use energy from the hydrolysis of ATP to build more complex molecules from simpler ones. For example, translation is an anabolic pathway using energy to assemble larger polypeptide molecules from amino acids. Regardless of the type of metabolic pathway, each step is catalysed by a specific enzyme that speeds up the reaction (Figure 2.63). Metabolic pathways require several enzymes, as each rarely catalyses more than one reaction. Without enzymes, metabolic pathways would become congested as each reaction would occur too slowly. Cells exert control over their metabolic pathways by regulating enzyme activity. For example, a metabolic pathway is stopped by inhibiting one or more enzymes and started by increasing their concentration.

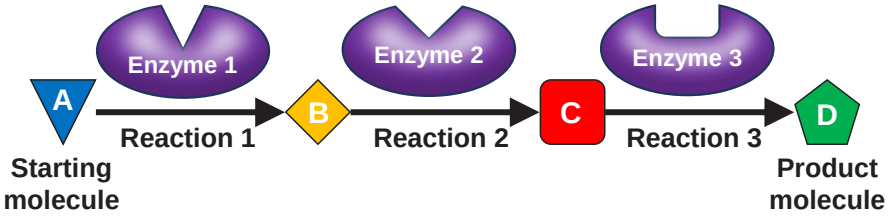


Figure 2.63: A typical metabolic pathway

Internal Membranes of Mitochondria and Chloroplasts

A cell is not just a bag of chemicals with thousands of different kinds of enzymes and substrates in a random mix. Instead, cells are compartmentalised, and their internal structures help bring order to metabolic pathways. In some cases, several enzymes of a metabolic pathway are assembled into a group that facilitates the sequence of reactions, with the product from the first enzyme becoming the substrate for an adjacent enzyme in the group, and so on, until the end product is released. In addition, some enzymes have fixed locations within membranes, while others move freely within the cytosol or fluid within an organelle. For example, in eukaryotic cells, the enzymes for the third stage of aerobic respiration reside in the inner mitochondrial membrane, as shown in Figure 2.65. These enzymes catalyse sequential steps in a metabolic pathway that concludes with ATP synthesis. The structure of the mitochondria supports its function in facilitating aerobic respiration. The inner mitochondrial membrane is highly folded into finger-like projections called cristae that significantly increase its SA:V and the concentration of enzymes that facilitate ATP synthesis.

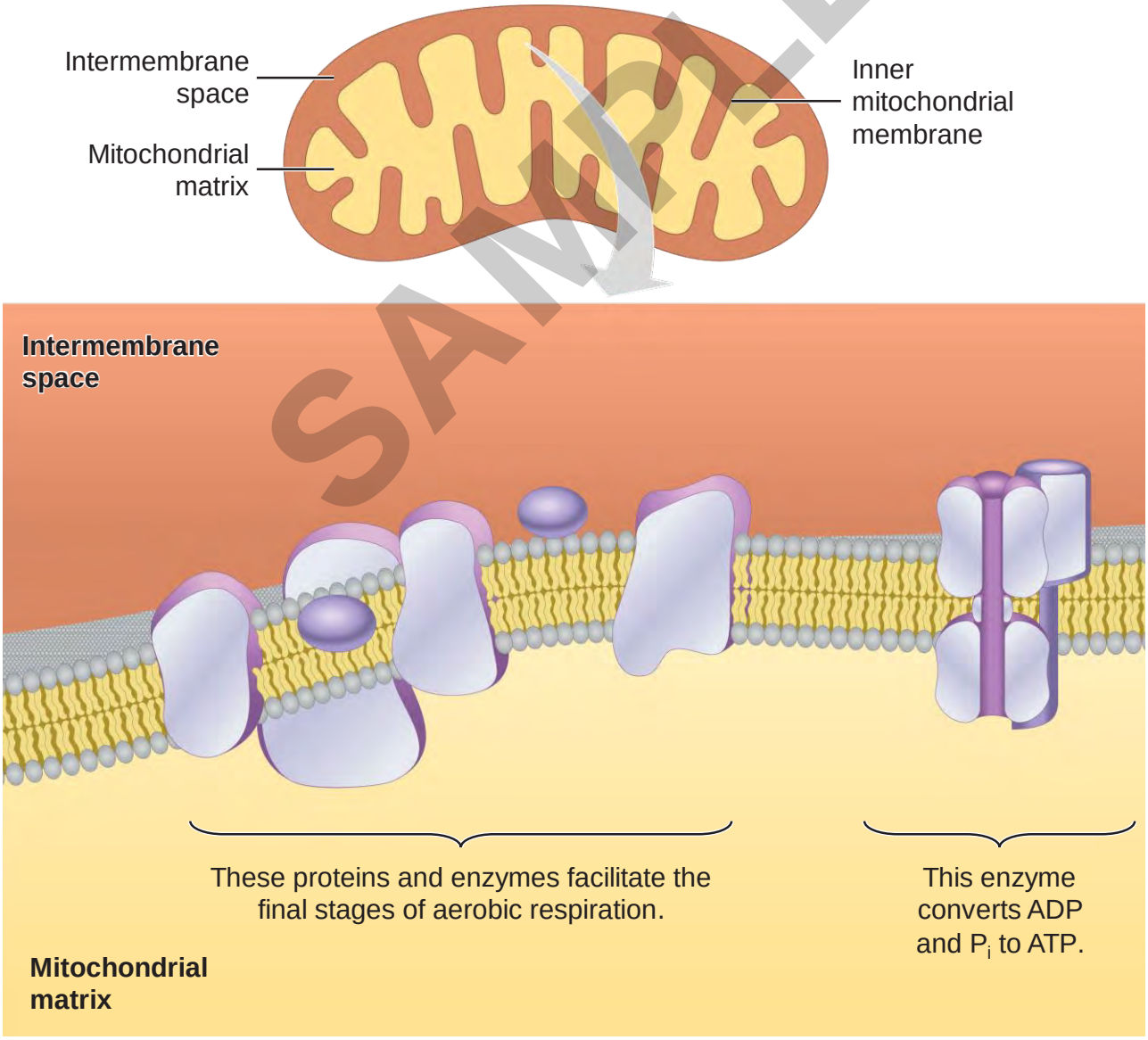


Figure 2.65: Enzymes in the inner mitochondrial membrane.

Similarly, the thylakoid membranes of chloroplasts facilitate a metabolic pathway in photosynthesis. Firstly, thylakoid membranes contain **photosystems**, light-harvesting complexes of proteins and various pigments like chlorophyll that capture light (Figure 2.66) for photosynthesis. Second, thylakoid membranes contain enzymes that synthesise ATP (Figure 2.67). The highly folded thylakoid membrane increases its SA:V, the concentration of pigments that capture light, and enzymes that facilitate ATP synthesis.

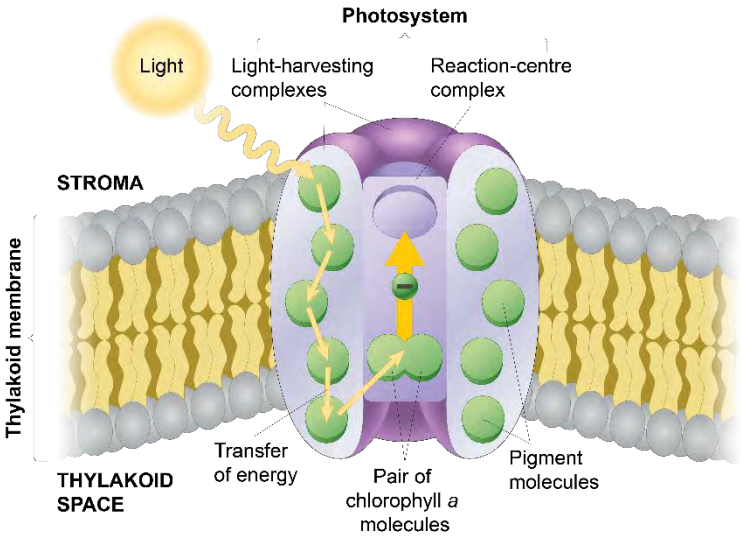


Figure 2.66: Photosystem structure

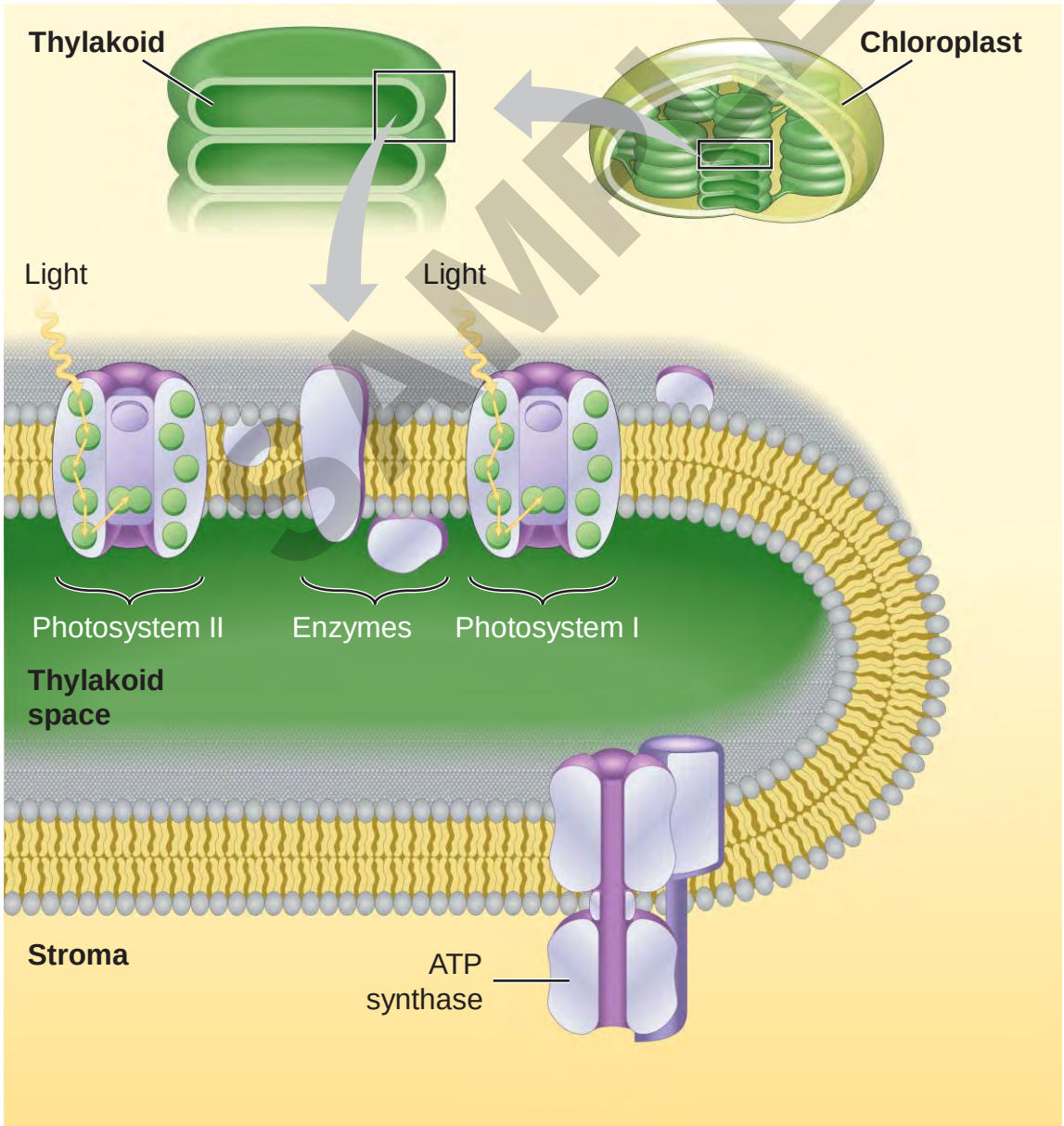


Figure 2.67: Enzymes in the thylakoid membrane of a chloroplast.

Environmental Factors and Metabolism

Metabolic pathways are affected by a range of environmental factors. Firstly, high temperatures denature enzymes, and low temperatures reduce the rate of metabolic reactions. Second, enzymes are sensitive to pH changes which distort their tertiary structure and alter the shape of their active sites, reducing their ability to bind substrates. For this reason, the enzymes in a metabolic pathway are often found in specialised compartments or organelles with a pH close to their optimum. Third, the reactions in a metabolic pathway proceed at their optimum rate, provided that neither enzyme nor substrate concentration is a limiting factor. Finally, the rates of reactions in metabolic pathways are affected by enzyme inhibitors. In most cases, cells use enzyme inhibition is used as a mode of metabolic control, called **feedback inhibition**. In such cases, one or more end products of a metabolic pathway will bind to and inhibit enzymes that catalyse reactions early in the same pathway. For example, Figure 2.68 shows an example of feedback inhibition operating on a five-step metabolic pathway to synthesise the amino acid isoleucine from threonine, another amino acid. As isoleucine accumulates, it slows down its synthesis by inhibiting the enzyme for the first step of the pathway. Feedback inhibition prevents the cell from making more isoleucine than is required, thus wasting raw materials.

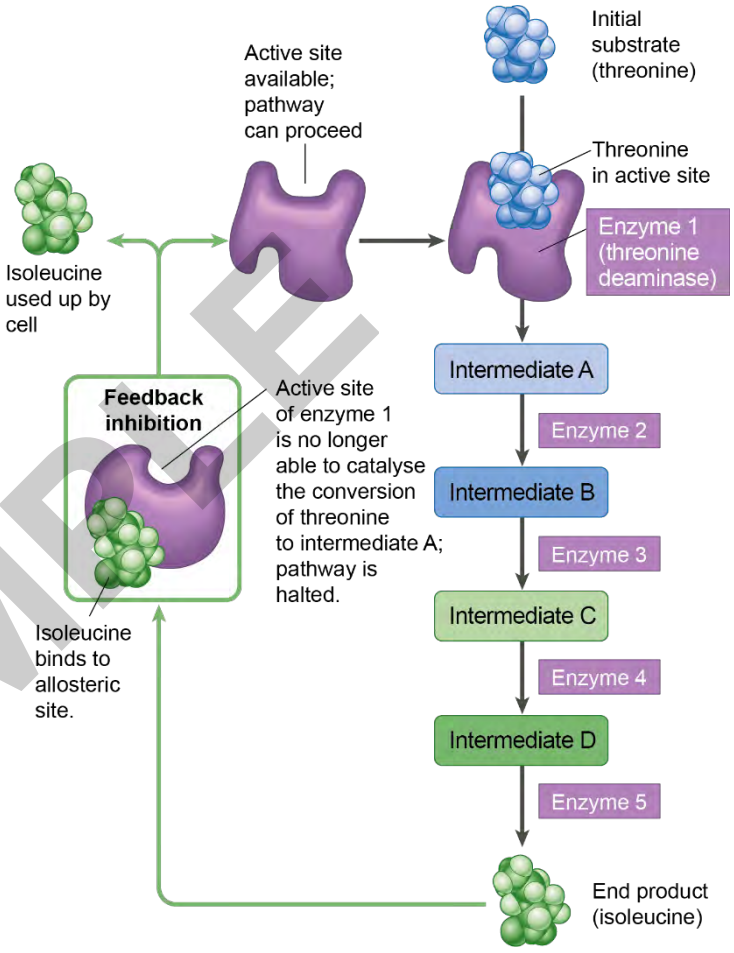


Figure 2.68: Feedback inhibition

In other cases, certain chemical substances taken in from the environment can dramatically affect metabolism by inhibiting enzymes key to one or more pathways. For example, cyanide compounds (CN^-) affect aerobic respiration by irreversible inhibition of cytochrome c oxidase (Cyt C in Figure 2.69), an enzyme key to ATP synthesis in most living things. Similarly, many antibiotics inhibit ribosome function, preventing the synthesis of enzymes that facilitate several key metabolic pathways in bacteria, including respiration.

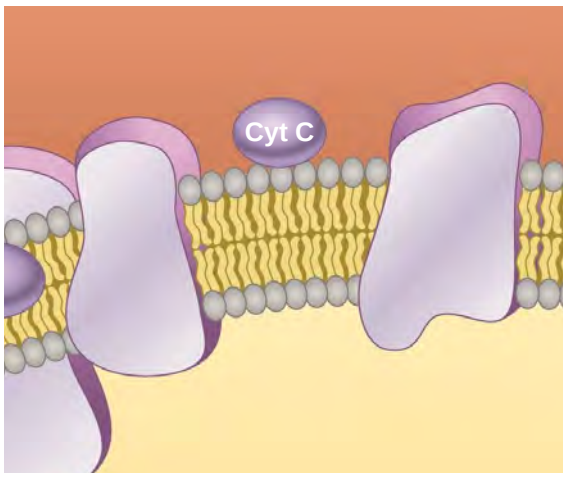
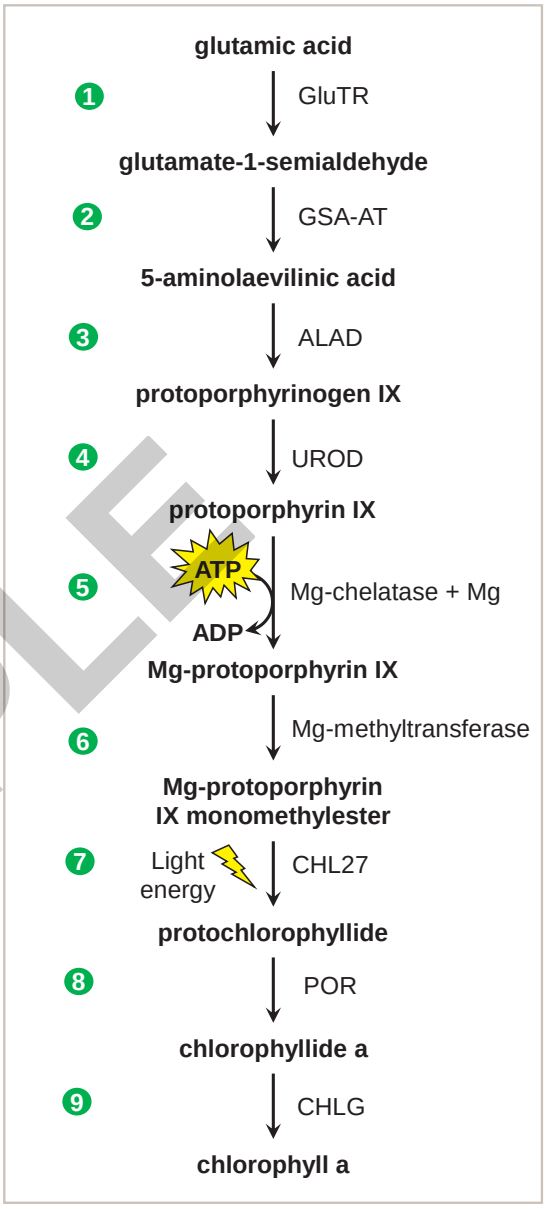


Figure 2.69: Inhibition by cyanide

Question 125

The diagram below illustrates the metabolic pathway that produces chlorophyll in plants.



(a) Explain why a different enzyme is required to catalyse each step in the metabolic pathway.

(2 marks) **KA1**

(b) **Step 2** produces 5-aminolevulinic acid (5ALA). Explain why plant growth rate increases when leaves are sprayed with low doses of 5ALA.

(2 marks) **KA1**

(c) **Step 5** produces Mg-protoporphyrin IX from magnesium (Mg) and protoporphyrin IX. State two factors limiting **Step 5**.

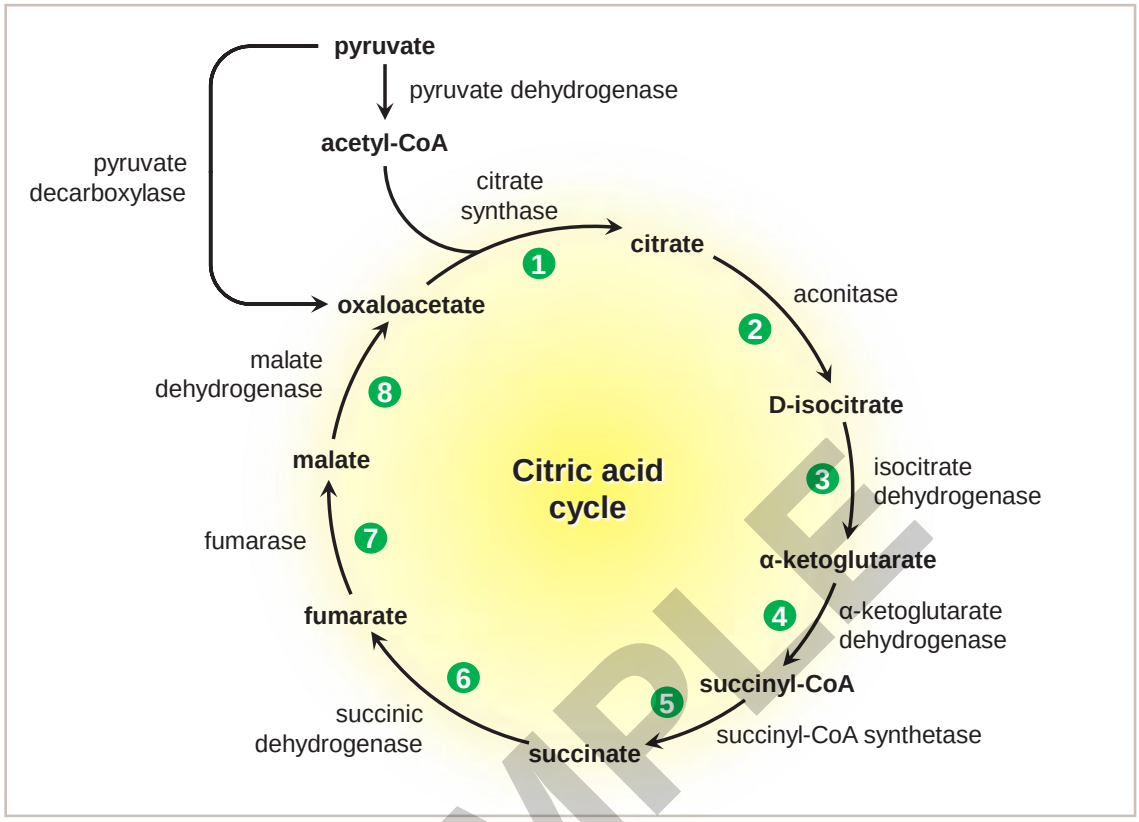
(2 marks) **KA2**

(d) Chlorophyll molecules produced in the pathway are embedded in thylakoid membranes. Explain how the structure of thylakoid membranes facilitates chlorophyll function.

(2 marks) **KA2**

Question 127

The diagram below shows the citric acid cycle, a metabolic pathway in aerobic respiration.



(a) The citric acid cycle involves more than ten different enzymes and substrates.

(1) Name the enzyme that catalyses the formation of malate.

_____ (1 mark) KA4

(2) Name the substrate of isocitrate dehydrogenase.

_____ (1 mark) KA4

(b) Acetyl-CoA inhibits pyruvate dehydrogenase.

State how this process regulates the citric acid cycle.

_____

_____ (1 mark) KA2

(c) Fluoroacetic acid is a herbicide that inhibits aconitase.

(1) State how fluoroacetic acid reduces the growth rate of weeds.

_____

_____ (1 mark) KA2

(2) State one possible consequence of using fluoroacetic acid on weeds.

_____

_____ (1 mark) KA2

2.7: Cell Division and Asexual Reproduction

Cells arise from pre-existing cells, and cell division leads to an increase in cell number.

Cell division in somatic cells is different from the cell division that produces gametes from germ-line cells.

Continuity of life requires the replication of genetic material and its transfer to the next generation through processes including binary fission, mitosis, meiosis, and fertilisation.

- Explain why the amount of DNA in a cell doubles before division.

The products of binary fission and mitotic division have the same number and type of chromosomes as the parent.

- Recognise, describe, and represent the process of binary fission in prokaryotic cells.
- Recognise, describe, and represent the process of mitosis in eukaryotic cells.
- Compare the products of binary fission and mitotic division.

Growth, the increase in cell number, and reproduction, the ability to produce more of their kind, are two characteristics that best distinguish living and nonliving things. These processes divide a pre-existing cell, called the **parent cell**, into two daughter cells. This chapter explores the process of cell division and how it ensures the continuity of life.

The Functions of Cell Division

Cell division serves several vital functions in living things. In unicellular organisms such as prokaryotes and some eukaryotes such as the amoeba shown in Figure 2.70 1, cell division produces a new organism (reproduction). In multicellular eukaryotes, cell division enables an organism to develop from a single cell—the fertilised egg or **zygote**. Following fertilisation, the zygote divides by cell division into two cells (Figure 2.70 2) and then four, forming an embryo that develops through cell division and differentiation into an adult organism. In addition, cell division facilitates renewal and repair in fully grown multicellular eukaryotes, replacing older or damaged cells that die from accidents or normal wear and tear ((Figure 2.70 3). Finally, in animals, cell division produces an army of white blood cells that facilitate the immune response.

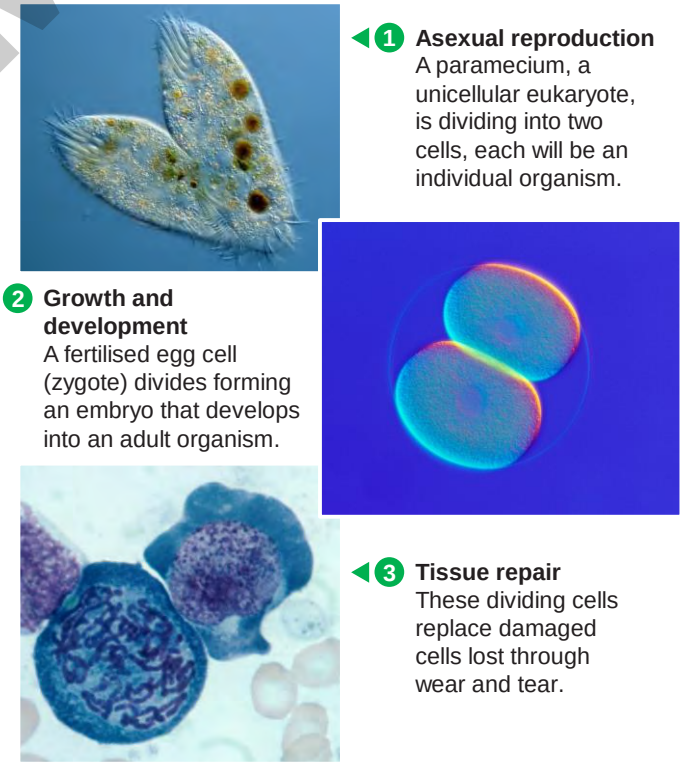


Figure 2.70: The functions of cell division

Mitosis

In eukaryotes, the division of a somatic cell occurs in two phases: **mitosis**, the division of the nucleus, and **cytokinesis**, the division of the cytoplasm. Mitosis occurs in four distinct stages: **prophase**, **metaphase**, **anaphase**, and **telophase,** with cytokinesis overlapping the latter two stages. In early prophase, the chromatin fibres become more tightly coiled, condensing into discrete chromosomes observable with a light microscope. Each replicated chromosome appears as two identical sister chromatids joined at their centromeres and all down their sides by cohesins. As the chromosomes condense, the nucleus breaks down; first, the nucleoli disappear and then the nuclear envelope fragments. In animal cells, a pair of centrosomes (Figure 2.72 1) begin producing microtubules called **spindle fibres** that extend outwards, forming a mitotic spindle structure. The centrosomes migrate away from each other, partly propelled by lengthening spindle fibres, until they reach opposite poles of the cell (Figure 2.72 2). In seed plants, spindle fibres are assembled by **microtubule organising centres (MTOCs)** on the cytoplasmic side of the nuclear envelope during prophase and are gradually organised into a mitotic spindle that resembles that of an animal cell at the end of prophase (Figure 2.72 3). Each of the two sister chromatids of a replicated chromosome has a **kinetochore**, a structure made up of proteins assembled on specific sections of DNA at each centromere. The chromosome's two kinetochores face in opposite directions. At the start of metaphase, some spindle fibres attach to the kinetochores and organise the chromosomes on the **metaphase plate**, a plane midway between the spindle's two poles (Figure 2.72 4). Anaphase begins when the cohesin proteins are cleaved, allowing the two sister chromatids of each pair to part suddenly. Each chromatid becomes an independent chromosome as it is moved toward opposite ends of the cell by shortening spindle fibres (Figure 2.72 5). By the end of anaphase, the two ends of the cell have identical and complete collections of chromosomes. At the start of telophase, two daughter nuclei form in the dividing cell. Nuclear envelopes are assembled around the chromosomes from fragments of the parent cell's envelope. The nucleoli reappear, and the chromosomes become less condensed. Any remaining spindle microtubules are broken down.

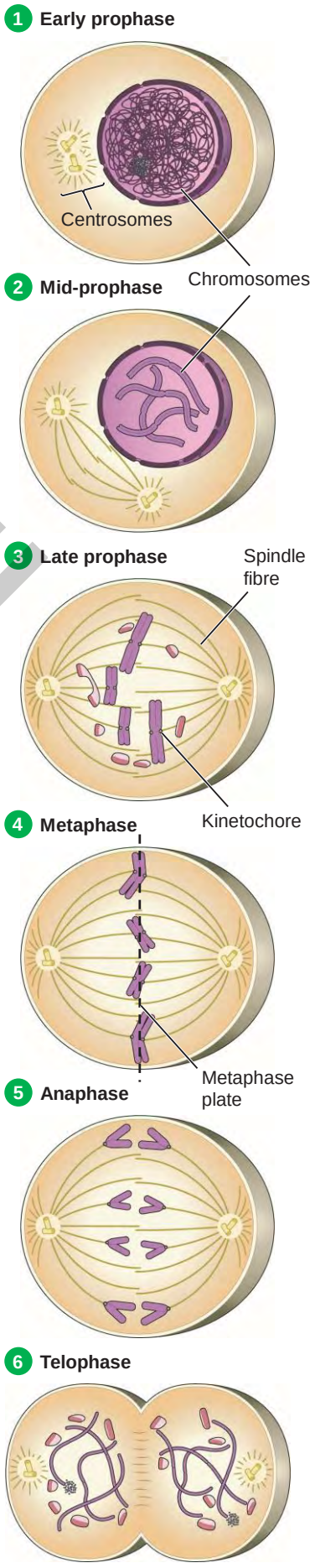


Figure 2.72: Mitosis in animal cells

Cytokinesis

Cytokinesis begins in anaphase and ends just after telophase. In animal cells, cytokinesis occurs through a process known as **cleavage**. The first sign of cleavage is the appearance of a **cleavage furrow**, a shallow indentation in the cell surface near the old metaphase plate (Figure 2.73 1). On the cytoplasmic side of the furrow is a **contractile ring** (Figure 2.73 1) of actin microfilaments associated with molecules of the protein myosin. The actin microfilaments interact with the myosin molecules, causing the ring to contract. The contraction of the dividing cell's ring of microfilaments is like the pulling of a drawstring that deepens the cleavage furrow until the parent cell is pinched in two, producing two wholly separated cells (Figure 2.73 2), each with a nucleus and a share of the parent cell's cytosol, organelles, and other cellular structures.

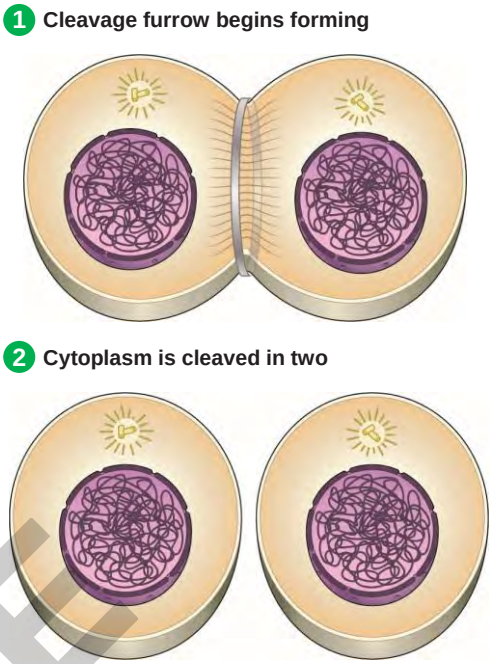


Figure 2.73: Cytokinesis in animal cells

Most plant cells are enclosed by a semi-rigid cell wall that prevents changes in cell shape from occurring during cytokinesis in animal cells. Rather than a contractile ring dividing the cytoplasm from the outside in, the plant cell's cytoplasm is partitioned from the inside out by constructing a new cell wall, called the **cell plate**, between the two daughter nuclei. During telophase, small vesicles, primarily derived from the Golgi body and filled with polysaccharide and glycoproteins required for cell wall synthesis, are transported along the cytoskeleton to the middle of the cell, where the contents are assembled into the cell plate (see Figure 2.74 1 and 2). The cell plate enlarges and grows outwards until its membrane fuses with the cell membrane along the perimeter of the dividing cell. A new cell wall develops from the cell plate as it matures, dividing the parent cell into two daughter cells, each with a cell membrane. The two phases of cell division in eukaryotes, collectively known as **mitotic cell division**, produce two daughter cells genetically identical to the parent. From a fertilised egg or zygote, mitotic cell division produces the billions or trillions of somatic cells that make up the body of a multicellular eukaryote human body. The same processes generate new cells to replace dead and damaged ones.

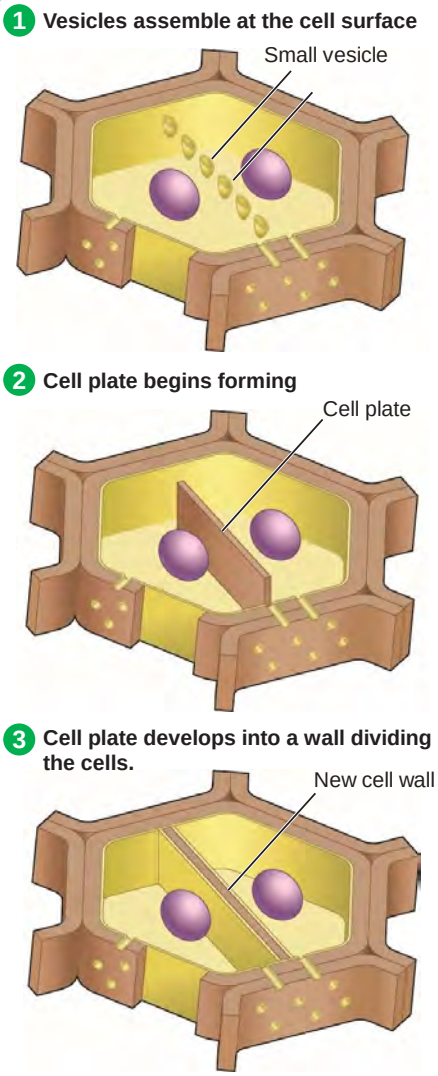


Figure 2.74: Cytokinesis in plant cells

Sets of Chromosomes

The DNA of a eukaryotic cell is packaged into chromosomes within the nucleus. One chromosome includes several hundred to a few thousand genes, each a precise sequence of nucleotides at a specific location along the length of a chromosome called its **locus**. Every eukaryotic species has a characteristic number of chromosomes. For example, human somatic cells have 46 chromosomes. Careful examination of the 46 human chromosomes from a single somatic cell reveals two chromosomes of each of 23 types, a feature that becomes clear when images of the chromosomes are arranged in pairs starting with the longest chromosomes, an ordered display called a **karyotype** (Figure 2.77). The two chromosomes of a pair, called **homologous chromosomes**, have the same length, centromere position, and gene loci. Both chromosomes of a homologous pair carry genes controlling the same inherited characters. For example, suppose a gene for eye colour is situated at a particular locus on a specific chromosome. In that case, its homologous chromosome will also have a version of the eye-colour gene at the equivalent locus. The occurrence of pairs of homologous chromosomes in each human somatic cell is a consequence of sexual reproduction. We inherit one chromosome of a pair from each parent. Thus, the 46 chromosomes in our somatic cells are actually two sets of 23 chromosomes—a maternal set from our mother and a paternal set from our father. The number of chromosomes in a single set is represented by n. Any cell with two sets of chromosomes is called a **diploid cell** and has a diploid number of chromosomes, abbreviated **2n**. For humans, the diploid number is 46 (2n = 46), the number of chromosomes in our somatic cells. In a cell in which DNA synthesis has occurred, all chromosomes are duplicated, and therefore each consists of two identical sister chromatids, associated closely at the centromere, as shown in Figure 2.78. Unlike somatic cells, gametes contain a single set of chromosomes. Such cells are called **haploid cells**, each having a haploid number of chromosomes (n). For humans, the haploid number is 23 (n = 23).

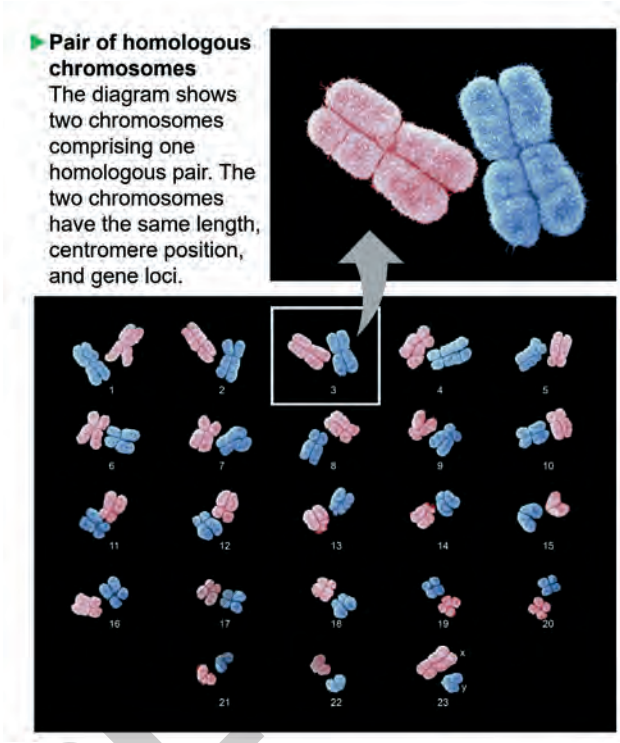


Figure 2.77: Human somatic cell karyotype

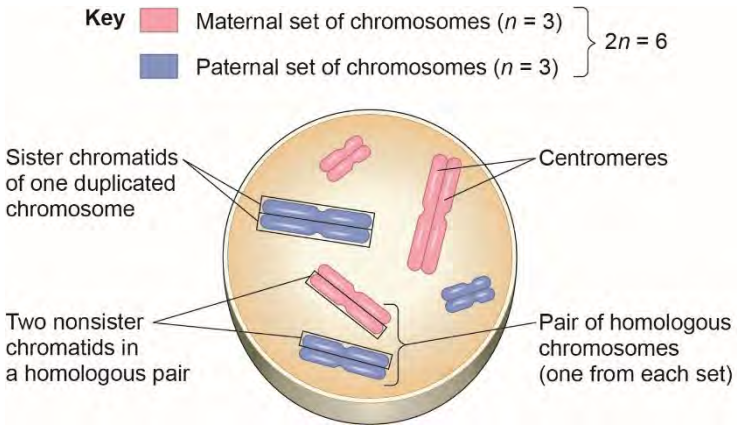


Figure 2.78: Homologous chromosomes

Meiosis

Several steps of meiosis closely resemble corresponding steps in mitosis. Meiosis, like mitosis, is preceded by chromosome duplication by DNA replication. However, in meiosis, this is followed by not one but two consecutive cell divisions, called **meiosis I** and **meiosis II**. These two divisions result in four daughter cells (rather than the two daughter cells of mitosis), each with only half as many chromosomes as the parent cell—one set rather than two. The overview of meiosis in Figure 2.80 shows that for a single pair of homologous chromosomes in a diploid cell, both members of the pair are duplicated, and the copies are sorted into four haploid daughter cells. Recall that sister chromatids are two copies of one chromosome, closely associated all along their lengths. The sister chromatids comprise one duplicated chromosome (see Figure 2.80). In contrast, the two chromosomes of a homologous pair, called **homologs**, are individual chromosomes inherited from each parent. Homologs appear alike under the microscope, but each may have a different version of a gene, called alleles, at corresponding loci. For example, one chromosome might have an allele for freckles, but its homologous pair may have an allele for the absence of freckles at the same locus. Before the start of meiosis I in germ cells, the chromosomes are decondensed and surrounded by a nuclear envelope. At the start of **prophase I**, the nuclear envelope is broken down, the chromosomes begin condensing, and each pairs with its homolog. The homologs align themselves gene by gene, allowing a process called **crossing over** to occur. During crossing over, the DNA molecules of nonsister chromatids are broken by enzymes, and the resulting fragments are re-joined, forming new combinations of alleles on each homolog (a process explored in more detail shortly). The overlapping points where crossing over occurs are

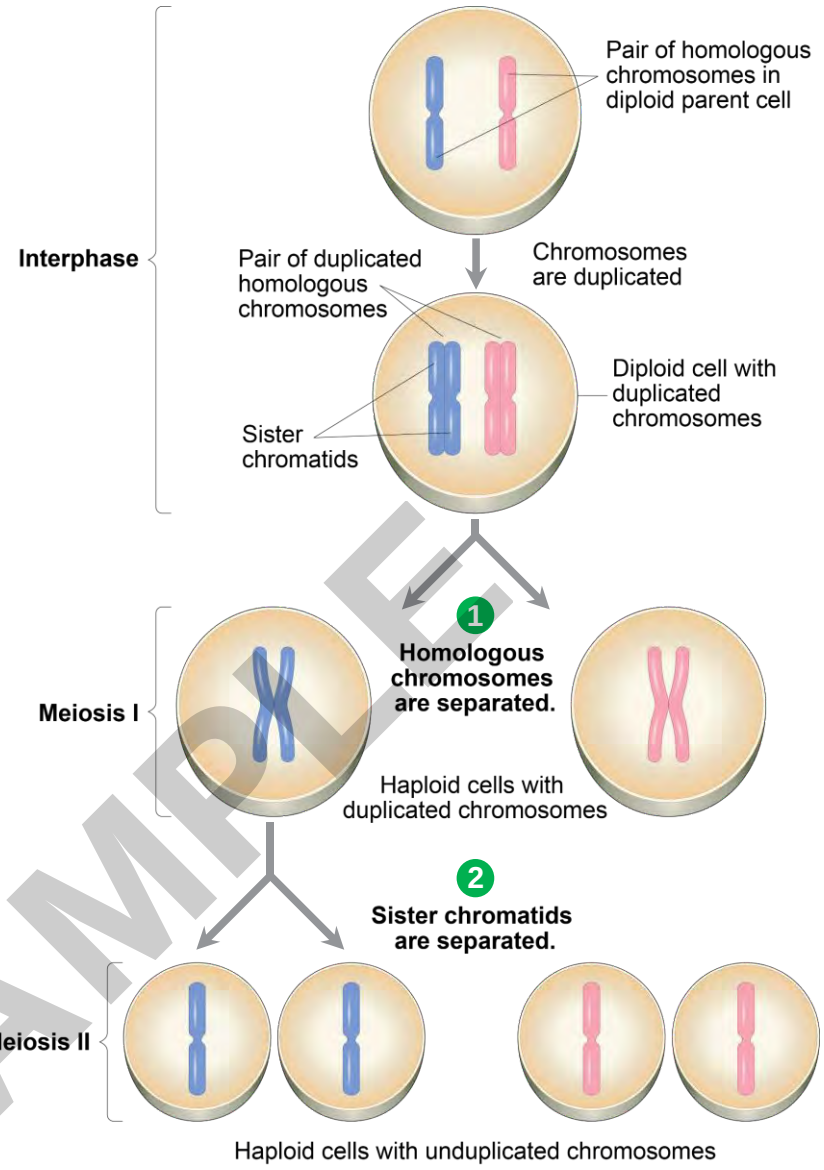


Figure 2.80: Meiosis I and II

called **chiasmata** (Figure 2.81❶). At the same time, centrosomes in animals or MOTCs in plants begin producing spindle fibres that attach to the kinetochores of each homolog. Spindle fibres then move the homologous pairs toward the metaphase I plate (Figure 2.81❷). During **metaphase I**, pairs of homologous chromosomes are arranged at the metaphase plate, with one chromosome of each pair facing each pole of the cell. Furthermore, each pair has lined up independently of other pairs; an arrangement called **independent assortment** (to be discussed later.) Both chromatids of one homolog are attached to spindle fibres from one pole, and the chromatids of the other homolog are attached to spindle fibres from the opposite pole (Figure 2.81❷). At the start of **anaphase I**, homologs are moved toward opposite ends of the cell by shortening spindle fibres (Figure 2.81❸). The sister chromatids of each homolog remain joined at the centromere, causing the two chromatids of each chromosome to move as a unit toward the same pole. When **telophase I** begins, each half of the cell has a complete haploid set of duplicated chromosomes. The sister chromatids of the duplicated chromosomes typically include regions of nonsister chromatid DNA from crossing over, as seen in Figure 2.81❹. In some eukaryotes, the chromosomes decondense and the nuclear membrane reforms from fragments of the parent cell's envelope. In most eukaryotic cells, **cytokinesis I** coincides with telophase I, forming two haploid daughter cells. In animal cells like those shown in Figure 2.81❹, a cleavage furrow forms, and in plant cells, a cell plate forms that develops into a cell wall dividing the cells.

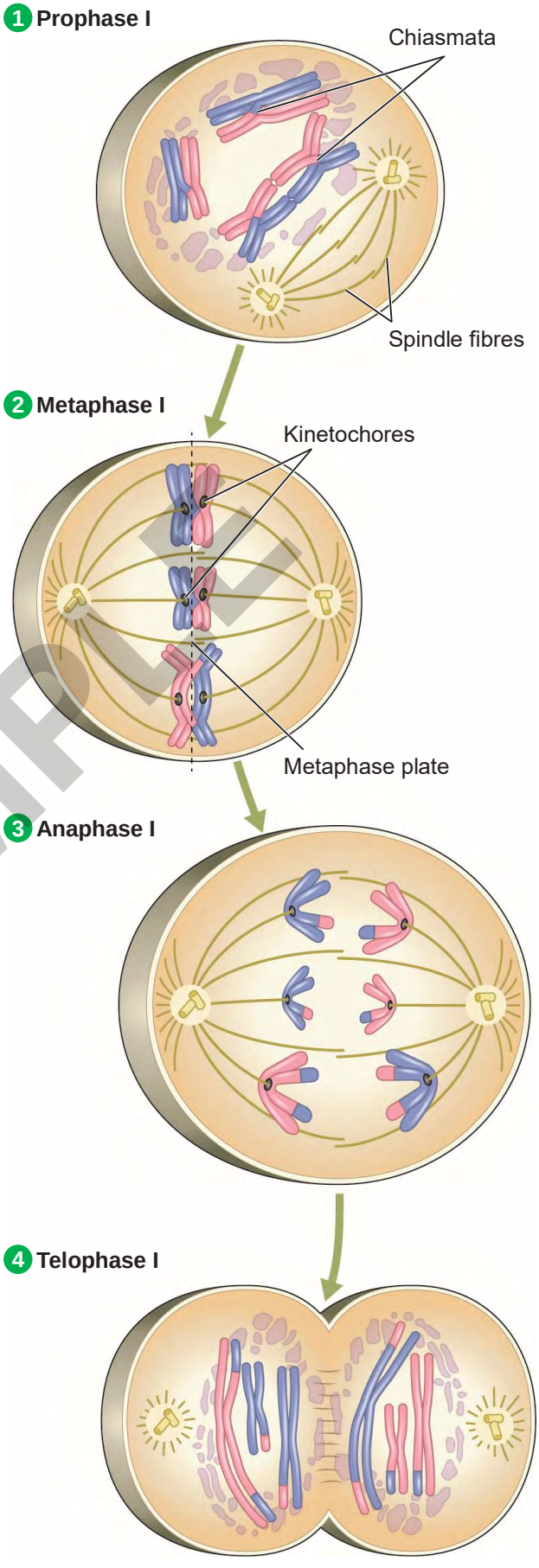


Figure 2.81: Stages of Meiosis I

Comparing Mitosis and Meiosis

Mitotic and meiotic cell division are similar processes in diploid cells with some key differences. **Firstly**, meiosis produces four cells and reduces the number of chromosome sets from two (diploid) to one (haploid), whereas mitosis produces two cells and conserves the number of chromosome sets. **Second**, meiosis produces cells that differ genetically from the parent cell and each other. In contrast, mitosis produces daughter cells genetically identical to their parent cell and each other. Three events unique to meiosis occur during meiosis I. The first is in prophase I where duplicated homologs pair up and crossing over occurs. In contrast, crossing over does not occur in mitosis. The second is the alignment of homologous pairs of chromosomes on the metaphase plate. At metaphase I of meiosis, pairs of homologs are positioned at the metaphase plate, rather than individual chromosomes, as in metaphase of mitosis. The third is the separation of homologs. At anaphase I of meiosis, the duplicated chromosomes of each homologous pair move toward opposite poles, but the sister chromatids of each duplicated chromosome remain attached. In anaphase of mitosis, by contrast, sister chromatids separate. The key differences between mitosis and meiosis are summarised in Figure 2.86.

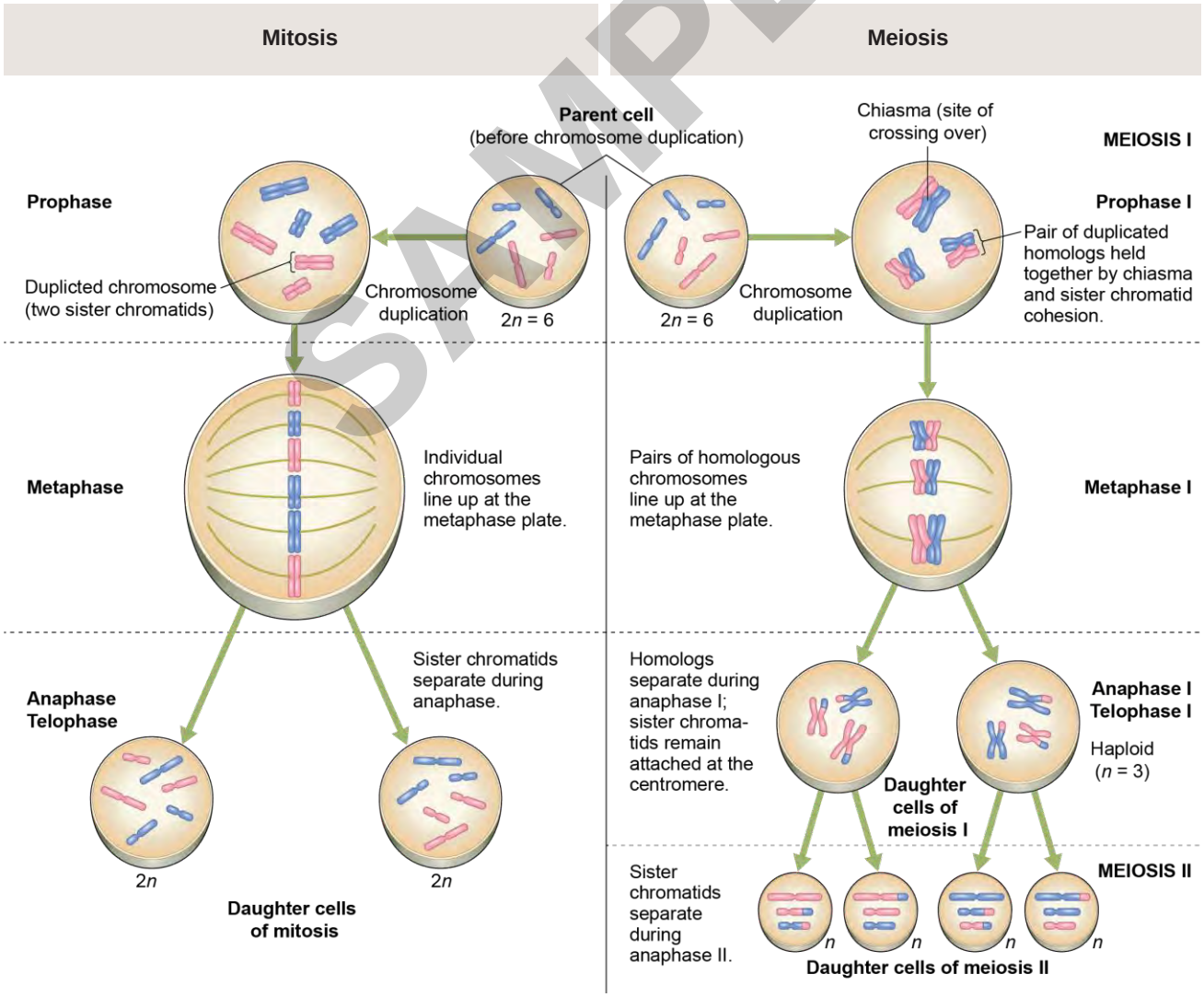


Figure 2.86: Comparing mitosis and meiosis

Question 140

The diagram below shows the production of human sperm cells from germ cells.

(a) Process Z increases the number of germ cells that undergo meiosis.

Name process Z.

_____ (1 mark) KA2

(b) Explain how the chromosome number is halved during meiosis I.

_____

_____

_____

_____ (2 marks) KA1

(c) Describe the events occurring in meiosis II.

_____

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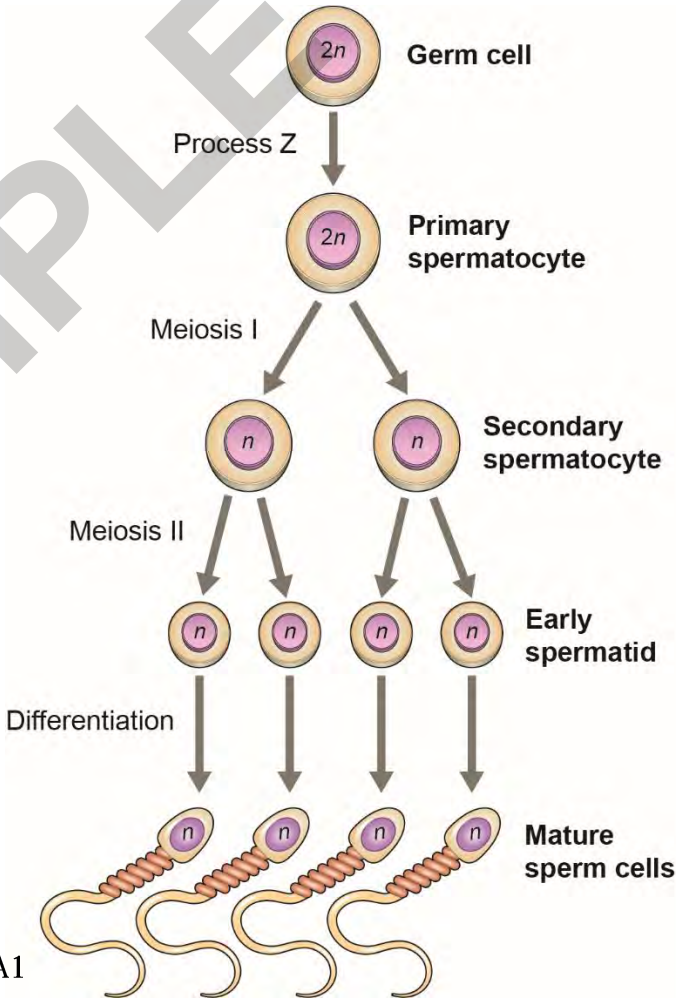
_____ (4 marks) KA1



(d) State two differences between meiosis I and II in spermatogenesis.

_____

_____

_____

_____ (2 marks) KA1

Question 141

The diagram below shows the production of human egg cells from germ cells.

(a) State when meiosis I begins in the life cycle of a human female.

_____ (1 mark) KA2

(b) A hormone is released at puberty which causes a primary oocyte to complete meiosis I.

Describe the events occurring in a primary oocyte during meiosis I.

_____

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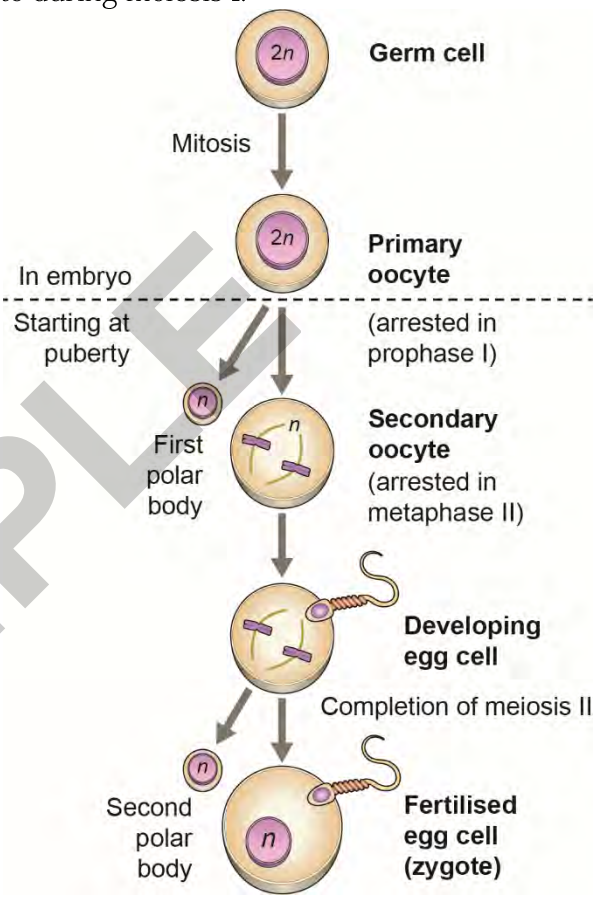
_____ (4 marks) KA1



(c) Meiosis I produces secondary oocytes that remain arrested in metaphase II.

During ovulation, a secondary oocyte is released that may be fertilised by a sperm cell.

(1) Suggest why secondary oocytes remain in metaphase II until penetrated by a sperm cell.

_____

_____ (1 mark) KA2

(2) Human females have two ovaries, each containing 1-2 million primary oocytes, around 500 developing into secondary oocytes released between puberty and menopause.

Explain how this process contributes to genetic variation in human offspring.

_____

_____

_____

_____ (2 marks) KA2

2.10: Cell Culture

Human beings culture cells for a variety of purposes.

- Describe techniques of cell culture and discuss the applications and limitations of contemporary examples.

Along with advances in DNA technology and biotechnology, scientists have developed and refined methods for growing unicellular organisms or whole multicellular organisms from single cells. The process of removing cells from their natural environment and growing them in a favourable artificial environment is called **cell culture**. Humans have cultured unicellular microorganisms for thousands of years, including yeast, to produce bread and alcohol. However, over the past hundred years, scientists have developed techniques for culturing plant and animal cells. This chapter explores the techniques, applications, and limitations of cell culture.

Cell Culture Techniques

The first step in producing cultured cells is to remove tissue cells from the target organism. Scientists typically extract tissue cells using sharp scissors or scalpels sterilised with alcohol or some other disinfectant to inhibit the growth of microorganisms that would contaminate the culture. Next, the extracted tissue is digested with enzymes to break down the connective proteins between cells, allowing them to separate. The isolated tissue cells are then transferred to a suitable **growth medium**, a reaction flask containing nutrients, respiratory gases, water, a suitable pH and other substances like antibiotics that promote the growth of cultured cells while inhibiting the growth of contaminating microorganisms (Figure 2.96). The type of reaction flask depends on whether the cell is anchorage-dependent. For example, most human cells are anchorage-dependent and must be grown in a flat flask or dish to provide a solid surface for cell growth. In contrast, anchorage-independent cell types may be grown in a liquid suspension. Finally, specific growth factors are added, and the cells are grown at their optimum temperature in an incubator.

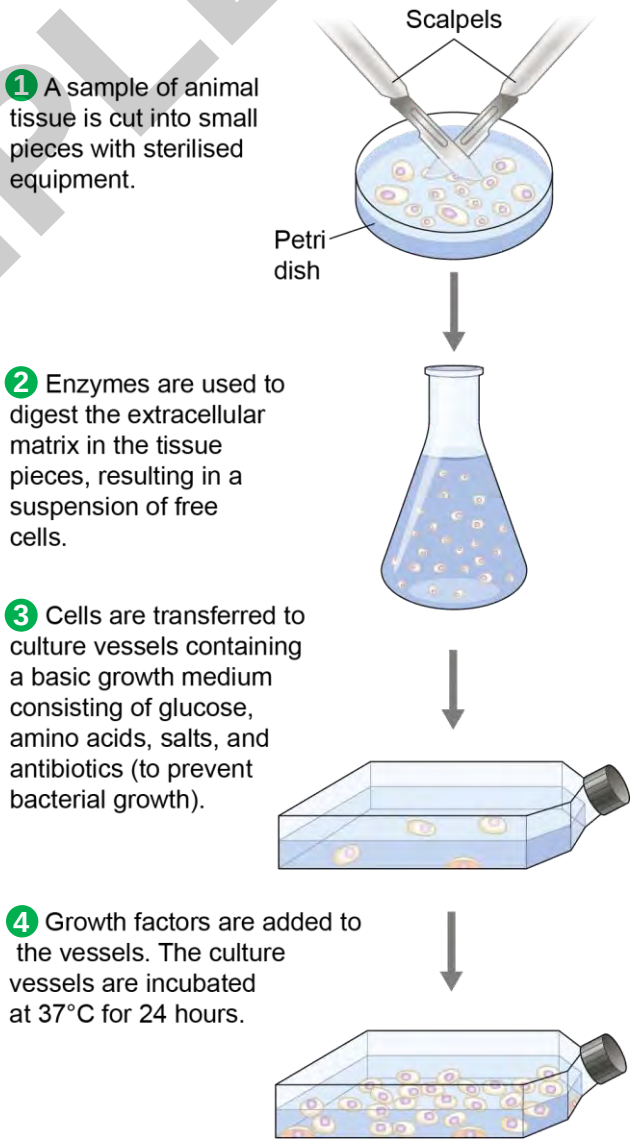


Figure 2.96: Cell culture techniques.

Applications and Limitations of Cell Culture

Cell culture produces cloned cells that are genetically identical to the "parent" cell. Cultured cells have a range of applications, from research to the production of therapeutic goods or other commercial products. One of the most important uses of cultured cells is biological research. Scientists culture different cell types to study their structures and various biological functions. For example, biologists deliberately mutate specific genes to study the effect of such mutations on metabolic pathways. This research has helped biologists better understand the effect of mutations on metabolism. Furthermore, scientists can culture cancer cells to study their modified structures and functions to understand cancer better and discover new treatment methods. Another active area of research is **vaccine production**. Scientists infect cultured cells with different viruses to produce vaccines that save millions of lives each year. Cultured animal cells have also been used to test the efficacy and safety of various commercial products, namely new drugs and cosmetics. Testing the short and long-term effects of different concentrations of a drug or cosmetic helps pharmaceutical scientists to identify the appropriate concentration to achieve the desired outcome while avoiding toxicity. Cell culture also plays a significant role in the development of new life. For example, fertilised egg cells and embryos produced with **in vitro fertilisation (IVF)** are cultured before being implanted in the mother's uterus. Perhaps the most critical area of research using cell culture is the production of **stem cells**, undifferentiated cells that can reproduce indefinitely and, under appropriate conditions, differentiate into specialised cells of one or more types, giving them great potential for regenerating damaged tissues. Stem cells derived from human embryos can differentiate into all cell types in culture depending on the types of growth factors present. Figure 2.97 illustrates some possible cell types produced with stem cells. Cell culture has also been used extensively in agriculture. Scientists can now produce new plants from differentiated tissue cells, allowing them to produce cloned plants for food production. However, cell culture is limited by several factors. In addition to high costs, microbes and other cell types can easily contaminate a cell culture, reducing the validity of experimental data. Next, the rapid growth of cells in culture introduces unwanted mutations and genetic variation. Finally, non-cancerous cell lines growing in culture typically divide 20 to 50 times before they stop dividing and are no longer functional, limiting their long-term use and increasing replacement costs.

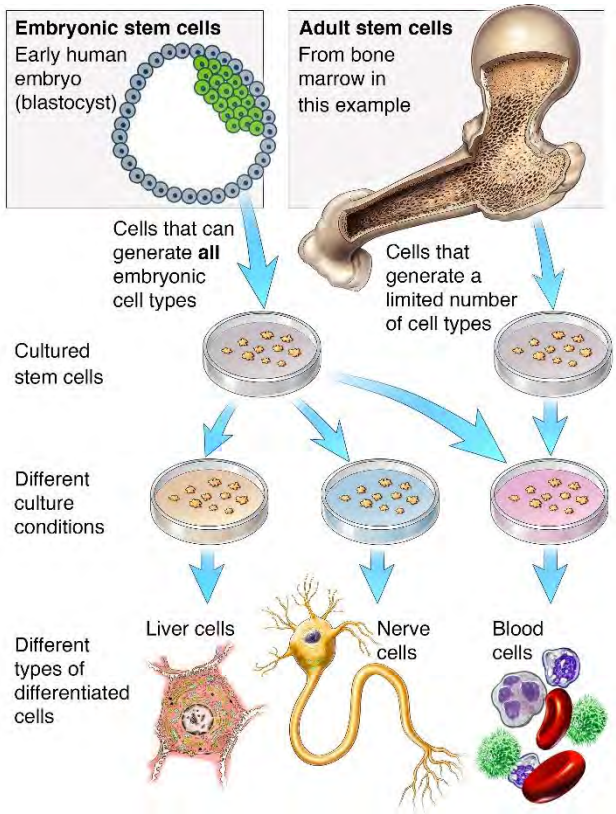


Figure 2.97: Cultured stem cells

3.1: Tolerance Limits

Organisms survive most effectively within their tolerance limits. Factors for which organisms have tolerance limits include:

- body temperature
- water availability
- blood glucose level
- carbon dioxide concentration in the blood and tissues.

There are impacts on an organism when conditions fall outside its tolerance limits.

Scientists estimate that nearly two million animal species inhabit our planet's diverse range of ecosystems. However, the geographical distribution of animals is not uniform; each is adapted to live with different environmental factors, including temperature, pH, salinity, and water and nutrient availability. Consequently, each animal species has a set of **tolerance limits** for certain factors before one or more cellular functions are impaired. This chapter introduces the factors for which animals have tolerance limits and explores the impacts on the organism when the limits are exceeded.

Body Temperature

Animals are complex multicellular organisms. However, like all living things, an animal's biological functions are determined by the metabolic reactions occurring in its cells. Enzymes control most of these metabolic reactions, and each has a tertiary structure that facilitates binding to one or more substrates. When the temperature of the cytosol is at the optimum for the enzyme, the substrate is rapidly converted to products that ensure the continuity of metabolic pathways. However, when the temperature is above or below the optimum, an enzyme's tertiary structure is altered (Figure 3.01) and no longer binds to its substrate effectively. Consequently, the rates of enzyme-controlled reactions in cells are slowing metabolism to a level that cannot sustain life. For this reason, animals preserve their body temperature within tolerance limits reflecting the optimum temperature for enzyme activity. For example, humans maintain an internal body temperature within narrow tolerance limits of 36°C and 38°C, reflecting the optimum temperature for enzyme activity (37°C).

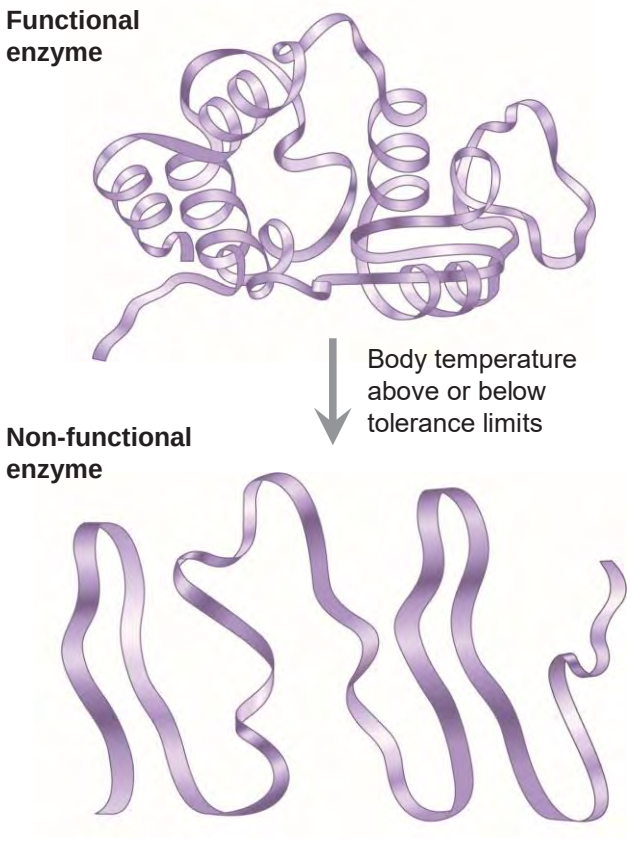


Figure 3.01: Effect of temperature on enzyme structure

Water Availability

Water fills animal cells and tissues, and its presence is vital for the continued function of cells. Water functions primarily as a solvent, filling the cytosol and organelles of animal cells and providing the aqueous medium and thermal stability required for enzyme activity. In addition, water functions as a chemical reactant in many metabolic reactions, such as the breakdown of ATP to release energy. For these reasons and more, animals must maintain a relatively constant volume of water in their cells and replace any water lost through metabolism or excretion by eating or drinking. However, maintaining a constant volume of water in animal cells is complicated, as water flows into and out of cells by osmosis, a process influenced by the **total solute concentration** or **osmolarity** of the cytosol and tissue fluid outside cells. Osmolarity is the total number of moles of solute per litre of solution. Therefore, osmolarity decreases when water is absorbed and increases when water is lost.

Animals must maintain osmolarity within narrow tolerance limits to prevent too much water from entering or exiting a cell. For humans, maintaining this fluid balance requires that the osmolarity of cells and tissues be kept between 275 and 295 mOsm kg^{-1}. When osmolarity is above the tolerance limit, cells become **hyperosmotic**, causing them to swell and burst as water flows in. Conversely, when osmolarity is below the tolerance limit, cells become **hypoosomotic**, causing them to shrivel as water flows out. In both examples, illustrated in Figure 3.02, the animal cell's functions are temporarily impaired or permanently damaged. In addition, specific solutes, including sodium and calcium ions, must be kept at concentrations that permit the regular activity of muscles, neurons, and other body cells. For this reason, animals carry out **osmoregulation**, the process of regulating and controlling solute concentrations to balance water gain and loss.

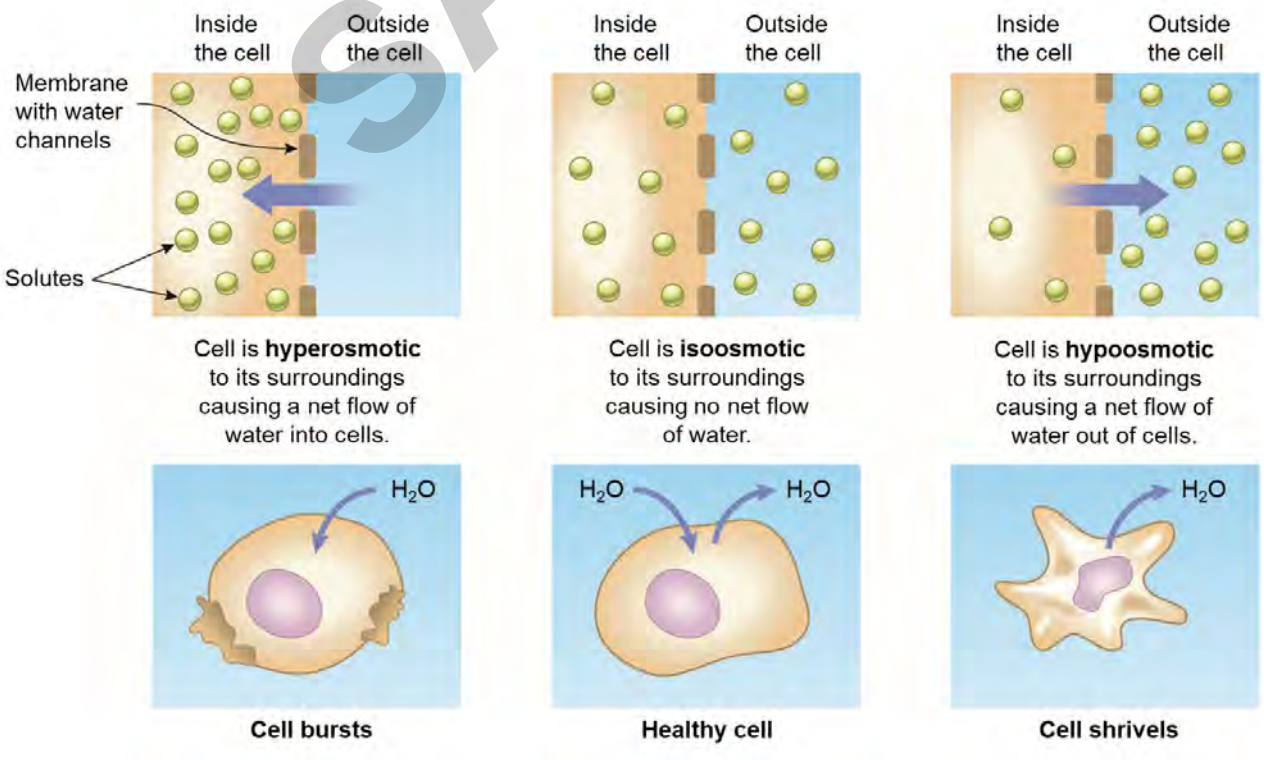


Figure 3.02: Effect of osmolarity on cell structure.

Blood Glucose Level and pH

Glucose is an essential nutrient in animals and the primary substrate of respiration. Most food types contain glucose which is released into the blood during digestion. Once inside the blood, glucose is transported to tissues around the body, supplying cells with their respiratory substrate. Because glucose is a major fuel source for cellular respiration, its concentration is maintained within narrow tolerance limits. In humans, blood glucose level is maintained within tolerance limits of 70 and 110 milligrams per 100 mL of blood. When blood glucose falls below tolerance limits, an animal can become **hypoglycaemic**, a condition associated with dizziness, confusion and fatigue due to the supply of glucose failing to meet the cell's respiratory demands. Conversely, when blood glucose levels are above the tolerance limits, a condition called **hyperglycaemia** develops, which causes a range of symptoms that impact the animal, including blurred vision, excessive thirst and fatigue.

Aerobic respiration produces carbon dioxide, a substance that rapidly diffuses out of tissue cells and into the tissue fluid and blood, as shown in Figure 3.03. Carbon dioxide diffuses into red blood cells, where more than half is transformed into carbonic acid (H_2CO_3), which dissociates into hydrogen ions (H^+) that reduce blood pH. The overall process is summarised below.

The reaction equation directly correlates carbon dioxide levels and blood pH. As the rate of cellular respiration increases, more carbon dioxide diffuses into the blood, where much of it is transformed into hydrogen ions that lower blood pH. Animals must maintain blood pH within narrow tolerance limits to maintain the tertiary structures of enzymes, allowing them to bind their substrates with high affinity. In humans, blood pH is kept between 7.35 and 7.45. When blood pH is below the tolerance limit due to excess carbon dioxide, a person develops **hypercapnia** and **respiratory acidosis** with symptoms including breathlessness, rapid breathing, headache, inflammation, disorientation, and loss of consciousness. Conversely, when blood pH is above tolerance limits due to a lack of carbon dioxide, a person develops **hypocapnia** and **respiratory alkalosis** with symptoms including tingling sensation in the limbs, abnormal heartbeat, muscle cramps, dizziness, fainting, and seizures. We have seen that animals have tolerance limits for factors such as body temperature, osmolarity, blood glucose level and blood pH, and there are impacts when conditions exceed these limits. Next, we look at the mechanisms animals use to monitor and maintain these factors within tolerance limits.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

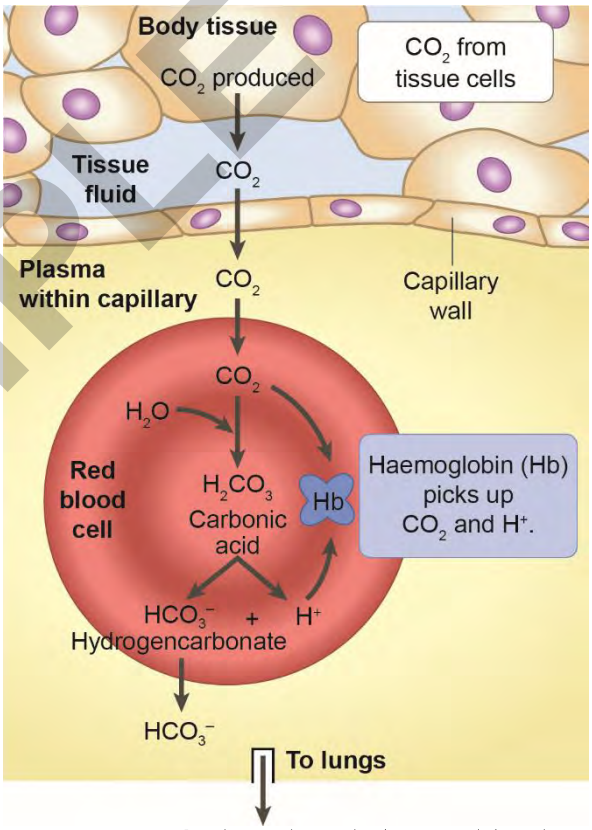


Figure 3.03: Carbon dioxide lowers blood pH.

The stimulus-response model relies on various receptor proteins and cells scattered throughout the body. These proteins and cells, discussed in Chapter 3.3, respond to a stimulus which is an internal factor outside of tolerance limits, such as human body temperature being above 38°C or blood glucose level being below 70 mg 100 mL^{-1}. Next, the stimulus is conveyed to a control centre; typically, the animal's central nervous system, that coordinates the appropriate response to return the internal factor within tolerance limits. Finally, the control centre uses electrical signals or hormones to stimulate an effector, typically a muscle or gland, to enact the response that returns the factor within tolerance limits. For example, consider the human body's response to changes in body temperature (Figure 3.07). The diagram shows receptors detecting a decreased body temperature in the brain's hypothalamus. The brain acts as the control centre, sending signals to sweat glands that secrete sweat, helping cool the body and reduce its temperature within tolerance limits. Conversely, when body temperature is below tolerance limits, the brain signals the skeletal muscle to shiver, a heat-generating response that increases body temperature within tolerance limits.

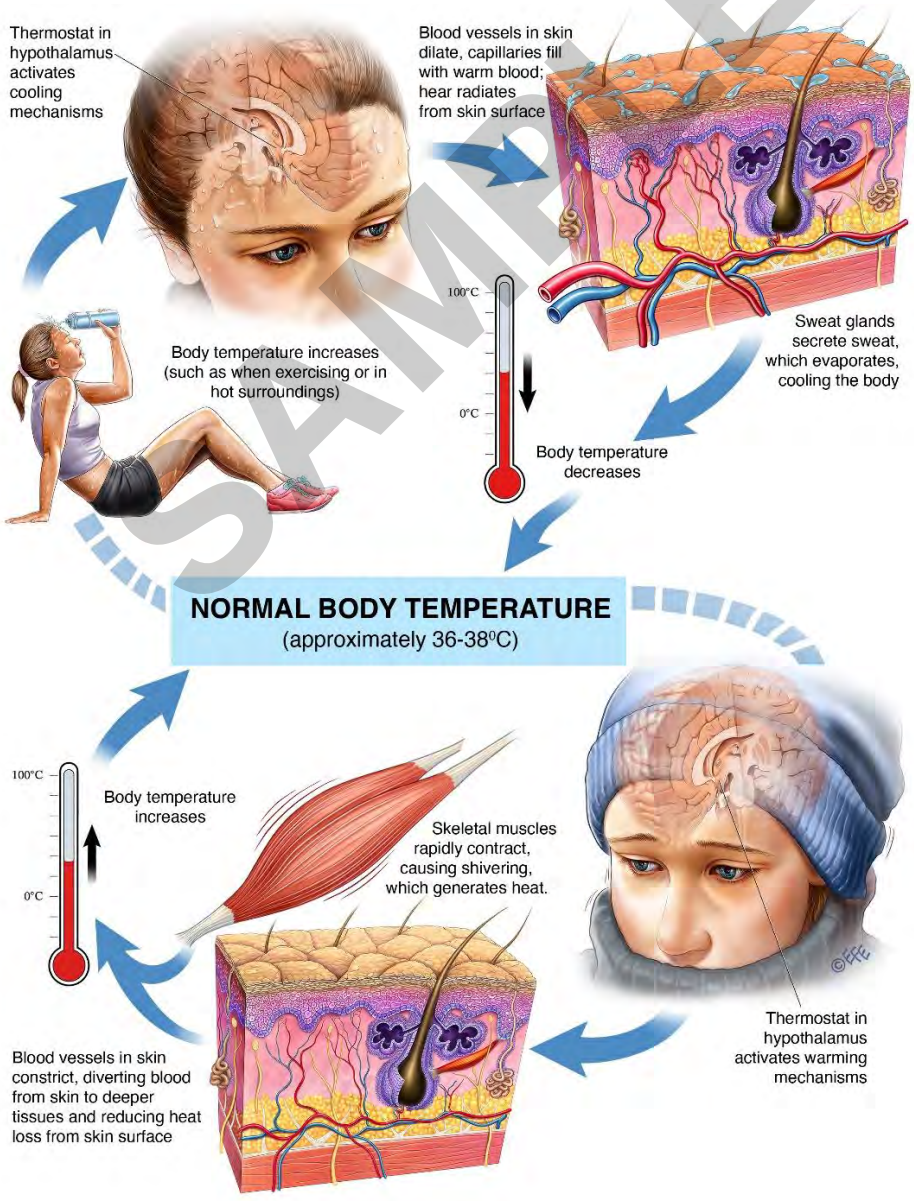


Figure 3.07: Stimulus-response in thermoregulation.

Another example of stimulus-response is seen in the body's response to changes in blood osmolarity. As seen in Figure 3.08, an increase in osmolarity due to water loss is detected by receptors in the brain's hypothalamus. The brain acts as the control centre, sending signals to areas of the brain that stimulate thirst, helping hydrate the body. In addition, the brain sends signals to the kidneys (effectors), stimulating water reabsorption, a response that reduces osmolarity within tolerance limits.

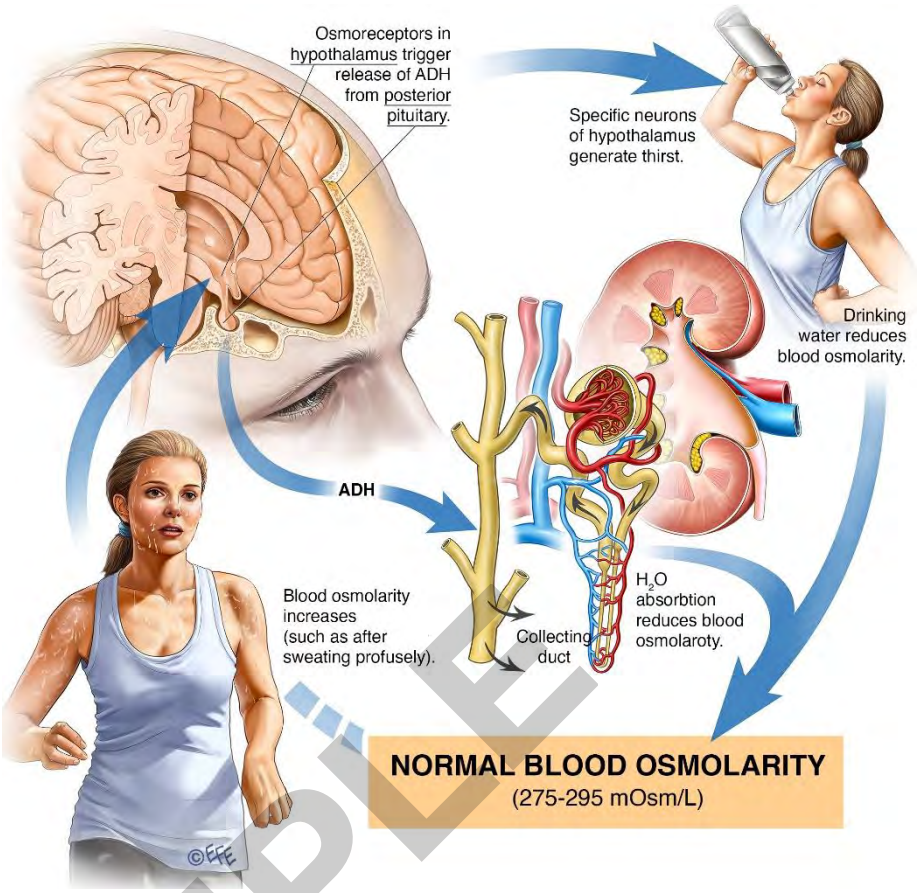


Figure 3.08: Stimulus-response in osmoregulation.

Similarly, stimulus-response mechanisms regulate blood pH that fluctuates during aerobic exercise due to increased respiratory carbon dioxide. As seen in Figure 3.09, physical exercise increases the rate of cellular respiration and carbon dioxide production. The increase in carbon dioxide level causes a decrease in blood pH that is detected by receptors in the heart, brain and blood vessels. The medulla oblongata in the brain acts as the control centre, sending signals to the lungs (effectors) that increase the ventilation rate, expelling excess carbon dioxide more rapidly and increasing blood pH within tolerance limits.

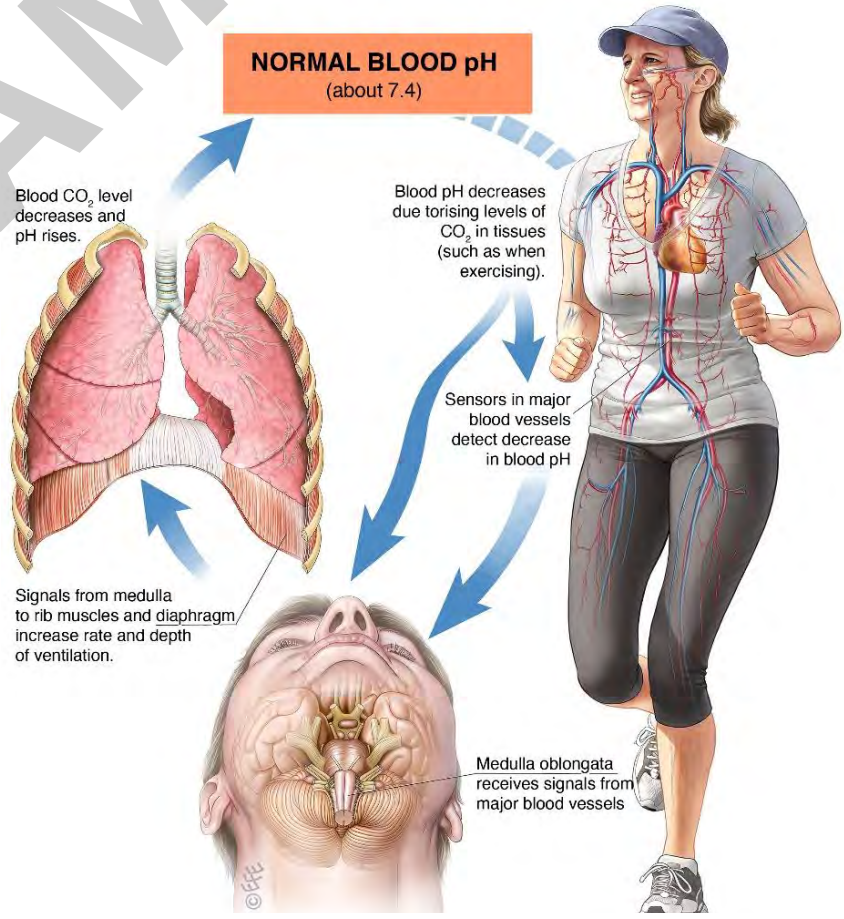


Figure 3.09: Stimulus-response in regulation of blood pH.

Blood glucose regulation (Figure 3.10) is also under stimulus-response control. For example, blood glucose levels increase above the tolerance limit after eating. This stimulus is detected by beta cells, receptors in the pancreas that respond by increasing insulin production and secretion. Insulin binds to complementary receptors on tissue cells, stimulating glucose absorption by tissues and reducing blood glucose levels within tolerance limits. Conversely, blood glucose levels may be below tolerance limits before a meal. This stimulus is detected by alpha cells, receptors in the pancreas that detect the stimulus and respond by increasing glucagon production and secretion. Glucagon binds to target cells in the muscles and liver that break down stored glucose, releasing it into the blood and returning blood glucose levels within tolerance limits. In blood glucose regulation, the alpha and beta cells act as receptors for the stimuli and effectors that secrete substances that facilitate the response.

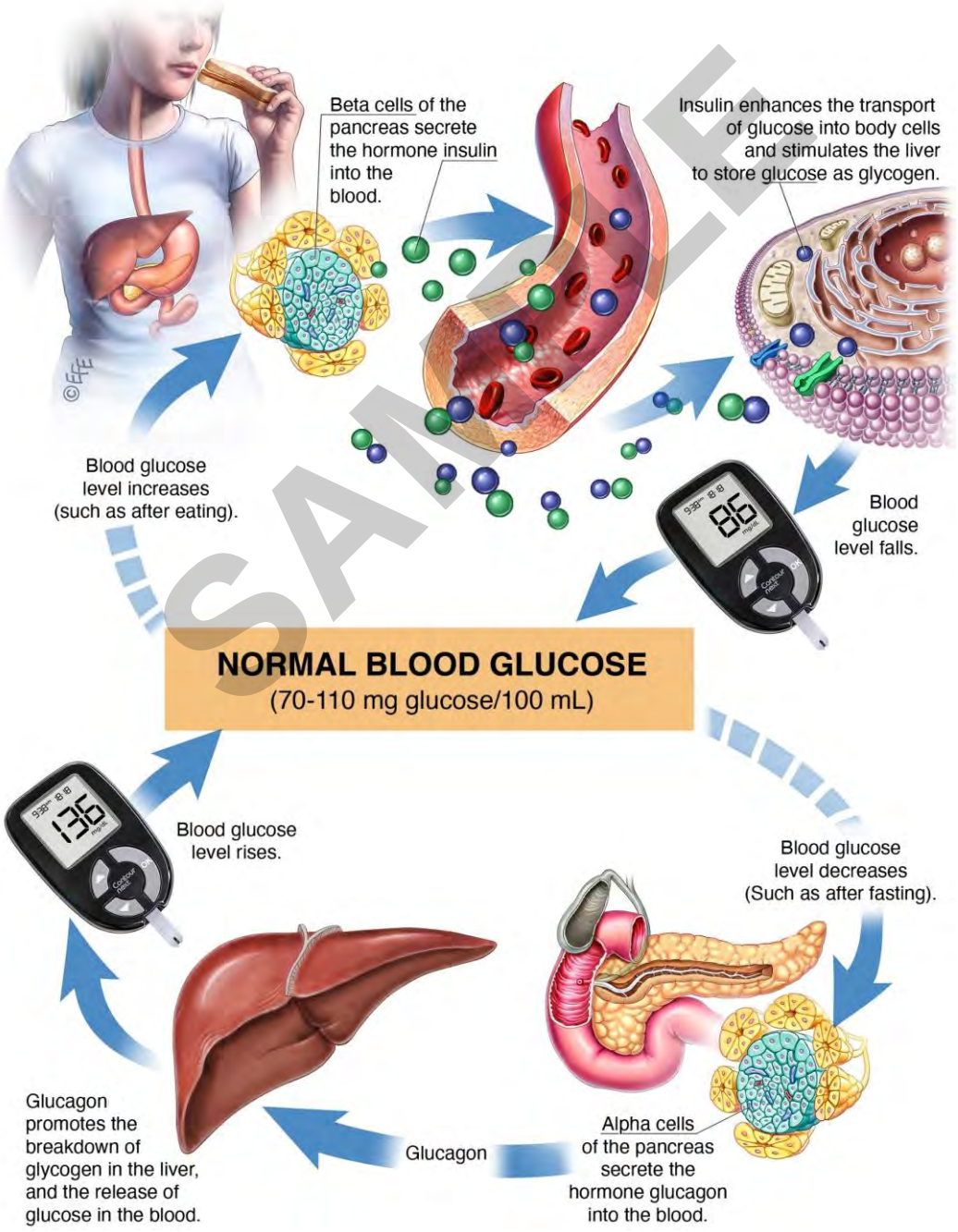


Figure 3.10: Stimulus-response model in blood glucose regulation.

3.3: The Nervous System

The nervous system is composed of the central nervous system and peripheral nervous system.

- Compare the structure and function of sensory neurons, interneurons, and motor neurons.
- Describe the structure of a nerve pathway from receptor to effector.
- Describe the role of synapses and neurotransmitters.
- Describe the role and pathway of reflex responses.

The previous chapter introduced homeostasis and the mechanisms animals use to regulate internal factors in the face of changing environmental conditions. This chapter explores the nervous system's role in homeostasis, specifically detecting environmental stimuli and transmitting information between sensory receptors, the control centre, and effectors.

The Nervous System

The **nervous system** is an organ system in animals capable of detecting sensory stimuli, transmitting the stimulus to the control centre for processing and transmitting the appropriate response to effectors. Our starting point for exploring the nervous system is the **neuron**, a cell type found throughout the nervous system capable of receiving and transmitting sensory information from the environment. Most of a neuron's organelles, including its nucleus and endomembrane system, are in the **cell body** (Figure 3.13). The cell body of a typical neuron is covered with numerous highly branched extensions called **dendrites** that receive signals from other neurons. A typical neuron has a single **axon**, the extension that transmits signals to other cells. Axons are typically much longer than dendrites, and some, such as those extending from a giraffe's spinal cord to the muscle cells in its feet, are more than a metre long! The specialised structure of axons allows them to use pulses of electrical current to transmit information, even over long distances. The base of an axon usually divides into many branches, and the ends of the branches transmit information to another cell at a junction called a **synapse** (explored shortly). The part of each axon branch that forms this specialised junction is a **synaptic terminal**. At most synapses, chemical messengers called **neurotransmitters** pass information from the transmitting neuron to the receiving cell.

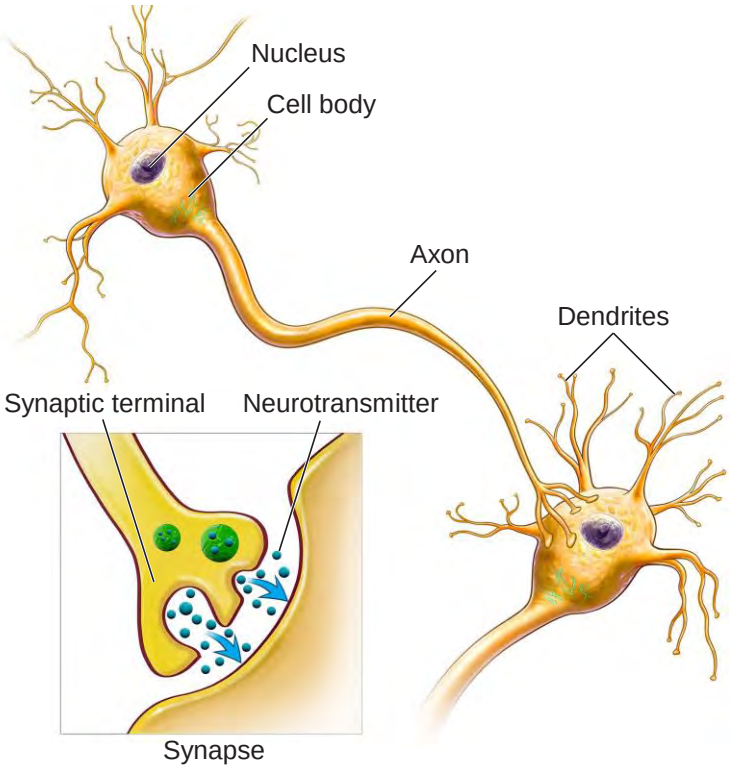


Figure 3.13: Neuron structure

Synapses and Neurotransmitters

The transmission of information from neurons to other cells occurs at synapses. Most synapses are **chemical synapses**, which rely on the release of a chemical neurotransmitter by the **presynaptic neuron** to transfer information to the **postsynaptic cell**. While at rest, the presynaptic neuron synthesises the neurotransmitter at each synaptic terminal, packaging it in multiple vesicles. The arrival of signals at the synaptic terminals of the presynaptic neuron causes vesicles to fuse with the membrane and release the neurotransmitter into the synapse via exocytosis. Neurotransmitters released from the presynaptic cell diffuse across the **synaptic cleft**, with a gap of less than 50 nm separating the presynaptic neuron from the postsynaptic cell. Upon reaching the postsynaptic membrane, the neurotransmitter binds to and activates a specific complementary receptor in the membrane. This series of events at the synapse is summarised in Figure 3.14.

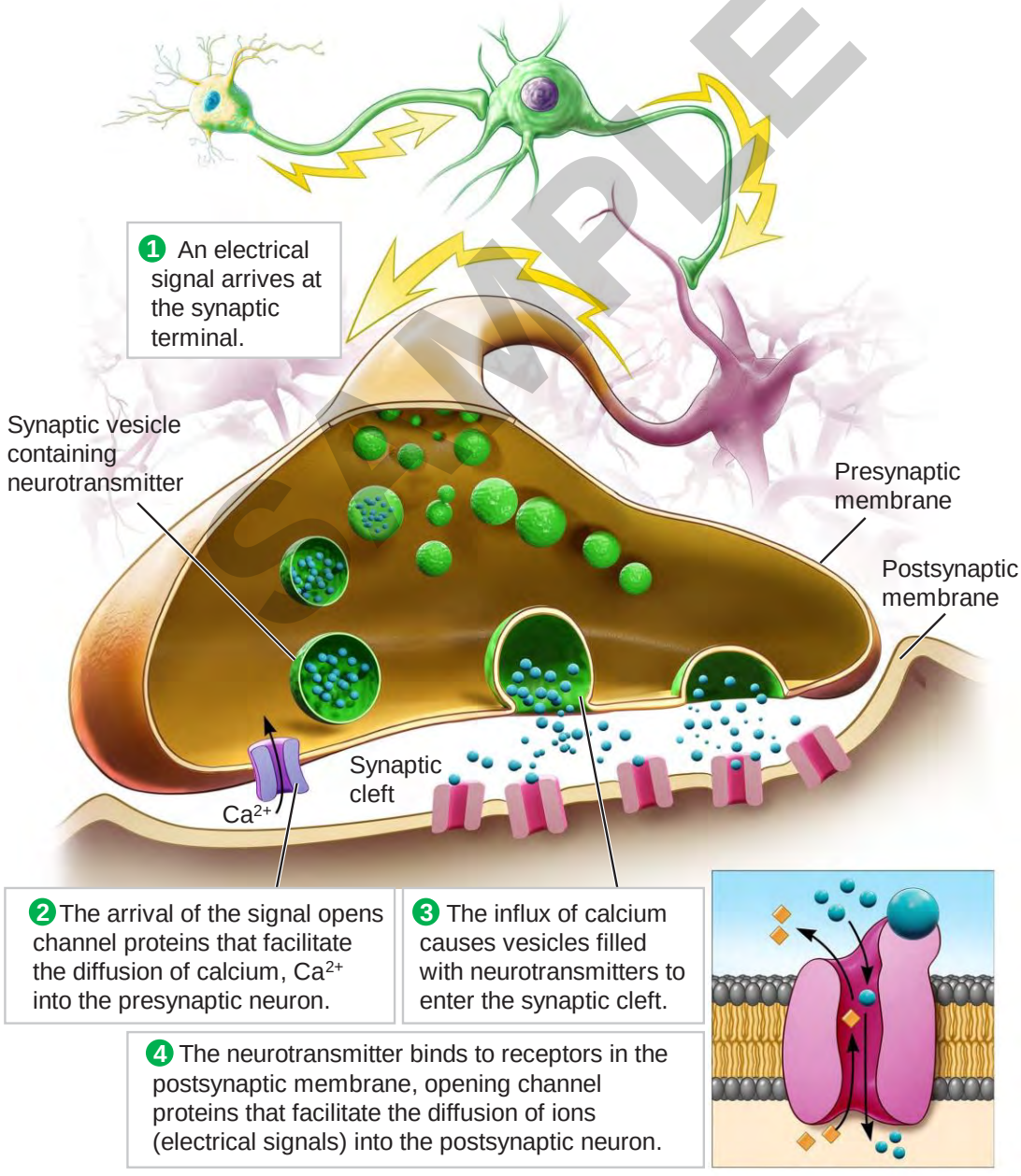


Figure 3.14: A chemical synapse

Types of Neurons

The nervous system processes sensory information in three stages: **sensory input**, **integration**, and **motor output**. In all but the simplest of animals, each stage of information processing is handled by three specialised populations of neurons. **Sensory neurons** transmit information about external stimuli, such as light and sound, and internal conditions, such as blood pressure, to the control centre, which may include a **brain** or simpler clusters of neurons called **ganglia**. Inside the control centre, a network of **interneurons** forms the local circuits connecting neurons that carry out integration, the analysis and interpretation of sensory input. Neurons in the control centre then transmit the response as electrical signals along **motor neurons** to effectors such as muscles or glands that carry out a response. The three types of neurons transmit electrical signals in the same way. For example, a sensory neuron transmits sensory information along its length, like a motor neuron transmits information controlling muscle movement. As shown in Figure 3.15, the structures of the three neurons vary from simple to quite complex, depending on their role in information processing. For example, sensory and motor neurons have a **myelin sheath**, an insulating structure that helps these cells transmit electrical signals rapidly over long distances. Sensory neurons are distinguished from motor neurons by their central cell body positioned around halfway along the length of the axon. Interneurons are much shorter than sensory and motor neurons, and their axons are never myelinated. However, unlike sensory and motor neurons, some interneurons have highly branched dendrites the length of their axons, receiving input through tens of thousands of synapses.

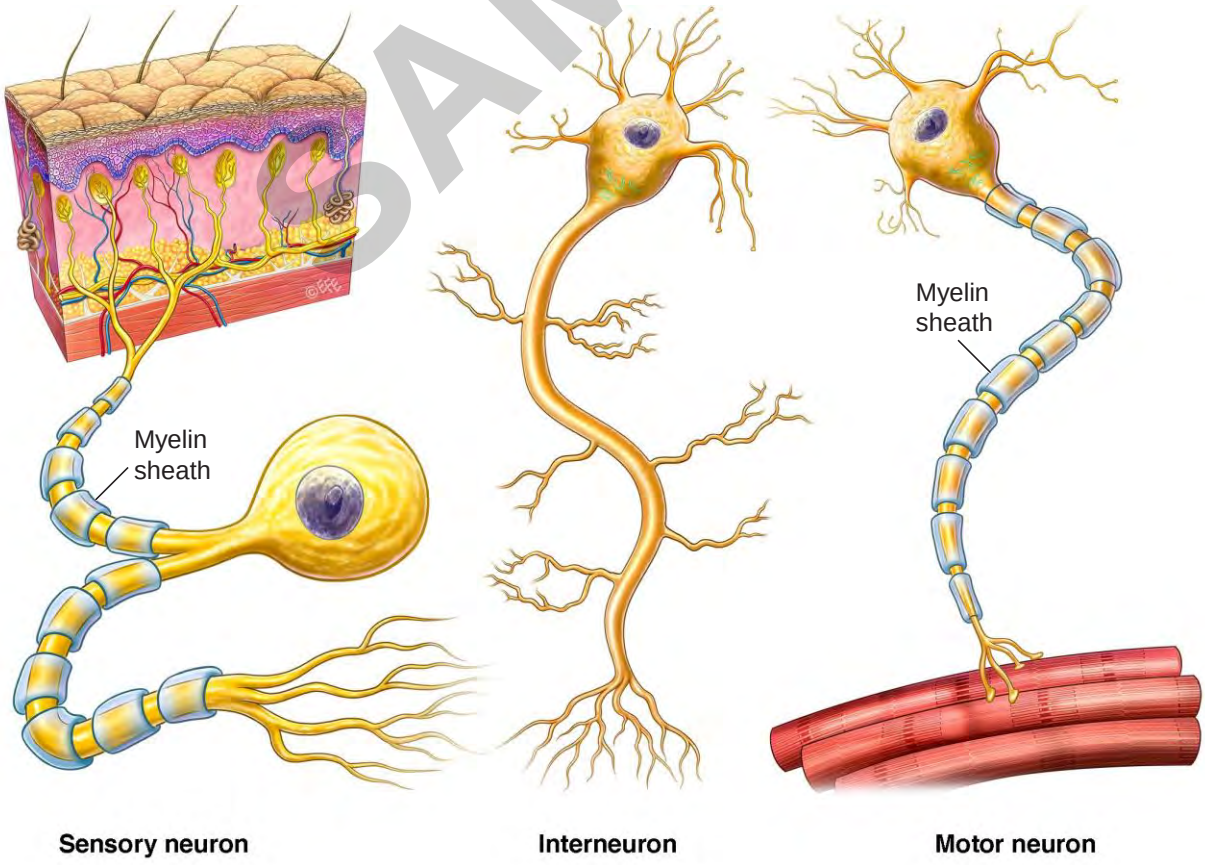


Figure 3.15: Three populations of neurons

Sensory Receptors

The peripheral nervous system contains several sensory receptors specialising in detecting and transmitting sensory input from the internal and external environment. The term sensory receptor describes a sensory cell or organ and the subcellular structure that detects a stimulus. Each sensory cell is either a neuron or a cell that regulates a sensory neuron. Some sensory cells exist singly; others are collected in sensory organs. Some sensory receptors respond to stimuli from within the body, such as blood pressure and body position. Other receptors detect stimuli from outside the body, such as heat, light, pressure, or chemicals. Some of these receptors are sensitive to the smallest possible unit of stimulus. Most light receptors, for example, can detect a single quantum (photon) of light. All sensory processes begin with a stimulus representing a form of energy such as light, sound or heat. A sensory receptor cell converts stimulus energy to a change in membrane potential that initiates electrical signals, pulses of electrical current that flow away from the receptor cell along sensory neurons to the CNS. Decoding of this information within the CNS results in sensation. For example, olfactory receptor cells in the nose detect specific odorants in the air and initiate signals that travel along sensory neurons to the olfactory bulb in the brain, which decodes the signal and produces the sensation of the smell of a rose (Figure 3.17)

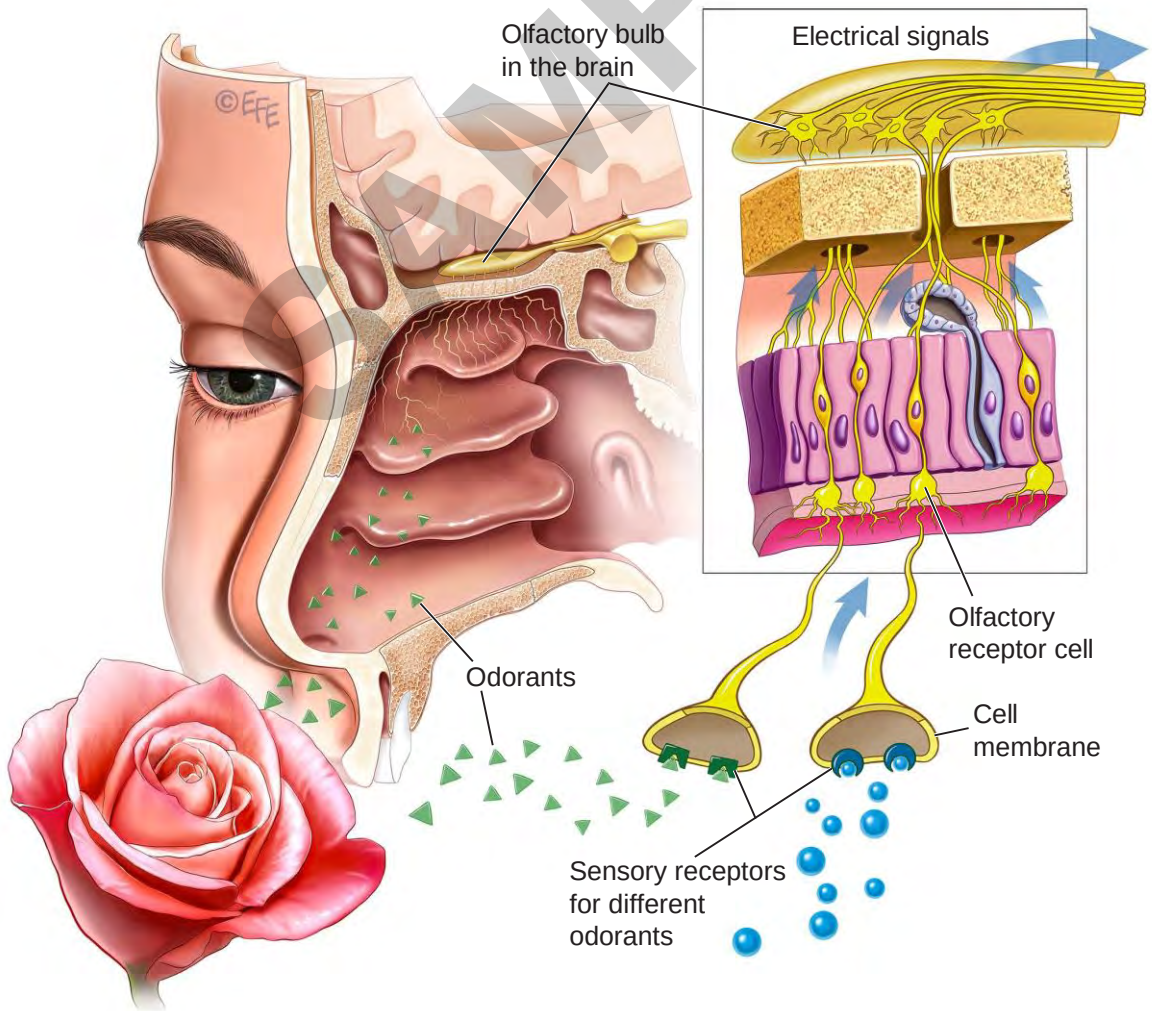


Figure 3.17: Sense of smell.

Nerve Pathways

The nervous system plays an essential role in homeostasis by detecting internal and external stimuli, integrating the information, and coordinating one or more responses through effectors. Crucial to these processes are **nerve pathways** connecting sensory organs in the PNS to the control centre in the CNS and from the CNS to effectors in the PNS, as depicted in Figure 3.18. A nerve pathway begins with sensory reception, the detection of a stimulus by specialised sensory receptors. Although animals use a range of sensory receptors to detect widely varying stimuli, the effect in all cases is to open or close ion channels that initiate the flow of electric signals along sensory neurons to the processing centre in the CNS. Next, interneurons in the CNS integrate the signals and coordinate the response transmitted along motor neurons to effectors that carry out the response. For example, touch and smell receptors activate when a shark contacts an object in the water. These receptors transmit sensory information about the object to the shark's brain. Finally, circuits in the shark's brain integrate the input and initiate one of two response pathways. If food is detected, the brain sends motor output commands to skeletal muscles that cause the jaws to bite down. If no food is detected, the brain sends instructions to skeletal muscles to continue swimming.

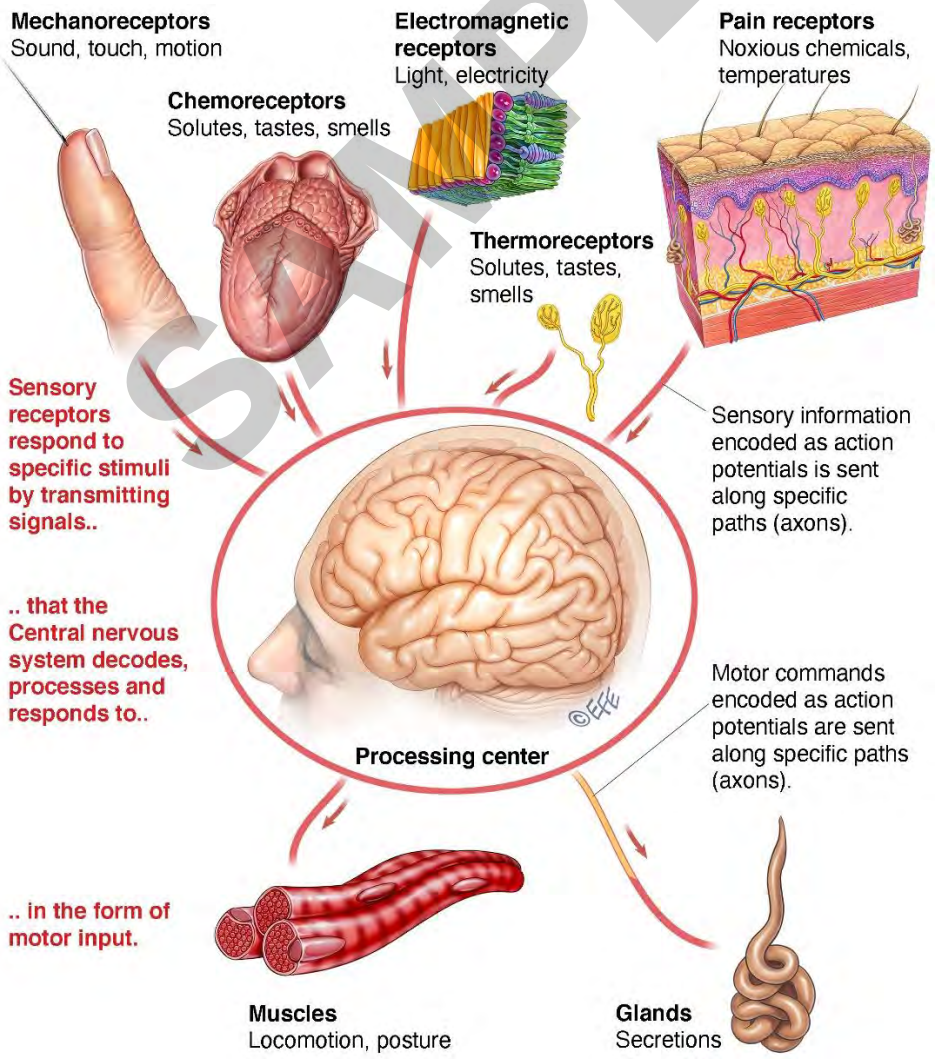


Figure 3.18: Sensory inputs and motor outputs in a nerve pathway.

Reflexes

In vertebrates, the brain and spinal cord act independently as part of the simple nerve pathways that produce **reflexes**, the body's automatic responses to certain stimuli. Reflexes protect the body by providing rapid, involuntary responses to particular stimuli. Reflexes are rapid because sensory information activates motor neurons without the information having to travel from the spinal cord to the brain and back. For example, if you accidentally put your hand on a hot object, a reflex withdraws your hand from the object even before your brain processes pain. Similarly, the knee-jerk reflex provides an immediate protective response when you pick up an unexpectedly heavy object. If your legs buckle, the tension across your knees triggers contraction of your thigh muscle, helping you stay upright and support the load. A reflex occurs via specialised nerve pathways that allow an organism to respond rapidly to a stimulus. In a simple reflex pathway, the stimulus activates sensory receptors that send signals along sensory neurons to the spinal cord. Interneurons in CNS then transmit the impulse directly to a motor neuron which activates an effector. Figure 3.19 shows the knee-jerk reflex in humans. A doctor assesses nervous system function by stimulating this reflex with a triangular mallet. Reflexes produce rapid responses as the signals are conducted along very few neurons and cross very few synapses. Reflexes serve various vital functions, including responding to environmental stimuli, avoiding and reducing tissue damage, maintaining balance and posture, finding food and mates, and evading predators.

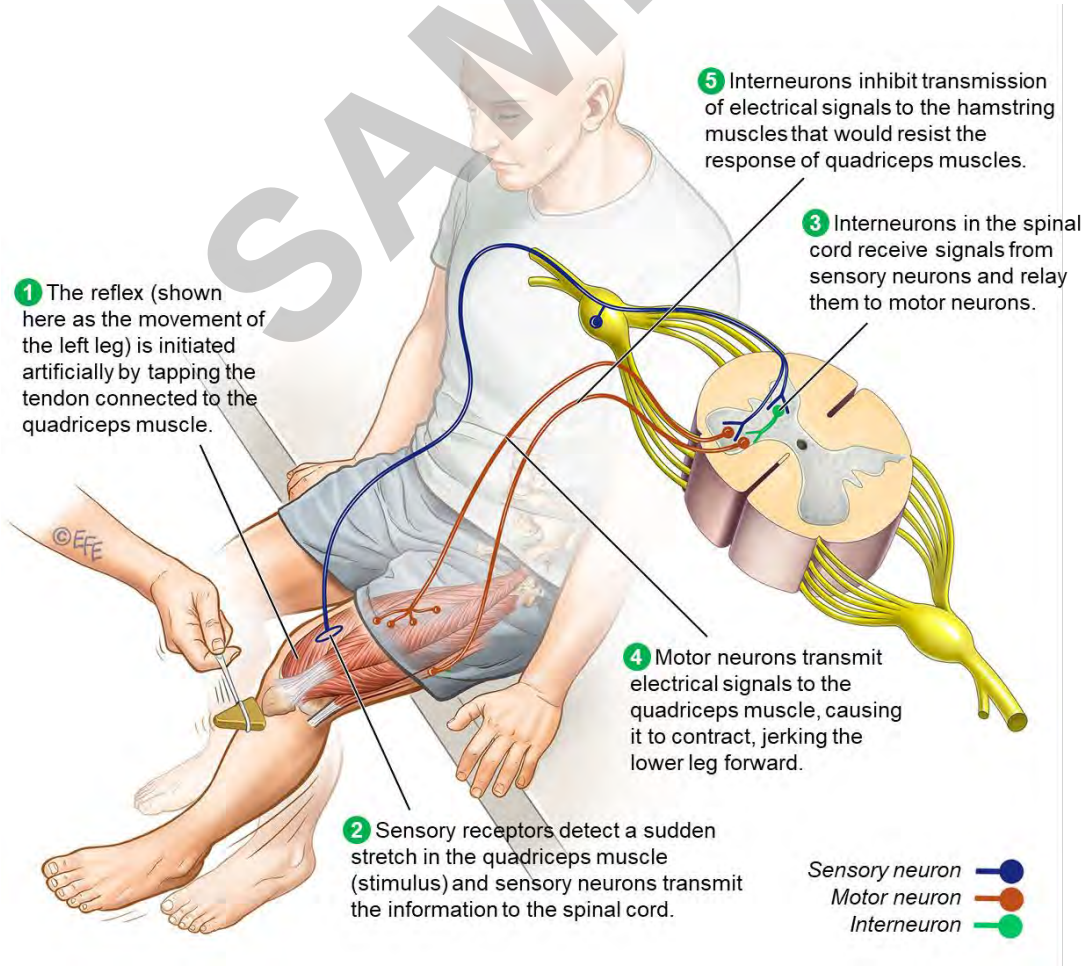
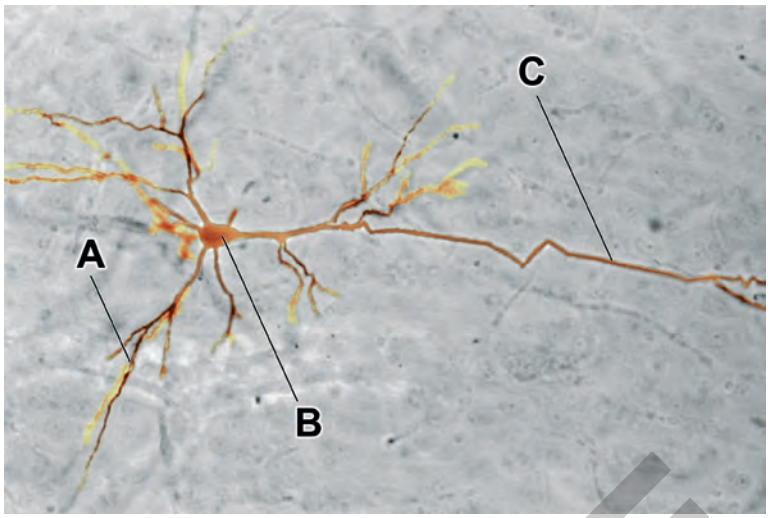


Figure 3.19: The knee-jerk reflex.

Question 159

The diagram below shows a light micrograph of a motor neuron.



(a) Name the components labelled **A** to **C** and give the function of each in a motor neuron.

(3 marks) **KA1**

(b) State the function of motor neurons in nerve pathways.

(1 mark) **KA1**

(c) The myelin sheath increases the speed of signals along motor neurons.

Multiple sclerosis (MS) is a disease in which the immune system destroys the myelin sheath.

State the likely effect of MS on nerve pathways.

(1 mark) **KA2**

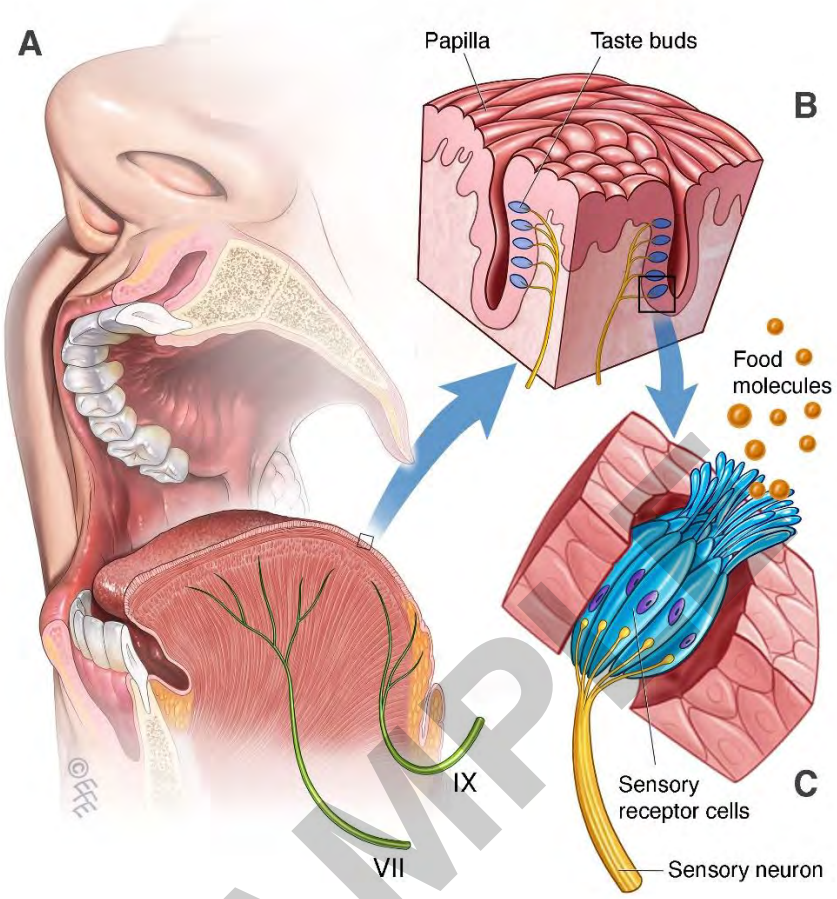
(d) Motor neuron disease is a rare disorder in which motor neurons lose function over time.

Suggest one likely symptom of motor neuron disease.

(1 mark) **KA2**

Question 164

The diagram below shows the human tongue.



The taste buds contain different sensory receptor cells.

(a) State the function of sensory receptor cells in the body.

(1 mark) **KA1**

(b) Describe the nerve pathway from the tongue to the brain that results in a person experiencing a sour taste.

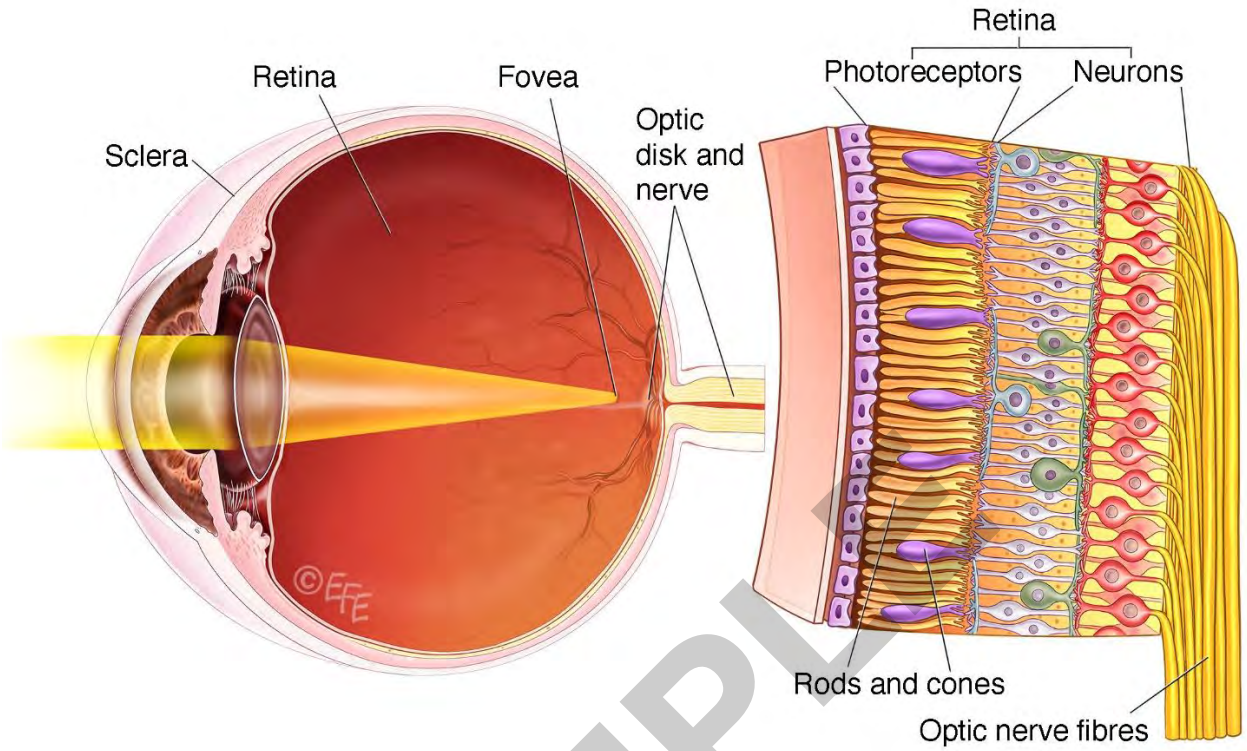
(3 marks) **KA2**

(c) Explain how the brain distinguishes between two tastes, such as sour and sweet.

(2 marks) **KA2**

Question 165

The diagram below shows the structure of the human eye.



(a) Light reflected from an object enters the human eye.

Describe how the light entering the eye transforms into information the human brain can interpret.

(3 marks) **KA2**

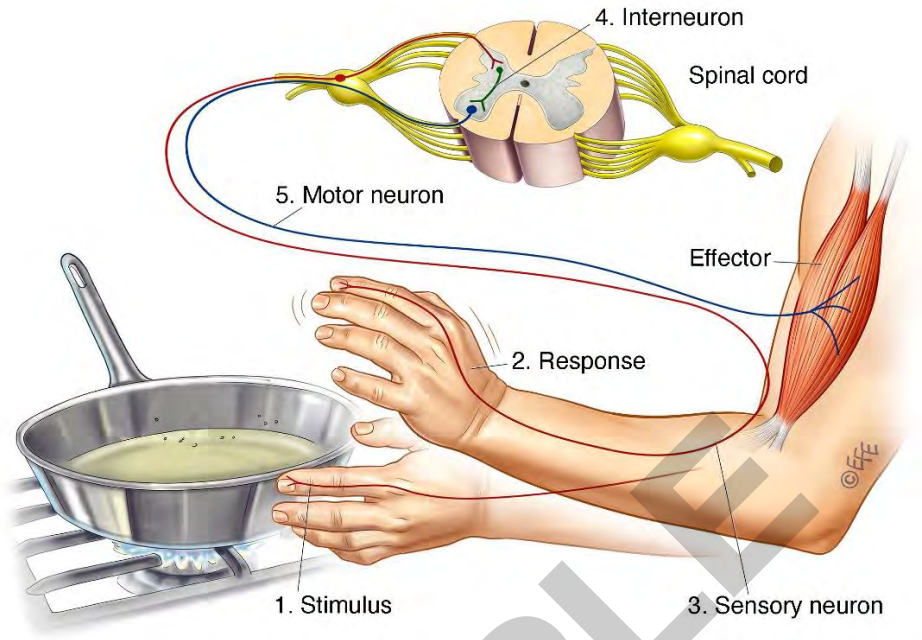
(b) The blink reflex is an automatic response when objects or irritants contact the cornea.

Describe the nerve pathway involved in the blink reflex.

(3 marks) **KA2**

Question 166

The diagram below shows a reflex response in a person who touches a hot object.



(a) State the importance of this reflex response.

(1 mark) **KA1**

(b) Describe the reflex response shown in the diagram.

(3 marks) **KA2**

(c) State two properties of reflex pathways ensuring the rapid transmission of signals.

(2 marks) **KA2**

3.4: The Endocrine System

The endocrine system releases hormones that are amino acid derivatives, peptides, proteins, or steroids.

Hormones travel to target sites via the blood.

Hormones can alter the metabolism of target cells, tissues, or organs.

- Compare the action of insulin and glucagon in blood sugar regulation.
- Describe how diabetes mellitus can result from a hormonal imbalance.
- Describe the action of thyroid stimulating hormone and thyroxine in metabolism.
- Describe the role of antidiuretic hormone (ADH) in osmoregulation.
- Discuss the links between osmoregulation, blood volume, and blood pressure.

Hormonal responses can be stimulated by either the nervous system or other hormonal messages.

- Describe the role of adrenaline in the 'fight or flight' response.

Describe the role of thyroid-stimulating hormone in the production of thyroxine.

The previous chapter explored the structure and function of the nervous system, a network of sensory organs and specialised cells called neurons that detect stimuli and transmit electrical signals along dedicated pathways. These signals regulate effectors, neurons, muscle cells, and endocrine cells that carry out the homeostasis responses. The other major system for communication and regulation in animals is the **endocrine system**, an interconnected network of organs and glands responsible for producing and secreting various chemical signals called **hormones** that circulate throughout the body in blood, reaching all cells. Each endocrine gland produces one or more hormones that elicit various responses. However, each hormone only elicits a response, such as a change in metabolism, in specific **target cells**, those with a receptor that binds the hormone with high specificity. Cells lacking a receptor for that hormone are unaffected. The structure and location of the human endocrine glands are shown in Figure 3.20.

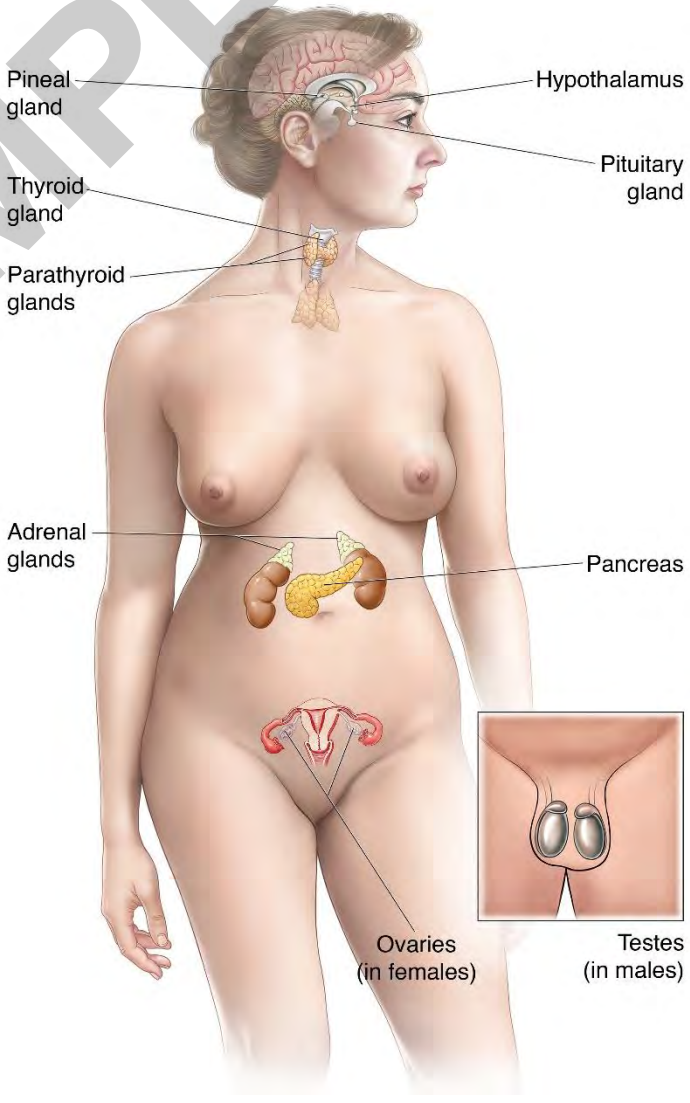


Figure 3.20: Human endocrine system

Hormones

Hormones are small or large molecules produced by specific **endocrine cells** grouped together in glands. After their production, hormones are stored until they are released into the extracellular fluid. Once secreted, hormones travel in the blood to target cells (Figure 3.21), mediating responses to stimuli such as changes in blood pressure and volume, metabolism and osmolarity.

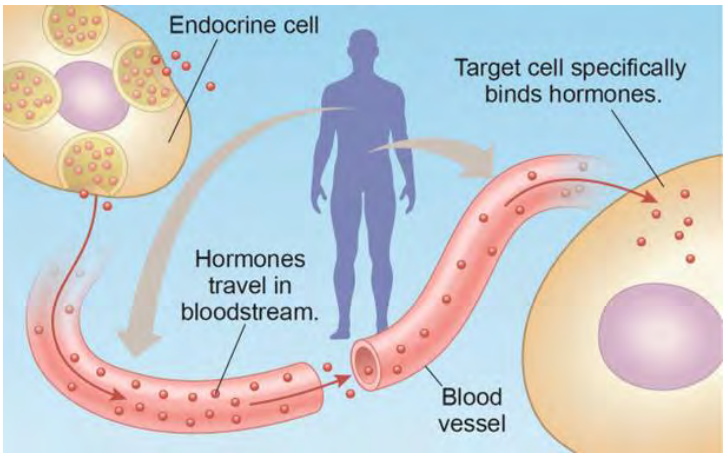


Figure 3.21: Chemical signalling by hormones

Hormones fall into four major chemical classes: **amino acid derivatives** or **amines**, **polypeptides**, **proteins**, and **steroids**, each varying in their solubility in hydrophilic and hydrophobic environments. Polypeptides, proteins, and most amine hormones are hydrophilic, steroid hormones, and some amines are hydrophobic. Hydrophilic and hydrophobic hormones differ in their response pathways. One key difference is the location of the receptor proteins in target cells. Hydrophilic hormones are secreted by exocytosis and travel freely in the blood. Being insoluble in lipids, they cannot diffuse through the cell membranes of target cells. Instead, these hormones bind to cell-surface receptors, initiating intracellular changes (Figure 3.22 1). In contrast, hydrophobic hormones exit secretory cells by simple diffusion and bind to hydrophilic transport proteins in the blood, increasing their solubility. After circulating in the blood, they diffuse into target cells and typically bind to receptors in the cytoplasm or nucleus (Figure 3.22 2). The binding of a hormone to its receptor protein triggers a cellular response such as the activation of an enzyme, a change in the uptake or secretion of specific molecules, or changes in gene expression that form part or all of the body's response to a stimulus.

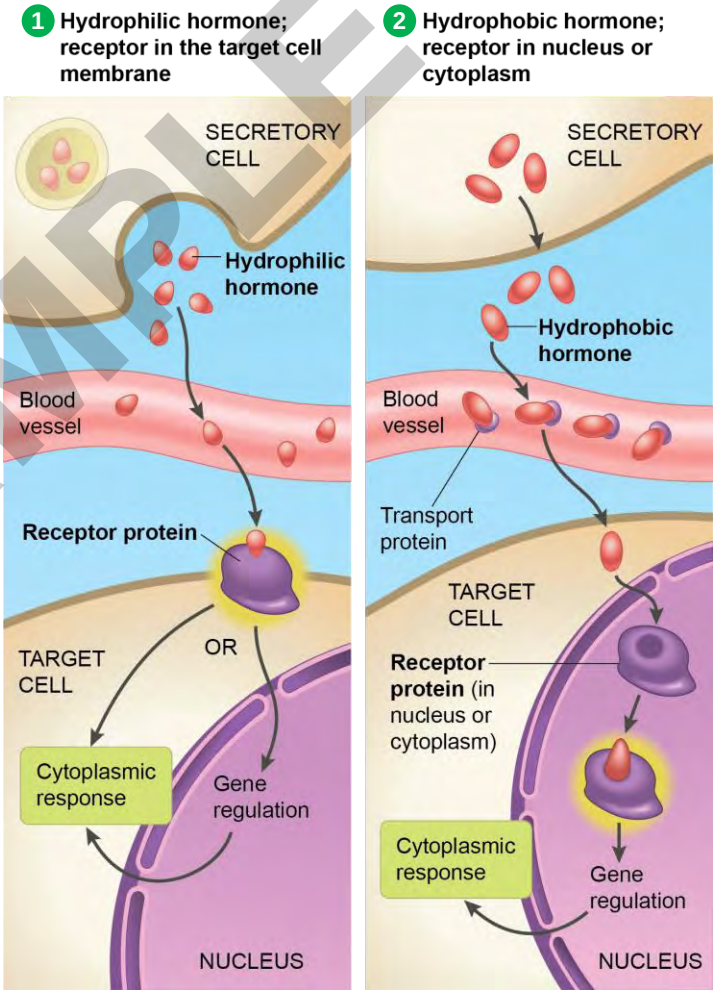


Figure 3.22: Hydrophilic and hydrophobic hormones

Hormone Pathways

Having explored hormone structure, recognition, and response, we now consider how regulatory pathways controlling hormone secretion are organised. In a simple **endocrine pathway** (Figure 3.23 1), endocrine cells respond to a change in some internal or external factor, the stimulus, by secreting hormone molecules. The hormone travels in the bloodstream to target cells, interacting with its specific receptor proteins expressed by target cells, triggering a particular cellular response that brings about the body's physiological response to the initial stimulus. For example, partially processed food containing acidic digestive juices enters the duodenum of the small intestine during digestion. The low pH of partially digested food is a stimulus detected by endocrine cells in the duodenum lining. In response, the endocrine cells secrete the hormone secretin, which diffuses into the blood and travels throughout the circulatory system before reaching the pancreas. Target cells in the pancreas have receptors for secretin and respond by releasing bicarbonate into the duodenum, an alkaline substance that neutralises stomach acid. Endocrine pathways are under negative feedback control, which links a response back to an initial stimulus. For instance, bicarbonate released in response to secretin increases pH in the intestine, eliminating the stimulus and stopping the release of secretin. Other hormone pathways incorporate components of the nervous system. For example, in a simple **neuroendocrine pathway** (Figure 3.23 2), the stimulus is received by a sensory neuron rather than endocrine tissue. The sensory neuron stimulates a neurosecretory cell that responds by secreting a hormone that diffuses into the blood and travels in the circulation to target cells. Examples of hormones secreted this way include thyroid-stimulating hormone (TSH) and antidiuretic hormone (ADH).

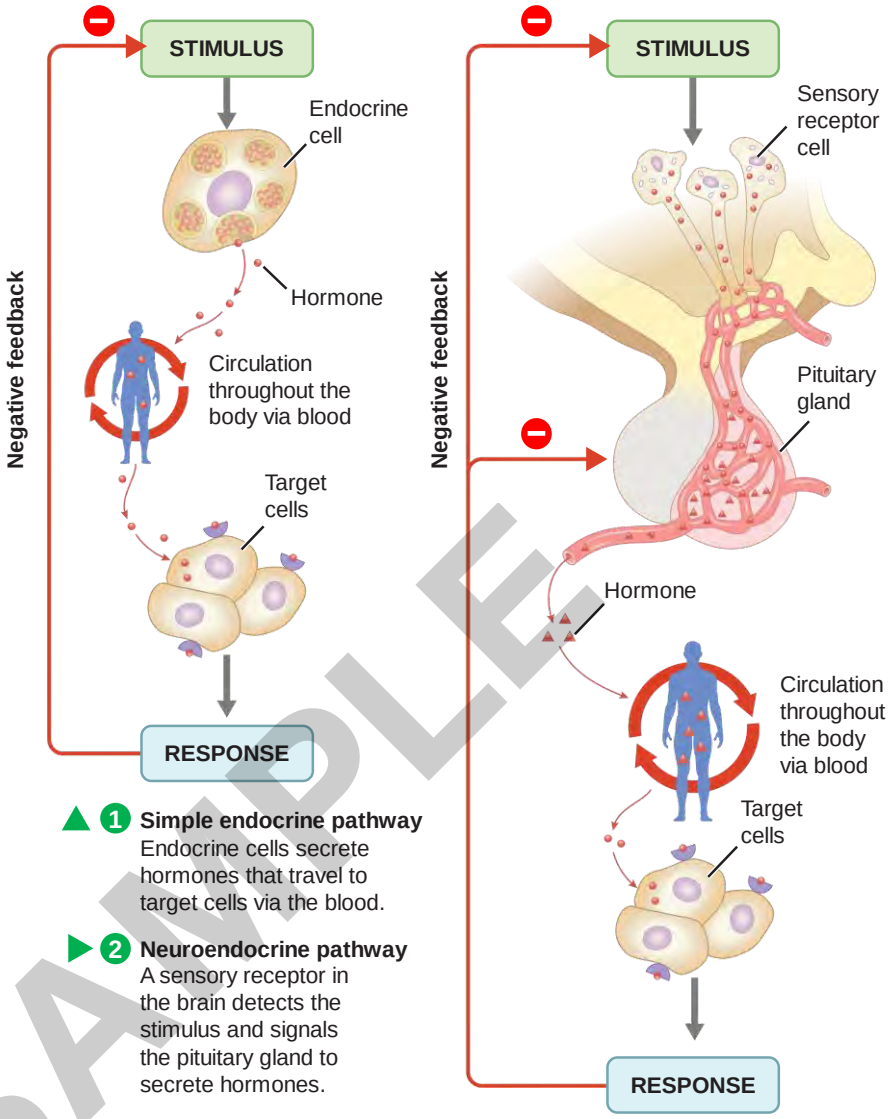


Figure 3.23: Endocrine pathways

Diabetes Mellitus

Several disorders can disrupt glucose homeostasis with potentially serious consequences, especially for the heart, blood vessels, eyes, and kidneys. The best-known and most prevalent of these is **diabetes mellitus**, a disorder caused by an insulin deficiency or decreased insulin response in target tissues. After its release from the pancreas, insulin diffuses into the blood and travels in the circulation to target cells. The binding of insulin to its specific cell-surface receptors initiates intracellular changes that increase the concentration of glucose transport proteins in the cell membrane (see Figure 3.26❶). In individuals with diabetes mellitus, blood glucose level rises, but tissue cells cannot absorb enough glucose to meet metabolic needs. Instead, fat becomes the primary substrate for cellular respiration. In severe cases, acidic metabolites formed during fat breakdown accumulate in the blood, threatening life by lowering blood pH and depleting sodium and potassium ions from the body. There are two main types of diabetes mellitus: type 1 and type 2. Each results in high blood glucose levels, but with very different causes.

Type 1 diabetes, also called insulin-dependent diabetes, is an autoimmune disorder in which the immune system destroys the pancreas' insulin-producing cells. The significant decrease in insulin production prevents tissue cells from absorbing glucose from the blood, leading to high blood glucose levels above tolerance limits (see Figure 3.26❷). Type 1 diabetes usually appears in childhood, and its treatment consists of insulin injections, typically given multiple times daily.

Type 2 diabetes, also called non-insulin-dependent diabetes, is characterised by a failure of target cells to respond normally to insulin. As a result, insulin is produced, but target cells fail to take up glucose from the blood, and the blood glucose level remains elevated (see Figure 3.26❸). Although heredity can play a role in type 2 diabetes, excess body weight and lack of exercise significantly increase the risk of developing this disorder. This form of diabetes generally appears after age 40 and accounts for more than 90% of diabetes cases.

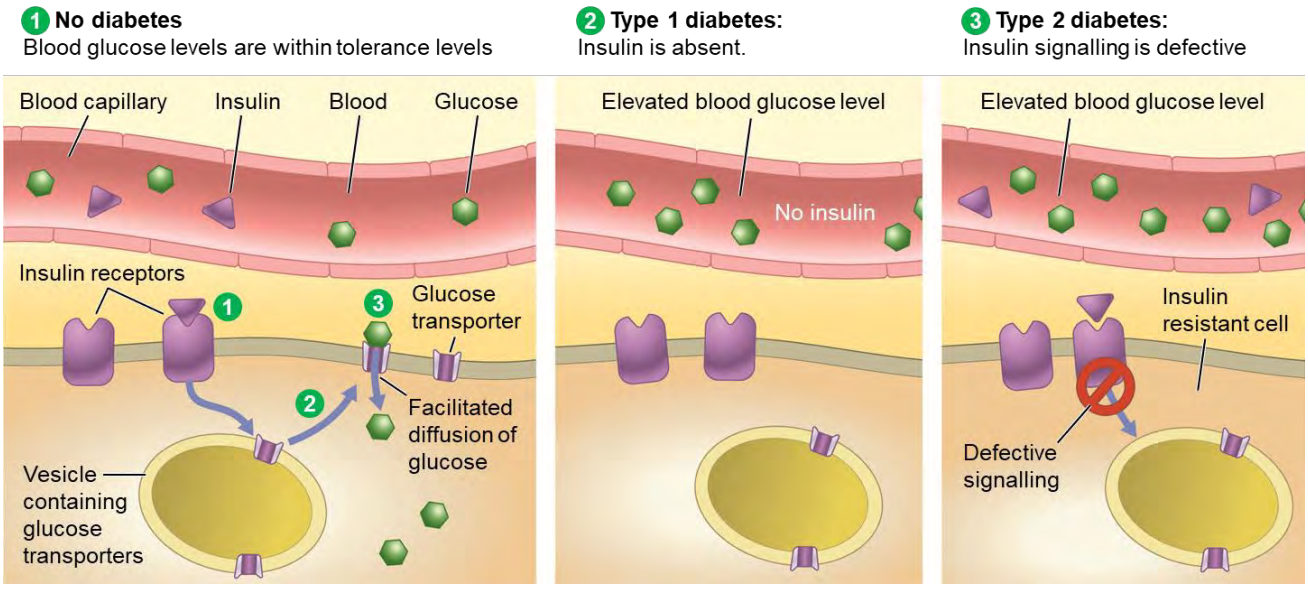
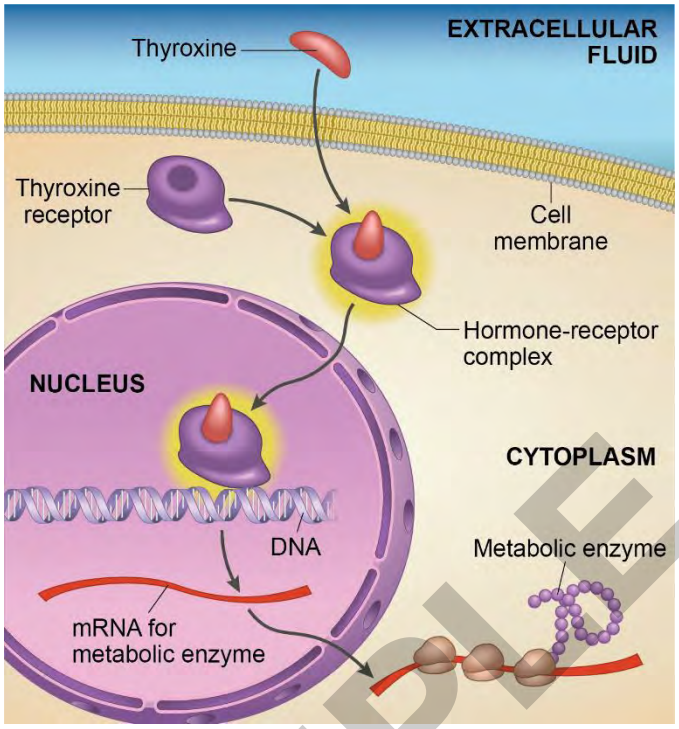


Figure 3.26: Diabetes mellitus types 1 and 2.

Question 173

The diagram below shows the action of thyroxine on tissue cells.



(a) Use the diagram to describe and explain the effect of thyroxine on metabolism.

(3 marks) **KA1**

(b) Thyroxine is synthesised using dietary iodine.

(1) State why iodine-deficient individuals frequently experience low body temperature.

(2 marks) **KA2**

(2) Predict and explain the effect of iodine deficiency on TSH levels in the blood.

(2 marks) **KA2**

Protocells

The Miller-Urey experiment has been repeated many times under various conditions, and the results show that Earth's early atmosphere and volcanic activity provided the environment for synthesising organic molecules. Scientists speculate a scenario in which high concentrations of organic compounds, including simple sugars, lipids, amino acids and nucleotides, formed near the openings of undersea volcanoes or hydrothermal vents (see Figure 4.02) beginning around 4 billion years ago. The lipids spontaneously assembled into vesicles in water, enclosing the simple organic compounds in a fluid-filled compartment with an internal chemistry different to its surroundings. Within these vesicles or **protocells**, the amino acids and nucleotides assembled into proteins and nucleic acids, self-replicating macromolecules capable of directing metabolism.

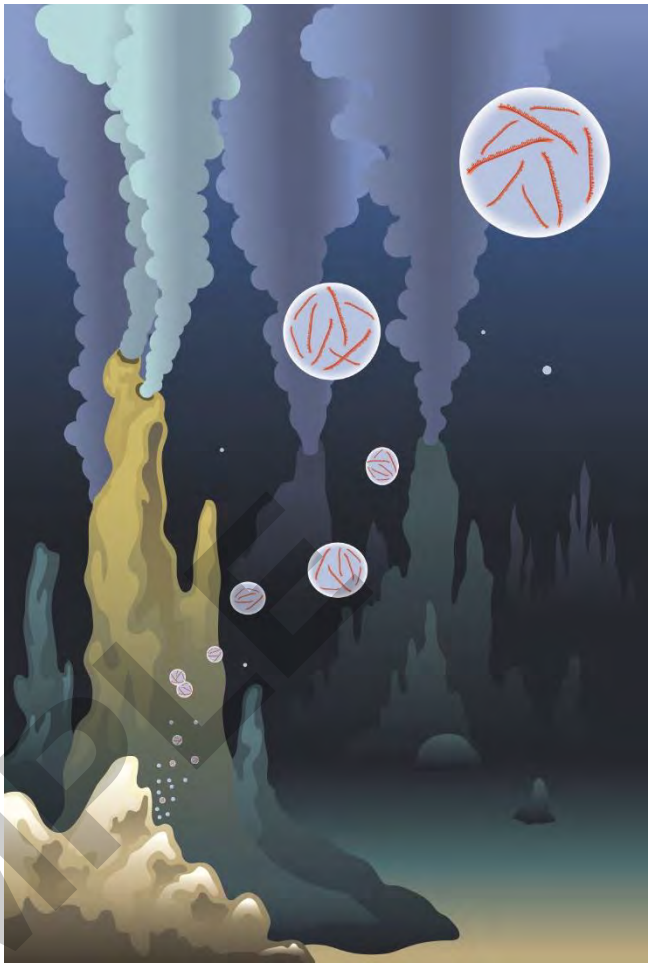


Figure 4.02: Protocell formation near hydrothermal vents

Recent experiments show that vesicles exhibit properties of life, including simple reproduction, metabolism and the maintenance of an internal chemical environment different to its surroundings. For example, vesicles can "reproduce" on their own, and they can increase in size ("grow") without dilution of their contents (see Figure 4.03). Finally, experiments have shown that some vesicles have a selectively permeable bilayer and can perform metabolic reactions, another essential requirement for life. Though speculative, a scenario in which the first protocells assembled from simple molecules in early Earth's atmosphere has led to predictions tested and confirmed in the laboratory.

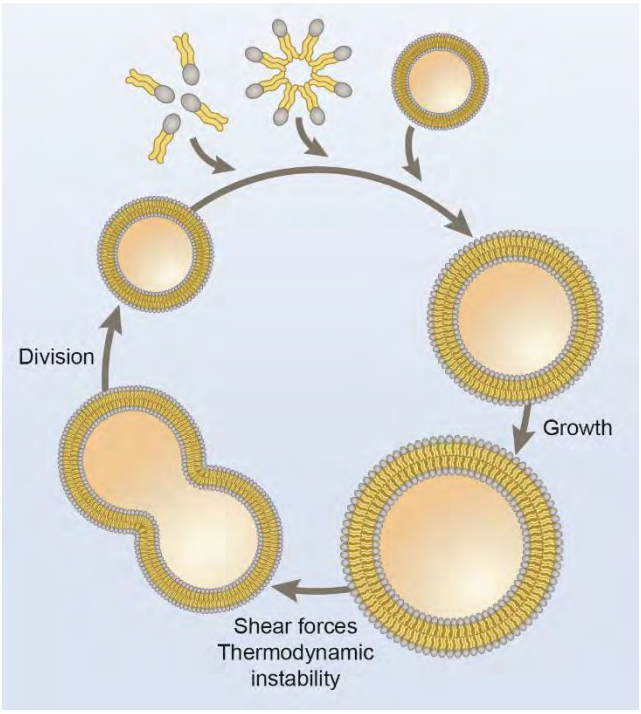
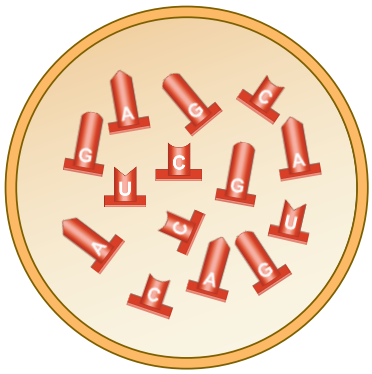


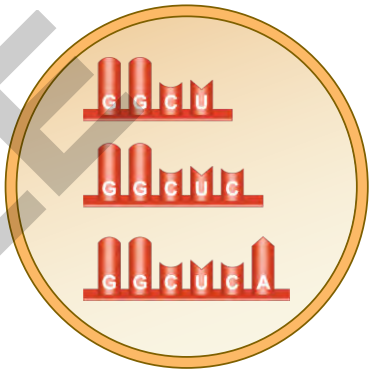
Figure 4.03: Vesicle growth and replication.

The RNA World

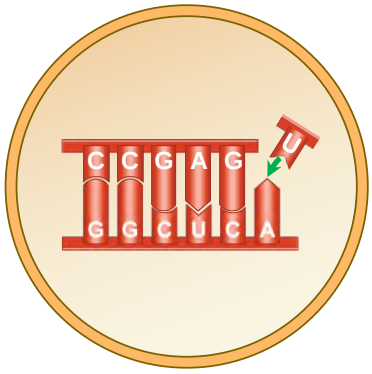
The Miller-Urey experiment and its subsequent repetitions produced nucleotides, the building blocks of nucleic acids, including DNA and RNA. However, most scientists believe the first genetic material in protocells was likely RNA, not DNA. RNA molecules have many properties protocells required for their survival and proliferation, including storing genetic information and acting as a catalyst. One class of RNA molecules, called **ribozymes**, synthesise complementary copies of short RNA strands when supplied with nucleotides (see Figure 4.04). Natural selection on the molecular level likely produced ribozymes capable of controlling metabolic reactions, including self-replication. Unlike double-stranded DNA, which forms a helix, single-stranded RNA molecules assume various specific three-dimensional shapes coded for by their nucleotide sequences. Some RNA molecules with specific nucleotide sequences had shapes enabling them to replicate faster and with fewer errors than other sequences. Occasionally, a copying error resulted in a molecule with a shape even more adept at self-replication. The RNA molecule with the greatest ability to replicate itself left the most descendant molecules. Thus, life as we know it may have been preceded by an "**RNA world**" in which small RNA molecules were able to replicate and store genetic information about the vesicles that carried them. On early Earth, vesicles with such self-replicating, catalytic RNA could grow, divide, and pass their RNA molecules to daughter protocells. Although the first such protocells likely carried only limited amounts of genetic information, specifying only a few properties, their inherited characteristics could have been acted on by natural selection. The most successful of the early protocells would have increased in number because they could exploit their resources effectively and pass their abilities on to subsequent generations. Once RNA sequences carrying genetic information appeared in protocells, many additional changes would have been possible. For example, RNA could have provided the template on which DNA nucleotides were assembled. Double-stranded DNA is less fragile than RNA, making it a more chemically stable repository for genetic information. Many new life forms developed once, a change evident in the fossil record.



1 The first protocells contained RNA nucleotides produced from inorganic molecules.



2 The nucleotides were assembled into short RNA strands, some of which had catalytic ability (ribozymes).



3 These ribozymes replicated the RNA strands, ensuring continuity of genetic information as protocells divided.

Figure 4.04: RNA synthesis in protocells.

The Fossil Record

Fossils are the remains or imprints of organisms from the past. Many fossils are found in sedimentary rocks formed from the sand and mud that settle at the bottom of swamps, lakes and seas (Figure 4.05). New layers of sediment cover older ones and compress them into layers of rock called **strata**. The fossils in a particular stratum show some organisms that populated the area when that layer formed. Beginning with the earliest traces of life, the **fossil record** opens a window into the world of long ago and provides glimpses of the evolution of life over billions of years. The fossil record shows significant changes in the kinds of organisms on Earth at different times. Many past organisms were unlike those living today, and several that were once common are now extinct. In addition, fossils document how new groups of organisms arose from previously existing ones. Such fossils are central to our understanding of evolution as they illustrate how new physical features arise and how long it takes for such changes to occur.

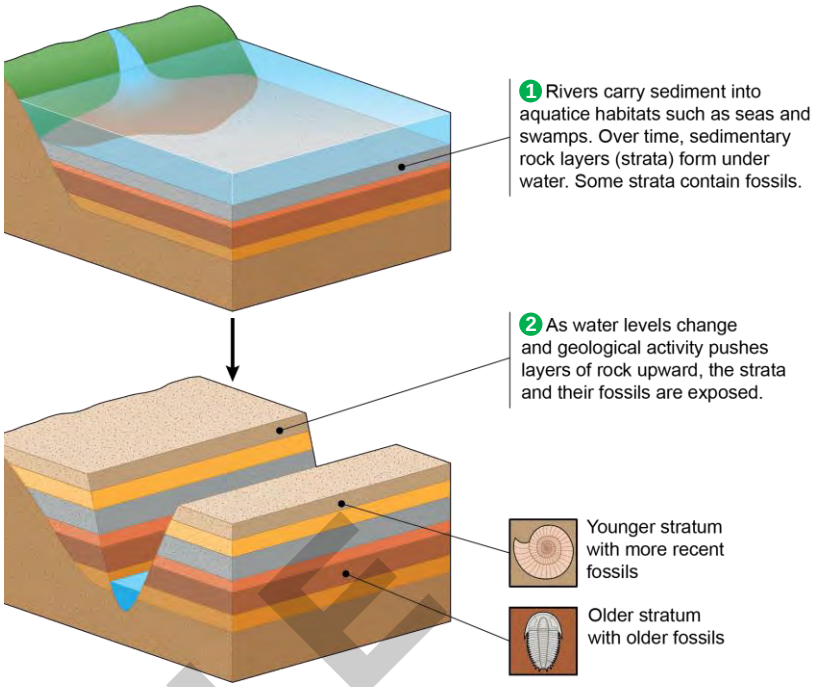


Figure 4.05: Formation of sedimentary strata with fossils.

Earth's first living things were single-celled prokaryotes that evolved from protocells. Early prokaryotes lived in the ocean and were primarily autotrophs. The earliest direct evidence of prokaryotes, dating to 3.5 billion years ago, comes from fossilised **stromatolites** (Figure 4.06), layered rocks that form when prokaryotes bind thin films of sediment together. Each layer contains fossilised photosynthetic prokaryotes, the ancestors of modern cyanobacteria. Stromatolites and other early prokaryotes were Earth's sole inhabitants for about 1.5 billion years, and their actions transformed life on our planet. Around 2.7 billion years ago, the oxygen produced by photosynthetic prokaryotes began diffusing from the oceans to the atmosphere, having an enormous impact on life. The rising oxygen concentration likely doomed many prokaryotes. Still, the survivors began using oxygen in cellular respiration, supplying the energy for the rapid growth and diversification of aerobic life on Earth. Among the survivors were the ancestors of eukaryotes, the first evidence of which is seen in the fossil record around 2 billion years ago.

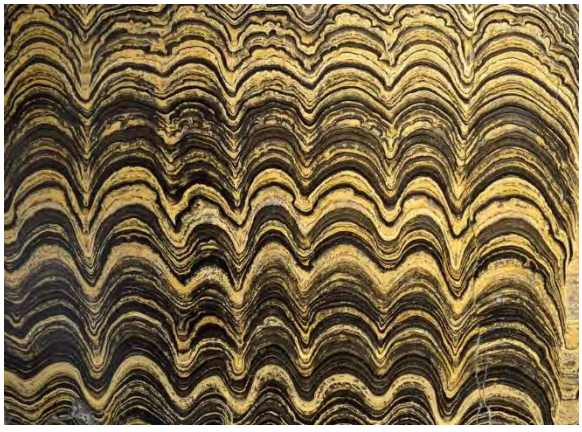


Figure 4.06: Fossilised stromatolite

Endosymbiotic Theory

The fossil record suggests eukaryotes evolved from their prokaryotic ancestors around 2 billion years ago. Current evidence indicates that eukaryotes originated from **endosymbiosis** when a prokaryotic cell engulfed a small aerobic cell that would evolve into the **mitochondrion**. The small, engulfed cell is an example of an **endosymbiont**, a cell that lives within another cell called the host cell. Over time, the host and endosymbionts became a single organism. Although all eukaryotes have mitochondria or remnants of these organelles, they do not all have chloroplasts and related organelles. Therefore, the **serial endosymbiosis hypothesis** predicts that mitochondria evolved before chloroplasts through endosymbiotic events. As shown in Figure 4.07, both mitochondria and chloroplasts are thought to have descended from bacterial cells. The nucleus and endomembrane system likely formed through an infolding of the cell membrane, and the prokaryotic ancestors of the mitochondrion and chloroplast probably entered the host cell as undigested prey or internal parasites.

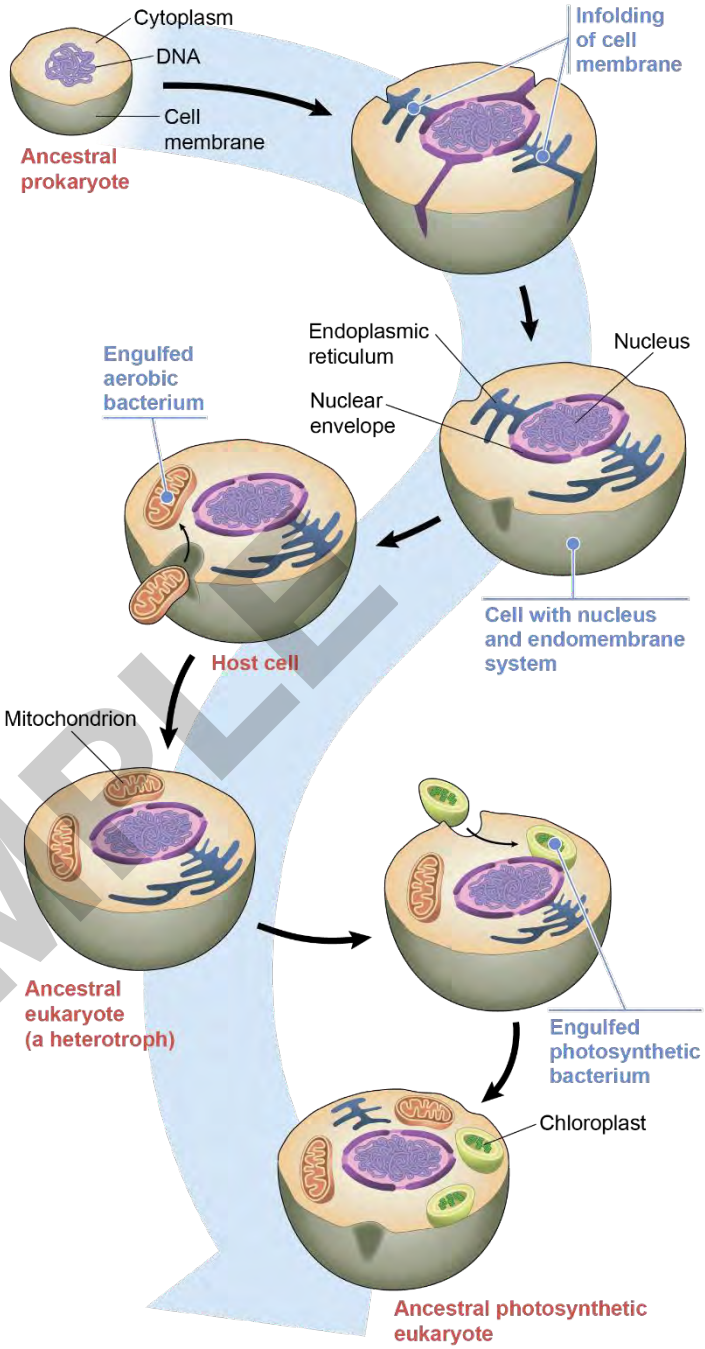


Figure 4.07: Serial endosymbiosis hypothesis.

A great deal of evidence supports the endosymbiotic origin of mitochondria and chloroplasts. **First**, the inner membranes of both organelles have enzymes and transport systems similar to those found in the cell membranes of living bacteria. **Second**, mitochondria and chloroplasts replicate by a similar splitting process to binary fission in certain bacteria. **Third**, mitochondria and chloroplasts contain a single circular DNA molecule that, like the chromosome of a bacterium, is not associated with histones or other proteins. **Fourth**, mitochondria and chloroplasts have ribosomes and other cellular machinery needed to transcribe and translate their DNA into proteins. **Finally**, in terms of size, the ribosomes of mitochondria and chloroplasts are more similar to bacterial ribosomes than to the cytoplasmic ribosomes of eukaryotic cells.

chromosomes and DNA. As shown in Figure 4.25, each gene is a sequence of nucleotides at a specific place, or **locus**, along a particular chromosome. However, the DNA at that locus can vary slightly in its nucleotide sequence. This variation in information content can affect the function of the encoded protein and, thus, an organism's inherited characteristics. For example, the purple-flower and white-flower alleles are two DNA sequence variations possible at the flower-colour locus on a pea plant's chromosomes. The nucleotide sequence of the purple-flower allele codes the synthesis of an enzyme that synthesises a purple pigment, and the white-flower allele sequence does not.

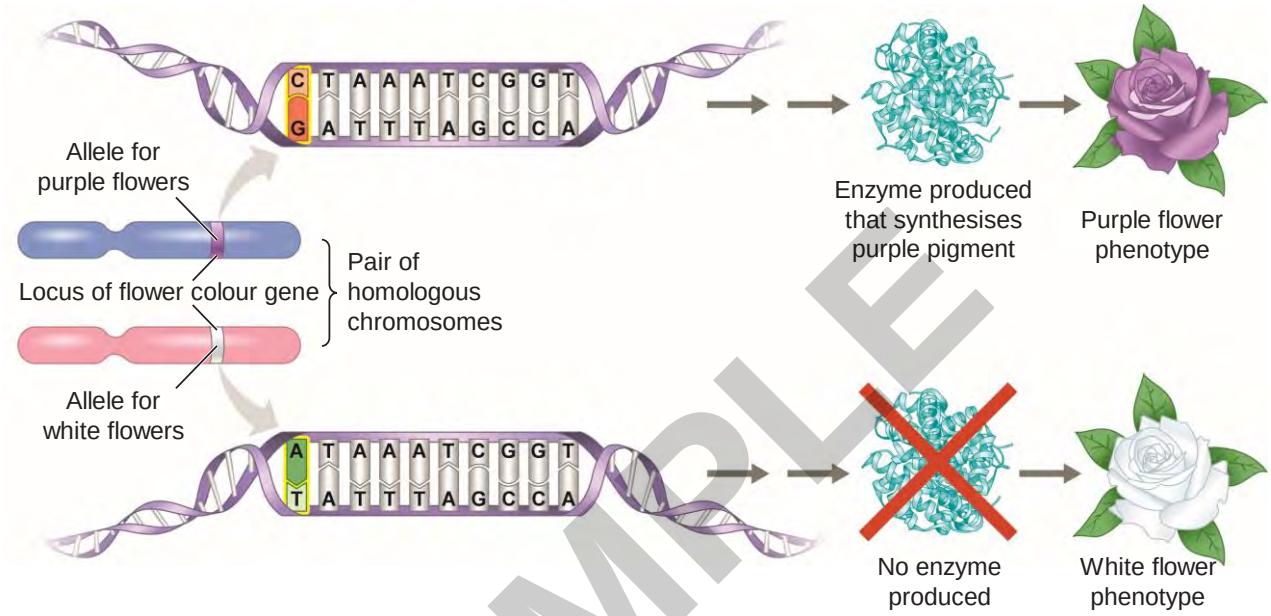


Figure 4.25: Alleles, alternate versions of a gene.

Phenotypic traits vary amongst members of a species. For example, humans vary in height, eye colour and blood type. Such phenotypic differences reflect **genetic variation**, differences between individuals in the composition of their DNA nucleotide sequences. Genetic variation originates when processes, primarily mutations, produce new alleles and genes. In some cases, a point mutation in a single gene can produce a new phenotype, as in Sickle-cell disease (see Figure 1.51), while in others, multiple mutations across several genes are required. Most species have low mutation rates, averaging around one mutation in every 100,000 genes per generation. However, prokaryotes and other organisms that reproduce asexually have many more generations per unit of time, so mutations can quickly generate genetic variation in their populations.

In organisms that reproduce sexually, most genetic variation results from the unique combination of alleles each individual receives from their parents. At the nucleotide level, all the differences among these alleles have originated from past mutations. Sexual reproduction then shuffles existing alleles and deals them randomly to produce individual genotypes. Three mechanisms contribute to this shuffling: crossing over, the independent assortment of chromosomes, and random fertilisation (see Chapter 2.8). Together, these three mechanisms ensure that sexual reproduction rearranges existing alleles into fresh combinations each generation, providing much of the genetic variation that makes evolution possible.

The concept of natural selection is based on differential success in survival and reproduction. Individuals in a population exhibit variations in their heritable traits, and those with traits that are better suited to their environment tend to produce more offspring than those with traits that are not as well suited. In genetic terms, natural selection results in alleles being passed to the next generation in proportions that differ from the present generation. For example, some cockroach populations have an allele that confers resistance to the insecticide DDT. This **insecticide-resistant allele** (see Figure 4.30) has a frequency of 0% in cockroaches collected in the wild before DDT was first used in the 1940s. However, the allele frequency has increased by more than 50% in most cockroach populations sampled after 1960, following 20 or more years of DDT use. The increase in the frequency of this allele most likely occurred because DDT is an effective poison acting as a selection pressure in exposed insect populations. Members of a cockroach population lacking this allele succumb to the poison before passing on their alleles through reproduction, while others survive and pass on their insecticide-resistant alleles. An organism's heritable traits can influence how well its offspring cope with similar environmental challenges. For example, a kangaroo may be a faster hopper, a trait that gives its offspring an advantage in escaping predators such as the dingo in Figure 4.31. When such advantages increase the number of offspring that survive and reproduce, the favoured traits will likely appear more frequently in the next generation.

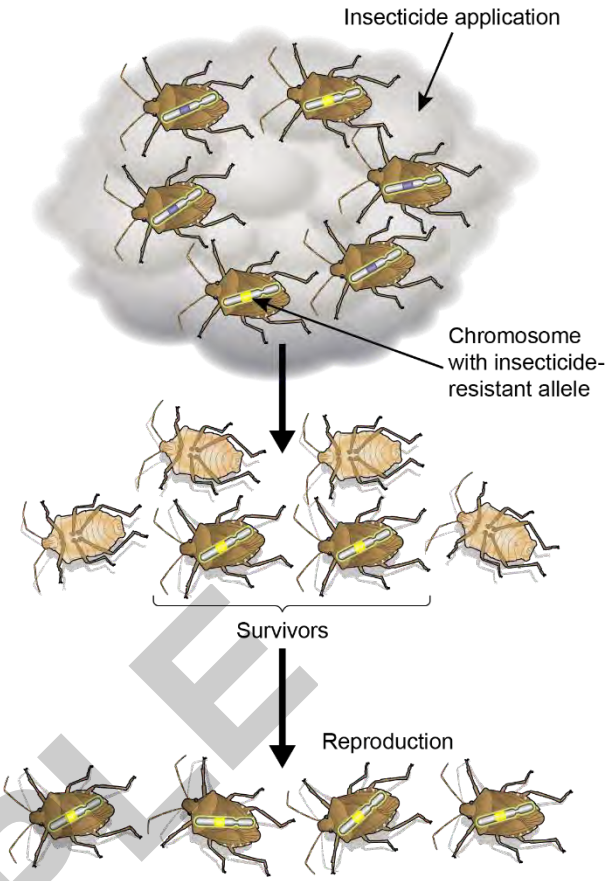


Figure 4.30: Natural selection in cockroaches

Figure 4.31: Natural selection in kangaroos.

Gene Flow

Natural selection and genetic drift are two phenomena affecting allele frequencies in a population's gene pool. A third phenomenon affecting allele frequencies is **gene flow**, whereby fertile individuals or their gametes migrate into or out of a population. For example, suppose two populations of silver gulls (*Chroicocephalus novaehollandiae*) are separated by a large body of water, as in Figure 4.38. The gene pools of the two populations have different allele frequencies, with the population to the left having 63% b and the population to the right having 0% b. One individual of genotype bb migrates away from its original population on the left and joins the distant population on the right, transferring its alleles and increasing the frequency of the b allele in its new population. This example of gene flow reduces genetic differences between the two populations of silver gulls.

Figure 4.38: Gene flow in silver gull populations.

Gene flow can have beneficial or detrimental effects on populations. In some cases, gene flow transfers alleles that improve the ability of populations to adapt to local conditions. For example, gene flow has resulted in the worldwide spread of insecticide-resistant alleles amongst mosquito populations. In other cases, gene flow transfers harmful alleles. For example, the migration of *Parus major* from mainland Netherlands introduces harmful alleles in Vlieland populations through gene flow. Natural selection reduces the frequency of these alleles in the eastern population, where mainland immigration is low, but their higher frequency in the central population decreases the survival rate of females, as shown in Figure 4.39.

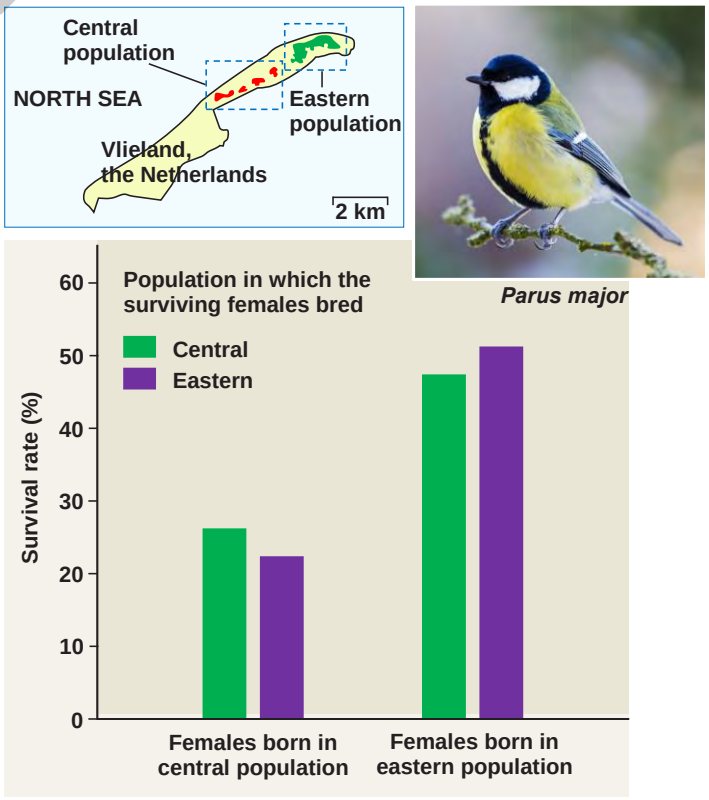
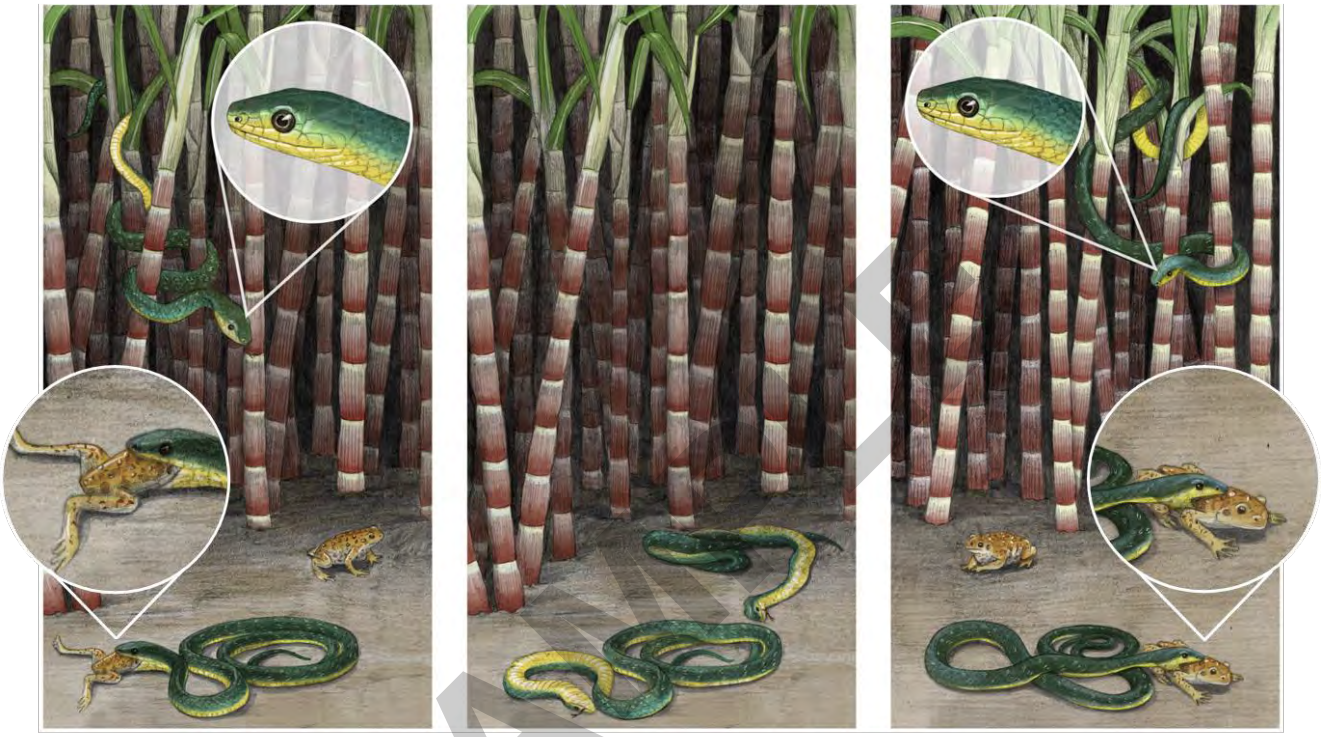


Figure 4.39: Gene flow in *Parus major*.

Question 194

A cane toad population was introduced to Australia in 1935 to control beetle populations that damaged sugar cane crops. Cane toads secrete venom from glands on each shoulder when threatened. The venom is often fatal if ingested as the toxins inhibit heart muscle function.

The diagram below describes one effect of the cane toad's introduction on the recent evolution of Australia's green tree snake population.



Before 1935, some green tree snakes had heads large enough to swallow cane toads.

These snakes were less likely to survive and reproduce.

Subsequent generations have smaller heads, incapable of swallowing cane toads.

(a) Identify the mechanism described in the diagram that decreases the frequency of alleles coding large heads in Australia's green tree snake population.

_____ (1 mark) **KA2**

(b) The cane toad is a selection pressure on Australia's green tree snake population.

(1) Define a selection pressure using the cane toad as an example.

_____

_____

_____ (1 mark) **KA2**

(2) Suggest one other selection pressure affecting Australia's green tree snake population.

_____

_____

_____ (1 mark) **KA2**

Allopatric Speciation

Allopatric speciation is a process by which a geographic barrier divides a parent population into isolated subpopulations. For example, the water level in a lake may decrease over time, resulting in two smaller lakes that are now home to separated populations, as in Figure 4.41. This geographic barrier between lakes prevents gene flow between the isolated populations. Instead, the two populations are exposed to different selection pressures in their respective lakes, resulting in evolution by natural selection. As a result, the two populations become reproductively isolated and evolve into separate species. Allopatric speciation also occurs when individuals colonise a remote area, and their descendants become geographically isolated from the parent population. For example, Darwin's finches (Figure 4.29) likely originated from an ancestral species that reached the Galapagos Islands from mainland South America around 2 million years ago. Populations of the ancestral species colonising each island were exposed to different selection pressures, and the geographic barriers reduced gene flow, leading to speciation.

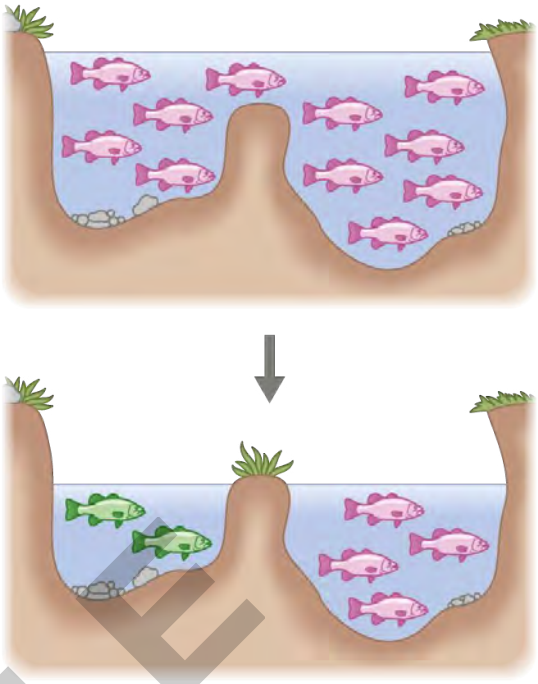


Figure 4.41: Allopatric speciation in fish

For allopatric speciation to occur, members of a population must become separated by a barrier sufficient to restrict gene flow. In small rodents, this could be a river or canyon, lizards, a mountain range, birds, an island, and shrimp, a land bridge. Once geographic isolation has occurred, the separated gene pools may diverge. As a result, different mutations arise, and natural selection and genetic drift alter allele frequencies differently in the separated populations. Reproductive isolation occurs due to genetic divergence resulting from natural selection or genetic drift. For example, a geologic event may isolate a plant population. Wind can no longer transfer gametes between the two sides of the canyon as it widens, causing the two populations to become reproductively isolated and separate species.

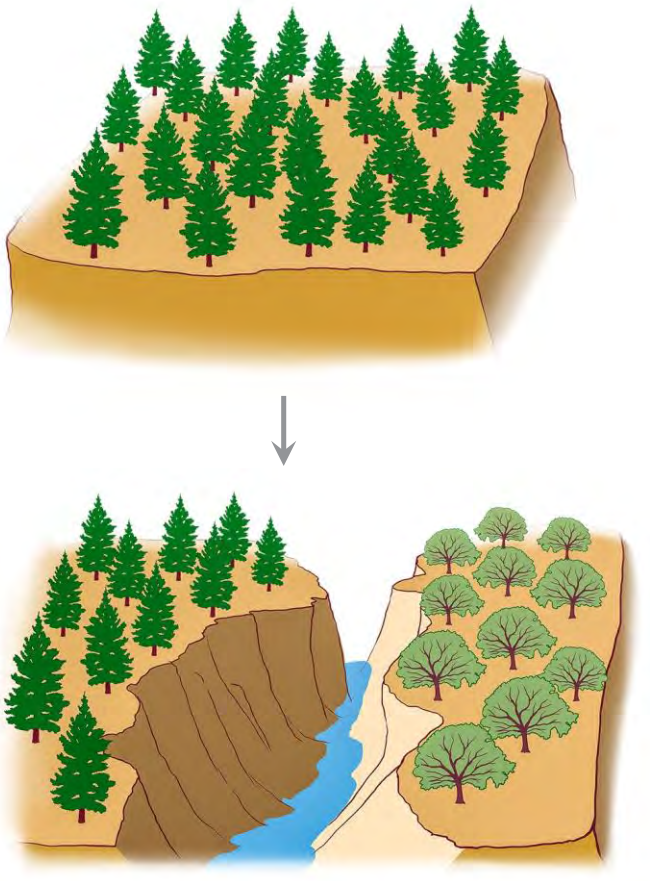


Figure 4.42: Allopatric speciation in plants

Sympatric Speciation

Sympatric speciation is a process by which a parent population evolves into reproductively isolated subpopulations in the same geographic area. For example, Figure 4.43 depicts an ancestral fish population evolving into separate species in the same lake. Over time, specific reproductive barriers develop between the parent fish population while its members remain in contact, resulting in the development of distinct species in the same lake. Although such contact and the ongoing gene flow make sympatric speciation less common than allopatric speciation, sympatric speciation can occur if such factors, including polyploidy, sexual selection and habitat differentiation, reduce gene flow.

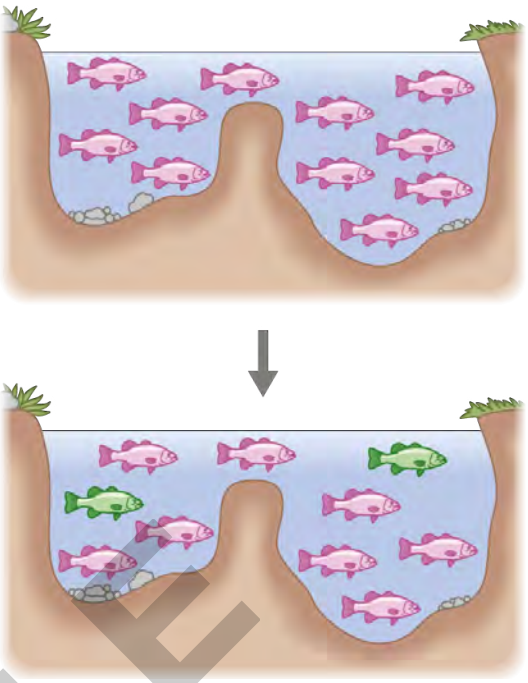


Figure 4.43: Sympatric speciation in fish

Polyploidy is a condition in which a mistake during cell division results in gametes containing extra sets of chromosomes. For example, cell division errors can double a cell's chromosome number from 2n to 4n, as in Figure 4.44. The resulting organism can produce fertile offspring by self-fertilising or mating with other organisms with the same chromosome number. In addition, the 4n offspring is reproductively isolated from the 2n members of the original population because their offspring would be sterile. A second type of polyploidy occurs when two different species interbreed and produce hybrid offspring. Most hybrids are sterile because their chromosomes are not homologous and cannot pair during meiosis. However, such a hybrid may be able to reproduce asexually. In subsequent generations, various mechanisms can change a sterile hybrid into a fertile polyploid incapable of interbreeding with either parent species. Polyploidy always results in sympatric speciation rather than allopatric speciation as the new biological species arise in the same geographic area as its parent population. Polyploidy accounts for more than 80% of all plant species alive today and a small number of animal species, including some salamanders, frogs, and leeches.

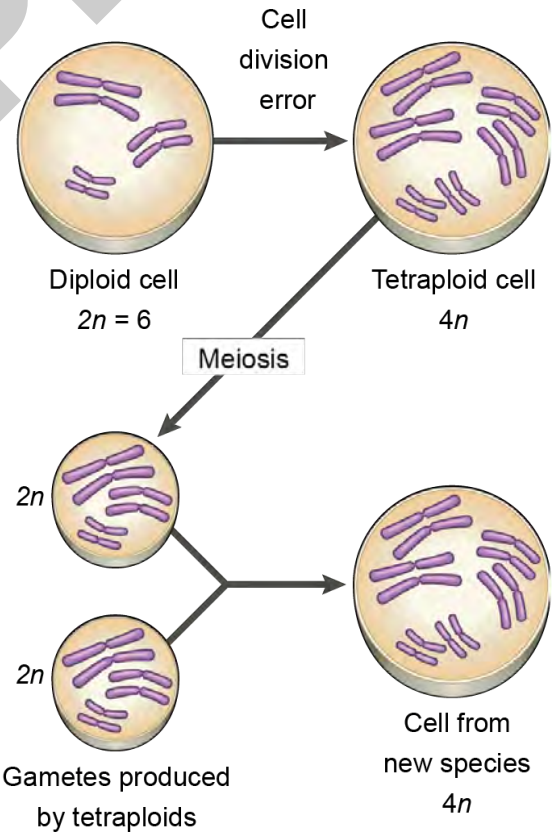


Figure 4.44: Polyploidy

Succession

Sympatric speciation may occur when subpopulations migrate to a new habitat where they are exposed to different selection pressures from their parent population. In some cases, new habitats arise when the previous occupants migrate away or become extinct. In others, new habitats arise following a severe environmental disturbance, such as a volcanic eruption producing bare land or a retreating glacier stripping away all existing vegetation. The disturbed area may be colonised by various species, which are gradually replaced by other species, a process called **ecological succession**. It is called **primary succession** when the process begins in a virtually lifeless area, such as on the bare rock produced by cooled lava or a retreating glacier. A recent example of primary succession is Muir Glacier, Alaska (Figure 4.46). The retreating expanse of ice and snow retreated over time, exposing bare rock that plants and animals soon colonised. During primary succession, the colonising species, called **pioneer species**, are often prokaryotes, lichens and mosses, small plants that grow from windblown spores. These organisms secrete acids that weather rocks, forming a thin soil with nutrients supporting lichens and mosses. Soil develops gradually as rocks weather, and organic matter accumulates from the decomposed remains of the early colonisers. Once the soil is present, the lichens and mosses are usually overgrown by **intermediate species,** including grasses, shrubs, and trees that sprout from seeds blown in from nearby areas or carried in by insects and birds. Eventually, an area is colonised by plants that become the community's dominant vegetation. Producing such a **climax community** through primary succession may take hundreds or thousands of years. The process of primary succession from bare rock is described in Figure 4.47.

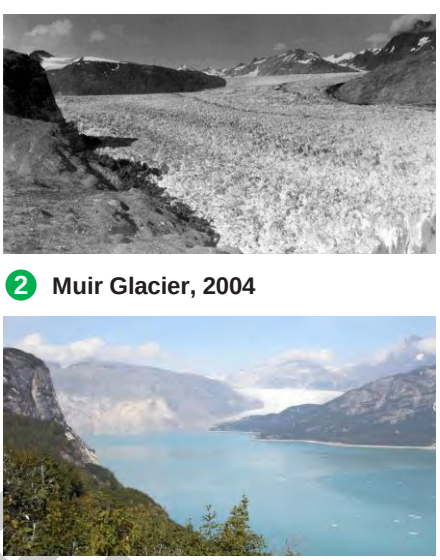


Figure 4.46: Primary succession (Muir Glacier)

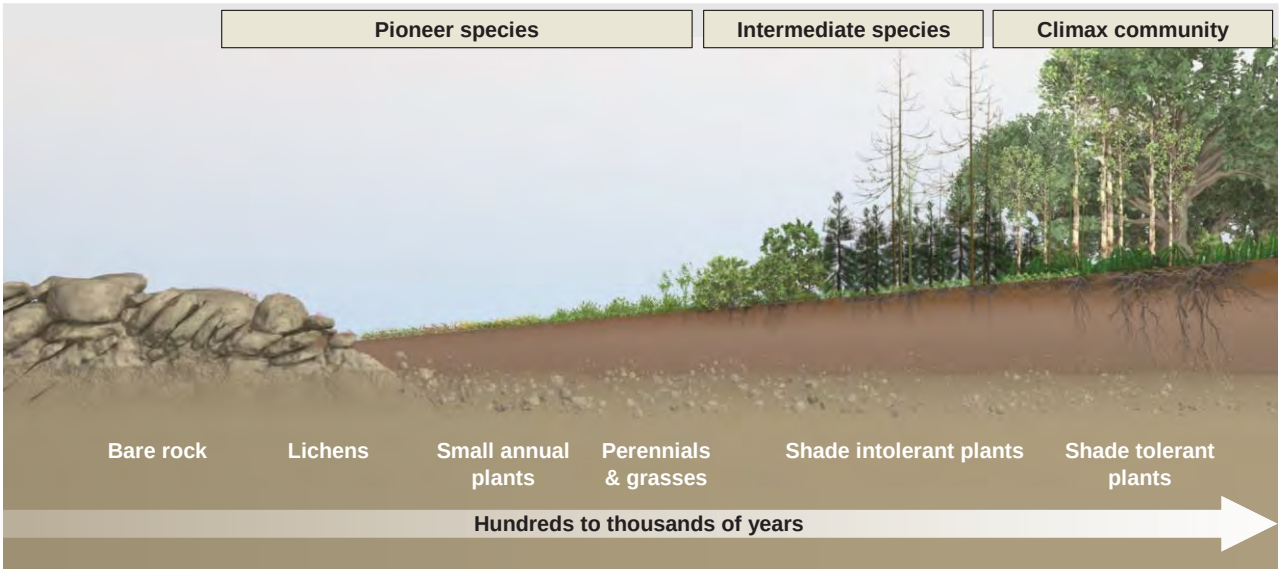


Figure 4.47: Primary succession from bare rock.

Figure 4.48 describes primary succession following volcanic eruptions that eject lava into the ocean.

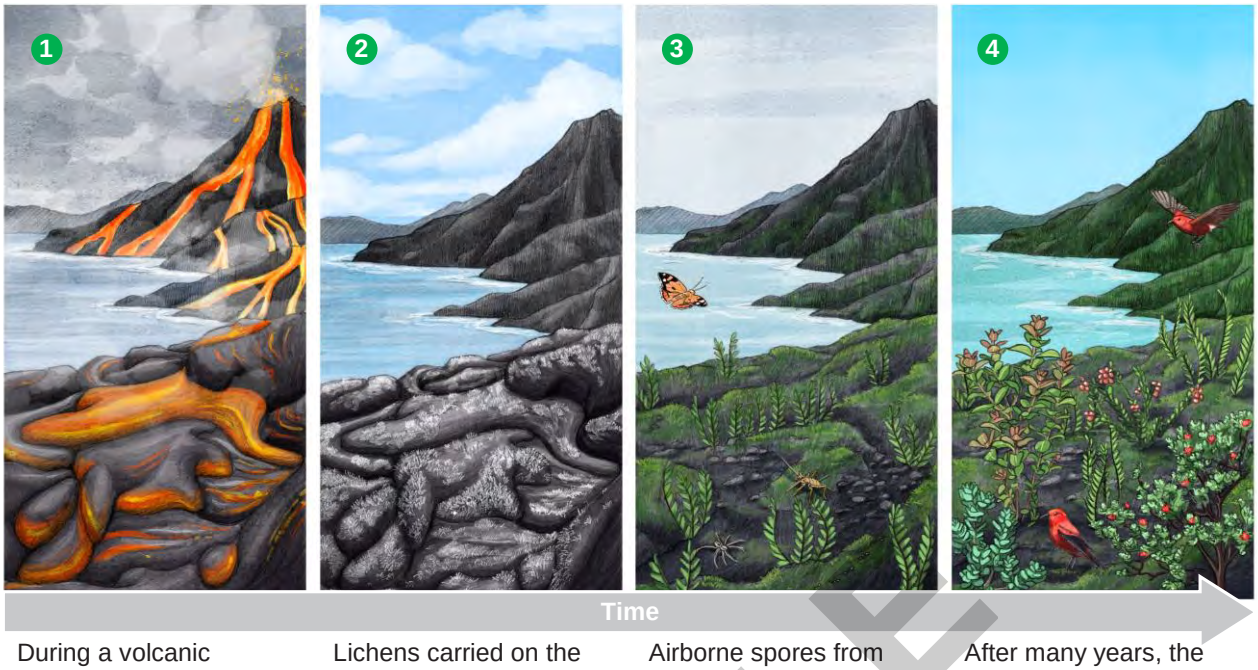


Figure 4.48: Primary succession on a volcanic island.

Secondary succession involves populations recolonising an area after a severe disturbance has removed most organisms in a community, but the environment remains habitable. For example, in the temperate broadleaf forests in Australia, bushfires (Figure 4.49) create patches of forest with a high nutrient content that are colonised by pioneer species germinating from seeds that lay dormant in the soil. Pioneer plant species grow rapidly in the fertile soil, and their increasing abundance is paralleled by a return of herbivores and lower-order consumers.

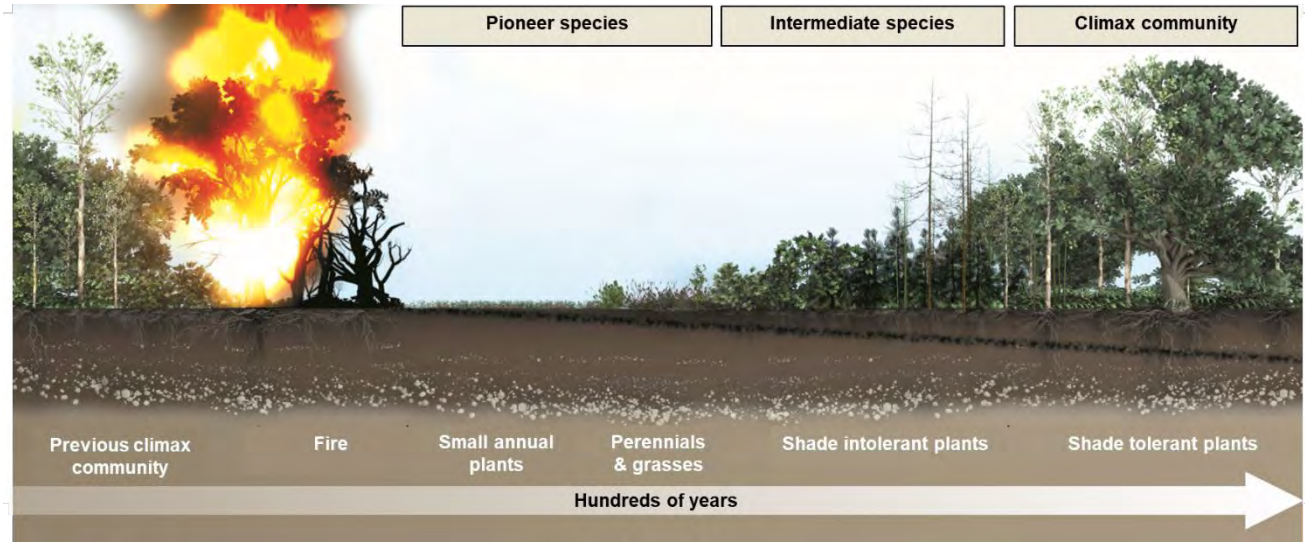
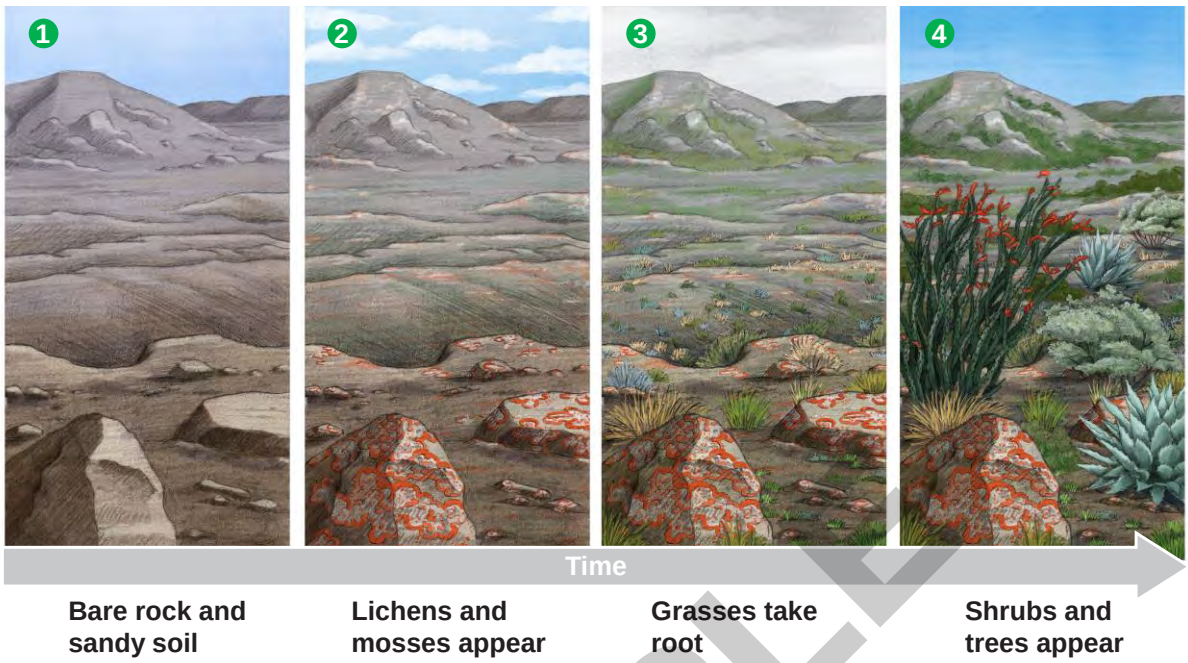


Figure 4.49: Secondary succession following a fire.

Question 208

The diagram below illustrates primary succession in a desert ecosystem.



(a) Describe the process of succession in the desert ecosystem.

(4 marks) KA2

(b) The species composition of the desert changes during each stage of primary succession.

Describe two factors that change the species' composition during primary succession.

(2 marks) KA2

4.7: Human Impact on Biodiversity

Human activities can create new and significant selection pressures on a gene pool, leading to species extinction.

Maintaining biodiversity is an ethical issue with long-term biological and/or environmental consequences.

- Describe how these activities have caused or may threaten the extinction of species.
- Give examples of human activities that lead to climate or environmental change.
- Recognise that humans have an obligation to prevent species extinction.

Biodiversity refers to the variety of living things found in an area and is considered at three levels: genetic diversity, species diversity, and ecosystem diversity. As previously explored, **genetic diversity** refers to the variety of alleles present in the gene pool of a population. **Species diversity** refers to the variety of species in an ecosystem. Species diversity is determined by several factors, including the number of species in a community, called **species richness**, and the abundance of each species in an area, called **species evenness**. In addition, species diversity depends on the local environmental conditions, including light intensity, climate, soil depth and type, competition, and resource availability. Finally, **ecosystem diversity** refers to the variety of ecosystems in a geographic area. The number of ecosystems depends on the region's topography and climate. Geographic areas with a broader variation in topography have greater ecosystem diversity as the varied terrain provides more habitats where species can find shelter and escape predation. For example, Figure 4.56 illustrates the three levels of biodiversity in the Daintree Rainforest in northeast Queensland. At the ecosystem level, there are a variety of habitats in the Daintree, including forests, mountains, streams and oceans, providing habitats for thousands of species. At the species level, thousands of animals, plants and microorganisms share the rainforest floor, including cassowary, northern quoll and tree kangaroos. At the genetic level, there is variation in the alleles at each chromosome locus within cassowary populations.

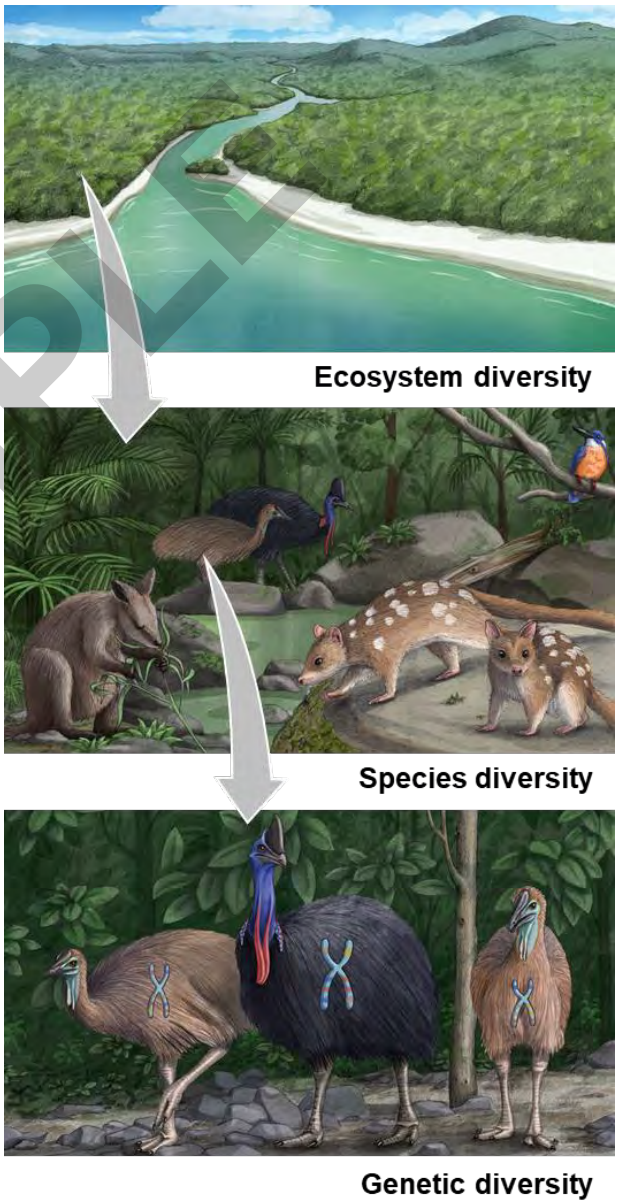


Figure 4.56: Three levels of biodiversity

Greenhouse Gases and Climate Change

Human activities, including burning fossil fuels and biomass, using fertilisers in agriculture and improper disposal of organic waste, release various gaseous waste products into the atmosphere. Many of these waste products are **greenhouse gases**, including carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), water vapour and others. Greenhouse gases in the atmosphere absorb much of the infrared radiation (heat) Earth emits after being heated by incoming solar radiation and radiate most of the energy back to Earth's surface, effectively trapping the heat and maintaining a stable atmospheric temperature. In the absence of this natural process, called the **greenhouse effect** (Figure 4.62), the average air temperature at Earth's surface would be below 0°C and most life as we know it could not exist.

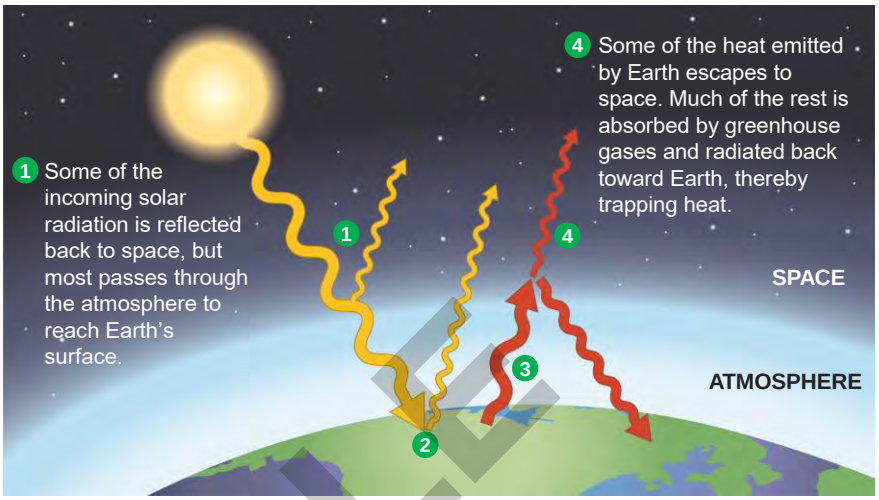


Figure 4.62: The greenhouse effect

Over the past two centuries, humans have emitted excessive quantities of greenhouse gases into the atmosphere, primarily carbon dioxide, which has enhanced the greenhouse effect. Over the past two centuries, the atmospheric concentration of carbon dioxide has increased due to the burning of fossil fuels and deforestation. The atmospheric carbon dioxide concentration increase concerns scientists because of its link to increased global temperature. As the concentrations of carbon dioxide and other greenhouse gases increase, more solar heat is retained, thereby increasing the temperature of our planet. So far, Earth has warmed by an average of 0.9°C since 1900. Much of this warming has occurred recently, with 18 of the 19 warmest years on record occurring since 2001 (Figure 4.63).

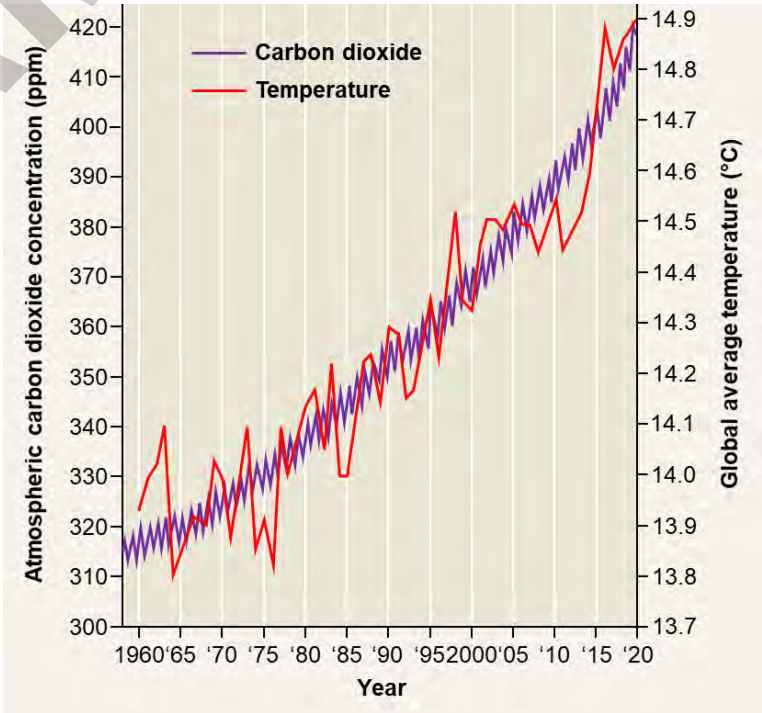


Figure 4.63: Increase in CO_2 and temperature

Question 15

The peppered moth (*Biston betularia*) is an insect found in the Northern hemisphere.

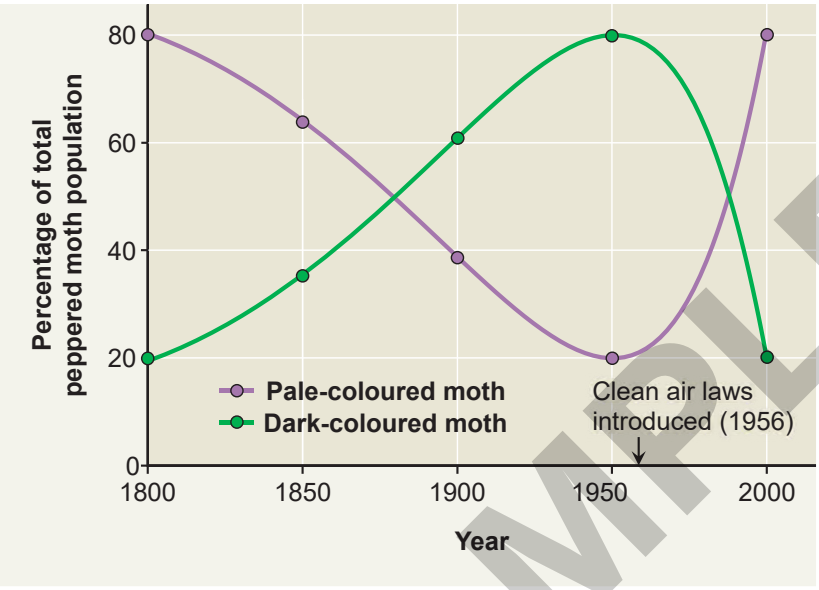
Peppered moths rest on trees during the day, where they are preyed upon by birds.

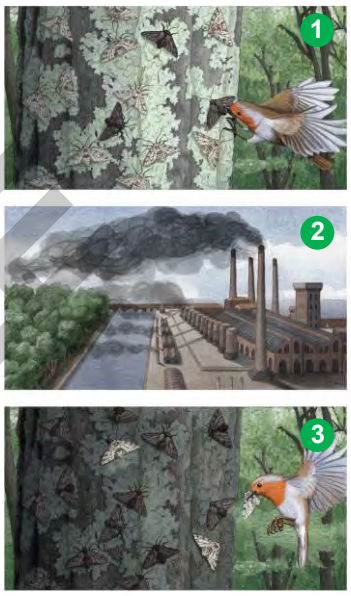
Two varieties of peppered moths were observed in Great Britain during the 19th century.

Before 1850, the trees were colonised by lichens which gave trees a pale speckled colour 1.

Industrial activity increased around 1850, and pollutants blackened the trees 2 and 3.

The graph below shows the population sizes of peppered moths in Great Britain since 1800.





(a) Describe the experimental results using the theory of evolution by natural selection.

(4 marks) **IAE3**

(b) Explain why some scientists may question the reliability of the data from this investigation.

(2 marks) **IAE4**

Question 18

Read the following passage:

More than 1000 species native to South Australia (SA) are threatened. The Department for Environment and Water works in partnership with community groups and government and non-government agencies in SA on projects that assist with conserving threatened species. One project, the Regional Species Conservation Assessment Project, was developed to manage threatened species and their habitats in South Australia.

The project provides a systematic and standardised way to assess and prioritise SA's native species using data collected from various sources and offers a framework for assigning conservation status and population trends for all native vertebrate animals and vascular plants. The data collected uses the best available science and information and the expertise of skilled people in various specialist areas to rank species according to their threat level and the likelihood of extinction.

The data from the project has enabled conservationists to identify and raise awareness of threatened and declining species, manage habitats, inform regional fire management plans, and help to identify threatening processes that affect a range of species.

Use your understanding of science as a human endeavour to:

(a) explain why collaboration between government departments, community groups and other agencies is required to protect threatened species in SA.

(2 marks) **KA3**

(b) explain the need for a framework assigning conservation status to threatened species.

(2 marks) **KA3**

(c) explain who should be responsible for maintaining and monitoring the framework.

(2 marks) **KA3**

Question	Part		Author's response	Marks
1	(a)		1: Phosphate 2: Sugar/deoxyribose	1+1
			3: Nitrogenous base/base 4: Sugar-phosphate backbone	1+1
	(b)		Two polynucleotide strands form a double helix.	1
			The strands are composed of repeating nucleotides.	1
			The strands are joined by hydrogen bonds between the nitrogenous bases.	1
2	(a)		Nucleic acid	1
	(b)	(1)	Nitrogenous base/ base	1
		(2)	The nucleotides are bonded together by chemical bonds between the sugar and phosphate groups, forming the sugar-phosphate backbone.	1
			The nitrogenous bases connected to the sugar-phosphate backbone face inwards, forming hydrogen bonds that connect the two polynucleotide strands in the DNA molecule.	1
3	(a)		The uprights are composed of the sugar and phosphate groups of adjacent nucleotides.	1
	(b)		Nitrogenous bases/bases	1
			A pairs with T, and G pairs with C.	1
4	(a)		The bacterial chromosome is a single unbound DNA molecule.	1
			The DNA molecule forms a closed loop.	1
	(b)		Cytosol/cytoplasm	1
5	(a)		The diagram shows a single loop of DNA.	1
			This is evidence that mitochondrial DNA is a single, circular DNA molecule.	1
	(b)		Mitochondria were once free-living prokaryotes.	1
	(c)		Nucleus and chloroplasts.	1+1
6	(a)		The diagram shows two open ends indicating the DNA molecule is linear and not a closed-loop like prokaryotic cells.	1
	(b)		Each chromosome is a single DNA molecule associated with proteins.	1
			The DNA is wound tightly around the proteins forming a mixture called chromatin.	1
			The chromatin is condensed and packaged into linear chromosomes.	1
	(c)		DNA in chloroplasts is arranged into a single circular chromosome, and DNA in the nucleus into multiple linear chromosomes.	1
			DNA in chloroplasts is not associated with proteins, and DNA in the nucleus is associated with proteins.	1
7	(a)		The relative percentages of adenine and thymine are approximately equal, as are those of guanine and cytosine.	1
			This is evidence that adenine is complementary to thymine, and guanine is complementary to cytosine.	1
	(b)		Sea urchins and humans have different chromosome numbers/different genes.	1
			Hence, each genome has a different number of each nucleotide/nitrogenous base.	1

	(c)		**Any two:**	1+1
			Increase in the global average temperature. Decrease in ocean pH. Increase in plant growth rate, altering transpiration rates that affect local climate. Increase in plant growth rate, reducing soil moisture.	
	(d)		To prepare accurate climate models for predicting the impact of environmental change. These predictions can help scientists and governments to develop strategies to minimise the adverse effects of such change.	1 1
	(e)		**Any two:**	1+1
			Use public transport when possible. Walk or ride your bike instead of driving. Use energy-efficient cars and electrical appliances that use solar power. Switch electrical appliances off when not in use. Do not use air conditioning unless it is necessary.	
219	(a)		**Any one:**	1
			Deforestation causes habitat loss, reducing the genetic and species diversity of pine trees and their animal inhabitants. Deforestation produces open land where smaller animals are readily preyed upon by large predators. Deforestation alters transpiration patterns, altering the local climate and reducing precipitation to levels that cannot support vegetation.	
	(b)	(1)	The pine tree population will decline as the global average temperature increases. Drought will reduce the growth rates of pine trees, and the lack of resin will lead to widespread parasitism by the pine beetle.	1 1
		(2)	Measuring pine beetle and weevil populations provides data that can be used to predict the level of threat faced by pine trees, allowing scientists and governments to develop strategies to minimise the threat.	1
		(3)	Reduces the number of pine trees that remove carbon dioxide from the atmosphere. Increases the decomposition rate of pine trees by soil microbes, discharging more carbon dioxide into the atmosphere through microbial respiration.	1 1
220	(a)		Coral bleaching will decrease the abundance of corals, reducing the habitats of other animals. This will reduce the genetic and species diversities of thousands of marine species.	1 1
	(b)		The frequency of infections will increase. The bacterial growth rate will increase with rising water temperatures, increasing the abundance of pathogens that infect corals.	1 1
	(c)	(1)	**Any one:**	1
			Turtles may become disorientated when navigating to nesting grounds. Nesting islands may become flooded by rising sea levels. Nesting islands may become damaged by severe storms.	
		(2)	The turtle populations are at risk of becoming entirely female, inhibiting sexual reproduction. The genetic and species diversities of green sea turtles will decrease significantly if breeding is inhibited.	1 1

Question	Part	Author's response	Marks
1		M	1
2		K	1
3		L	1
4		J	1
5		K	1
6		M	1
7		L	1
8		M	1
9		K	1
10		M	1
11	(a)	DNA replication	1
	(b)	A single nucleotide deletion changes the mRNA codon sequence downstream of the mutation. The primary structure/amino acid sequence of the protein is significantly altered. The polypeptide chain folds into an alternate tertiary structure which is non-functional.	1 1 1
	(c)	No effect Mutation 2 occurs in an intron which is not translated.	1 1
	(d)	The exons can be spliced together in a different order. Alternate splicing of pre-mRNA produces polypeptides/proteins with different primary structures/amino acid sequences.	1 1
12	(a) (1)	Primers provide a short sequence of nucleotides for DNA polymerase to add to.	1
	(a) (2)	One primer is required for each of the two polynucleotide strands of the DNA molecule/different nucleotide sequences on each polynucleotide strand of the gene.	1
	(b)	C The ratio of the control gene to the gene on chromosome 21 is uneven. Indicates there are extra copies of the gene from chromosome 21 present.	1 1 1
	(c)	**Any one:**	1
		The mother must provide informed consent. Possible harm to the mother or foetus. The mother must not be coerced into having the procedure.	

Notes