

Table 2.1.1 compares the nature and effects of competitive and non-competitive inhibitors.

## Using non-competitive inhibition by penicillin to inhibit bacteria

Penicillin is an antibacterial medicine that works by inhibiting the formation of bacterial cell walls. Penicillin is most effective against Gram-positive bacteria such as staphylococci and streptococci ([Chapter 10](#)). These bacteria have thick cell walls built of many linked peptidoglycan molecules. Penicillin is an irreversible inhibitor that covalently binds to the bacterial enzyme transpeptidase. Transpeptidase catalyses the formation of cross-links between the long polymers of peptidoglycan in the bacterial cell wall. The walls are left weakened and, when the bacteria divide, their new walls are not properly formed so that the cells burst and die.

## Using competitive inhibition to treat poisoning

Another example of an enzyme inhibitor that is used in medicine is fomepizole. Fomepizole is a competitive inhibitor of the enzyme alcohol dehydrogenase, which usually catalyses the **oxidation** of ethanol (alcohol) to acetaldehyde. Acetaldehyde is converted to harmless products in the liver and so it does not harm the body. But alcohol dehydrogenase also catalyses steps in the metabolism of ethylene glycol (antifreeze) to toxic metabolites that cause severe damage to the kidneys. If ethylene glycol is accidentally ingested, an injection of fomepizole blocks alcohol dehydrogenase so that toxic metabolites are not produced and the kidneys are not harmed.

| Competitive inhibitors | Non-competitive inhibitors |
|------------------------|----------------------------|
|------------------------|----------------------------|

|  |   |
|--|---|
| structurally similar to the substrate molecule   | structurally unlike the substrate molecule  |
| occupy and block the active site   | bind at a site away from the active site, reducing access to it   |
| if concentration of inhibitor is low, increasing the concentration of substrate will reduce the inhibition   | if concentration of substrate is low, increasing the concentration of substrate has no effect on binding of the inhibitor so inhibition stays high  |
| <p>examples include:</p> <ul style="list-style-type: none"> <li>• oxygen, which competes with carbon dioxide for the active site of ribulose bisphosphate carboxylase in photosynthesis</li> <li>• disulfiram, which competes with acetaldehyde for the active site of aldehyde dehydrogenase</li> <li>• ethanol, which can be used in preventing antifreeze poisoning because it is a competitive inhibitor of the enzyme alcohol dehydrogenase (refer to section ‘Using competitive inhibition to treat poisoning’)</li> </ul> | <p>examples include:</p> <ul style="list-style-type: none"> <li>• cyanide and carbon monoxide, which block cytochrome oxidase in aerobic respiration, leading to death</li> <li>• penicillin, which blocks the active site of an enzyme that synthesises the cell walls of some bacteria</li> </ul> |

**Table 2.1.1:** Comparing competitive and non-competitive inhibitors.

---

## 2.1.5 Controlling metabolic pathways

### End-product inhibition

**End-product inhibition** occurs when an enzyme in a metabolic pathway is inhibited by the product of that pathway. This prevents a cell over-producing a substance it does not need at the time. Many products may be needed by a cell at specific times or in specific amounts and over-production not only wastes energy but may also become toxic if the product accumulates.

In an assembly-line reaction, such as those described in Figure 2.1.9, each step is controlled by a different enzyme. If the end product begins to accumulate because it is not being used, it inhibits an enzyme earlier in the pathway to switch off the assembly line. In most cases, the inhibiting effect is on the first enzyme in a process, but in other cases it can act at a branch point to divert the reaction along another pathway.

When the end product starts to be used up, its inhibiting effect reduces, the inhibited enzyme is reactivated and production begins again. This is an example of **negative feedback**.

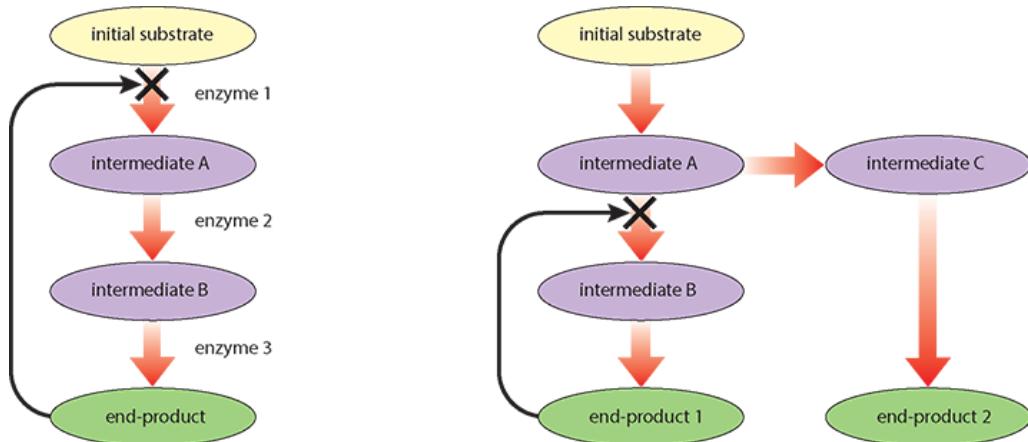
#### KEY POINTS

end-product inhibition is the control of a metabolic pathway by a product in or at the end of that pathway; the product inhibits an enzyme found earlier in the pathway.

negative feedback is a regulating mechanism in which a change in a sensed variable results in a correction that opposes the change.

## EXTENSION

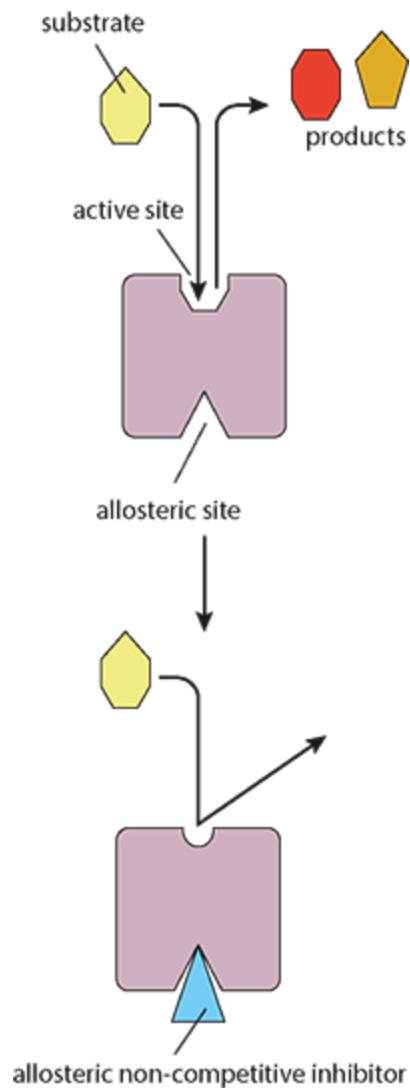
Negative feedback is also important in the control of several physiological processes including regulation of blood sugar levels and reproductive cycles.



The end-product inhibits the enzyme catalysing the first reaction in the series, so all the subsequent reactions stop.

The end-product inhibits an enzyme in the pathway, which causes a different enzyme to come into play and the pathway is diverted down a different route.

**Figure 2.1.9:** End-product inhibition.



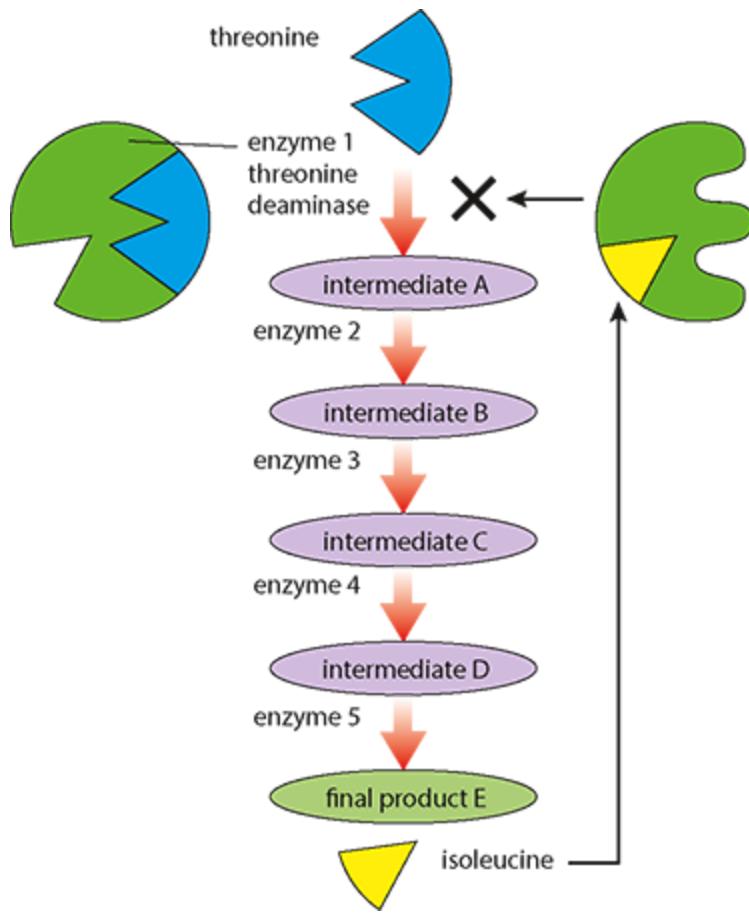
**Figure 2.1.10:** Allosteric control. Allosteric inhibitors prevent the active site functioning.

End-product inhibition may be competitive or non-competitive. Competitive inhibition will only work if the product is a similar shape to the normal substrate and there can be an induced fit of the product or inhibitor onto the enzyme. In most cases, the product will be a different shape and therefore there has to be non-competitive inhibition. In this case, the enzyme is known as an **allosteric enzyme**, the product is called an **allosteric**

**inhibitor** and the place where it binds to the enzyme is called the **allosteric site** (Figure 2.1.10).

### An example of end-product inhibition

Threonine is converted to isoleucine in series of five enzyme-controlled stages. Isoleucine is important in the immune system and also in the synthesis of proteins including hemoglobin. Isoleucine, as the end product of threonine metabolism, can inhibit threonine deaminase, the first of the five enzymes in the process (Figure 2.1.11). Isoleucine inhibits the enzyme by binding on the molecule at a site away from the active site. When it is attached, the active site of the enzyme is changed so that no further substrate can bind to it. As isoleucine concentration increases, more and more isoleucine molecules attach to this inhibition site on enzyme molecules and therefore inhibit further production of isoleucine. As their concentration falls, isoleucine molecules detach from the threonine deaminase enzyme molecules and are used in the cell. Once the inhibitor has been removed, the active site can bind new substrate and the pathway is reactivated.



**Figure 2.1.11:** The pathway that converts threonine to isoleucine – a specific example of end-product inhibition.

This mechanism makes the metabolic pathway self-regulating so that there is always sufficient isoleucine present in the cell.

## 2.1.6 Co-enzymes and co-factors

A **co-enzyme** is an organic, non-protein molecule that binds to an enzyme to allow it to catalyse a reaction. Co-enzymes cannot work alone, but they can be reused several times with an enzyme. Many co-enzymes are vitamins or are derived from vitamins. For example, pantothenic acid (also called vitamin B5) is needed for the synthesis of co-enzyme A, which is essential for fatty acid metabolism and in the link reaction of aerobic respiration (Section 2.2).

**Co-factors** are inorganic substances that promote enzyme activity. Many metal ions are co-factors.

### KEY POINT

Co-enzymes and co-factors both promote enzyme activity.  
Co-enzymes are organic molecules; co-factors are inorganic.

For example, cupric ions are co-factors needed to promote cytochrome oxidase activity and zinc is a co-factor in nearly 300 enzymes involved in metabolism. Chloride ions are allosteric activators for human amylases. As one chloride ion binds to amylase, it induces activation of the enzyme and increases the rate of reaction of the hydrolysis of starch.

### TEST YOUR UNDERSTANDING

- 6 Outline what is meant by activation energy.
- 7 Explain how an enzyme pathway can be switched off by an accumulation of the end product of the pathway.

- 8** Outline the way in which penicillin leads to the death of bacteria.
- 9** List three differences between competitive and non-competitive enzyme inhibitors.

## THEORY OF KNOWLEDGE

### Studying metabolic pathways

Metabolic pathways have been studied for centuries but one of the most significant advances was made by Eduard Buchner (1860–1917), who discovered enzymes at the start of the 20th century. At first, studies of whole animals were made, but more recently it has been possible to analyse metabolic pathways and their component reactions using modern techniques such as chromatography, X-ray diffraction, spectroscopy and radioactive isotopes. In the mid-20th century, the Krebs cycle (often called the citric acid cycle; see [Section 2.2](#)) and the glyoxylate cycle were discovered by Hans Krebs (1900–1981) and Hans Kornberg (1928–2019). But metabolic pathways are very elaborate. Many pathways are interrelated and together make up a complex metabolic network in a cell. These pathways are vital to homeostasis and cell function. Some pathways are connected by intermediate products, and products of one pathway may be substrates for another.

### To consider:

Most biochemical studies are made using carefully controlled experiments that look at one part of a pathway. To what extent can looking at component parts of a complex system give us knowledge of the whole?

# Links

- How do plants and algae convert light energy into organic compounds, or chemical energy, to use in their metabolism? ([Section 2.3](#))
- How does metabolism help to maintain constant internal conditions? ([Chapter 8](#))
- Why is compartmentalisation in cells important for the control of metabolism? ([Chapter 5](#))

## 2.2 Respiration

### LEARNING OBJECTIVES

In this section you will:

- understand that cell respiration is the controlled release of energy from organic substances to produce ATP (adenosine triphosphate)
- learn that cell respiration is a series of complex enzyme-catalysed reactions that involve hydrolytic breakdown of glucose and other molecules
- understand that much of the energy is released to the environment as heat
- discover that ATP from respiration is an immediately available source of energy in the cell
- understand that anaerobic respiration (called fermentation in microorganisms) gives a small yield of ATP from glucose and occurs in the cell cytoplasm
- recall that yeast cells respire anaerobically and are used in baking and brewing
- recognise that humans can respire anaerobically for a short time in the absence of oxygen, which produces lactate
- understand that lactate produced by some bacteria is useful in yoghurt production

- learn that aerobic respiration requires oxygen and gives a much larger yield of ATP from glucose
- learn that aerobic respiration starts in the cytoplasm and requires mitochondria

- learn that during cell respiration electron carriers are oxidised and reduced
- understand that phosphorylation of molecules increases their energy level making them less stable, and decarboxylation generates carbon dioxide
- understand that glycolysis does not use oxygen. It takes place in the cytoplasm and each glucose molecule is converted into two pyruvate molecules with a small net gain of ATP
- learn that during anaerobic respiration reduced NAD from glycolysis is oxidised and reduces pyruvate to avoid it building up in the cytoplasm
- understand how in aerobic respiration pyruvate is decarboxylated and oxidised. In the link reaction it is converted to an acetyl compound and then attached to coenzyme A to form acetyl co-enzyme A
- understand that in the Krebs carboxylic acid cycle the oxidation of acetyl groups is coupled with the reduction of hydrogen carriers and carbon dioxide is released
- learn that energy released during oxidation reactions is transferred by reduced NAD and FAD to the cristae of mitochondria

- learn that the transfer of electrons between carrier molecules in the electron transport chain (ETC) in the membrane of the cristae is coupled to proton pumps
- understand how during chemiosmosis, protons diffuse through ATP synthase to generate ATP
- discover how oxygen binds with free protons to form water, thus maintaining the H<sup>+</sup> (proton) gradient.

## GUIDING QUESTIONS

- What is the role of adenosine triphosphate (ATP) in the transfer of energy in cells?
- How do living organisms release the energy on which their cells depend?
- Why does aerobic respiration generate a much larger yield of ATP per molecule of glucose than anaerobic respiration?

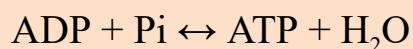
## 2.2.1 Cell respiration and ATP

All living cells need energy to stay alive. The energy is used to power all the activities of life including digestion, protein synthesis and active transport. A cell's energy sources are the sugars and other substances derived from nutrients, which can be metabolised in a series of chemical reactions to release the energy that holds their molecules together. Much of the energy is eventually transferred to the environment as heat.

**Cell respiration** is the gradual breakdown of nutrient molecules such as glucose and fatty acids in a series of enzyme-controlled metabolic pathways that ultimately release energy in the form of **ATP (or adenosine triphosphate)**.

### KEY POINT

ATP (adenosine triphosphate) is the immediately available energy currency of a cell. It is needed for every activity that requires energy. Cells make their own ATP in mitochondria. When energy is used, ATP is broken down to ADP (adenosine diphosphate) and inorganic phosphate. This conversion releases energy for use and a cyclic process reforms the ATP during respiration.



### ATP (adenosine triphosphate)

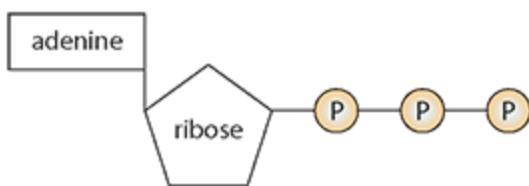
ATP is the molecule that acts as a source of energy for all living organisms. It is often called the universal energy carrier. It is an organic molecule that transports chemical energy for use in metabolic processes such as movement, active transport and the

synthesis of new molecules. Metabolic processes are chemical processes that occur in all cells to keep them alive.

ATP consists of three parts: a pentose sugar, a purine base (adenine) and three phosphate groups attached to the pentose sugar (Figure 2.2.1). Energy is released from ATP during a hydrolysis reaction in which the last phosphate group is removed. This leaves ADP (adenosine diphosphate) and an inorganic phosphate (shown as Pi).



ADP is converted back to ATP in the reactions of cell respiration.



**Figure 2.2.1:** The structure of a molecule of ATP.

Glucose is the most commonly used source of energy. Enzymes hydrolyse each glucose molecule in a number of stages, which release energy in small amounts as each covalent bond is broken. If there is insufficient glucose available, fatty acids or amino acids can be used instead.

The energy in glucose or other nutrient molecules can be released in a single reaction. This is what happens when glucose burns in the reaction known as combustion. In this case, the energy in the glucose is released as heat. In the series of reactions that occur during respiration, glucose is broken down gradually, with each step catalysed by a different enzyme. This releases energy in small amounts so that it can be used by cells. Nevertheless as energy is used much of it is lost to the surroundings as heat. We

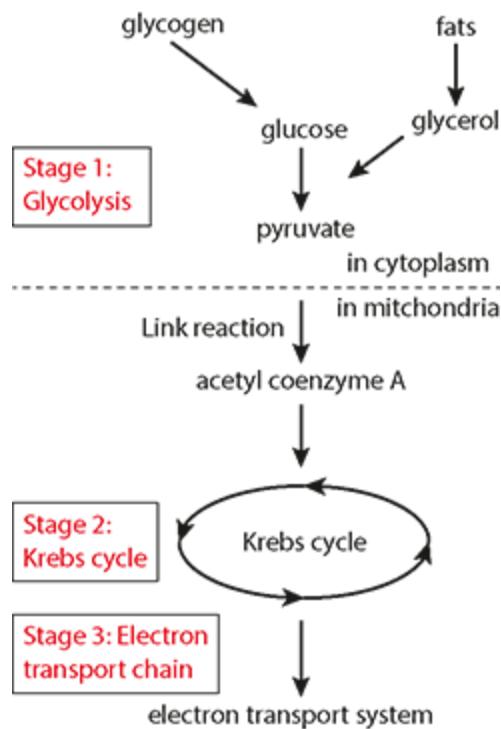
can experience this for ourselves when we use energy during exercise and our bodies become hot.

## Glycolysis

The first stage in cell respiration is **glycolysis**. Glucose that is present in the cytoplasm of a cell is broken down by a series of enzymes, to produce two molecules of a simpler compound called pyruvate. As this occurs, there is a net production of two molecules of ATP (Figure 2.2.2).



Glycolysis actually uses two molecules of ATP to get the process under way but produces four molecules of ATP in total, per molecule of glucose. Thus we say there is a net production of two ATPs.



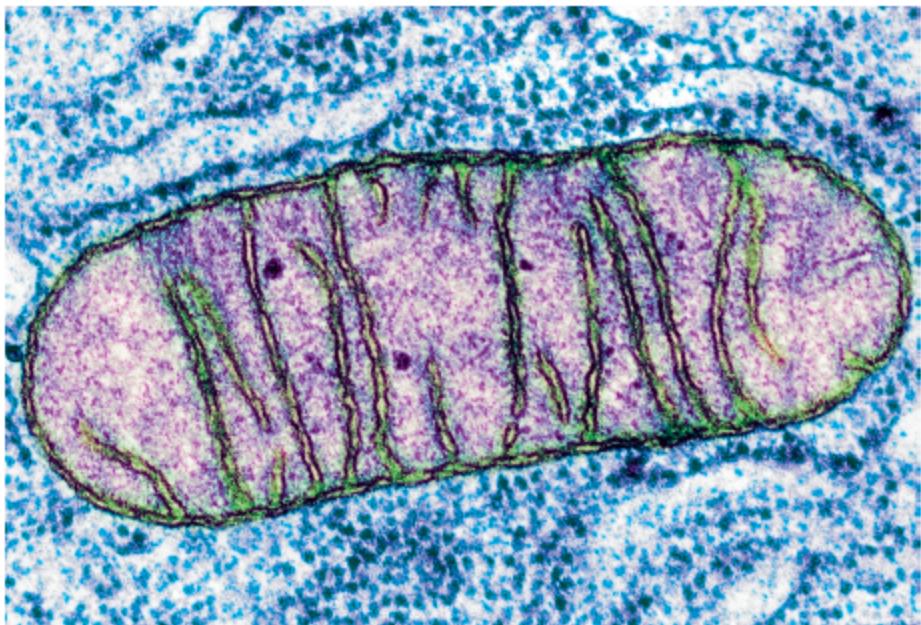
**Figure 2.2.2:** A summary of the stages in aerobic respiration.

## 2.2.2 Aerobic and anaerobic respiration

The next stage of cell respiration depends on whether or not oxygen is available. In the presence of oxygen, aerobic respiration can take place; without it, respiration must be anaerobic.

**Aerobic respiration** is the most efficient way of producing ATP. Aerobic respiration is carried out by cells that have mitochondria and it produces a great deal of ATP. A labelled micrograph of a mitochondrion is shown in Figure 2.2.2. Pyruvate molecules produced by glycolysis enter the mitochondria and are broken down, or oxidised, in a series of reactions that release carbon dioxide and water and produce ATP.

In the first step, two pyruvate molecules are transported into the mitochondria in the **link reaction**, as shown in Figure 2.2.1. Each pyruvate loses a carbon atom which forms carbon dioxide and a hydrogen atom, so that they become two molecules of acetyl CoA. Acetyl CoA then enters a stage called the **Krebs carboxylic acid cycle** (also known as the citric acid cycle) and is modified still further, releasing more carbon dioxide. The Krebs cycle takes place in the matrix of the mitochondria. Finally, on the inner membranes of the mitochondria, products of the cycle react directly with oxygen and the result is the release of large amounts of ATP. The original glucose molecule is completely broken down to carbon dioxide and water so the equation for aerobic respiration is often summarised as:



**Figure 2.2.3:** Coloured electron micrograph of a mitochondrion ( $\times 72\,000$ ).

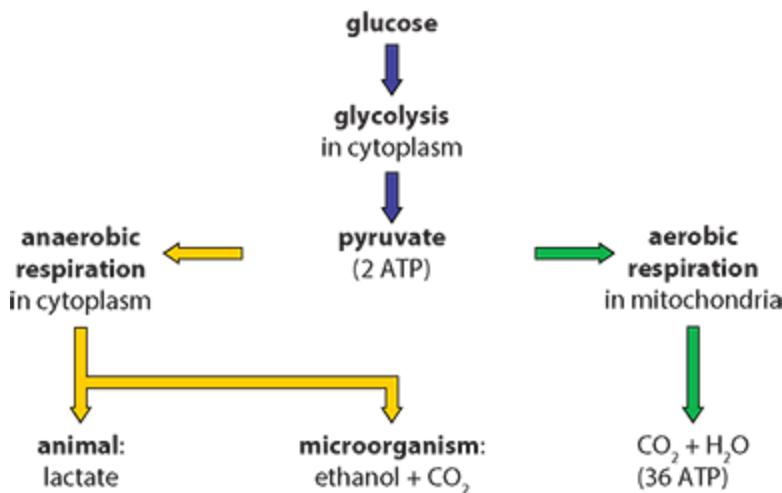
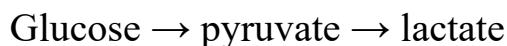


#### EXAM TIP

You should be familiar with the general structure of a mitochondrion (Figure 2.2.3), you don't need to remember all the details but thinking about where the reactions of respiration take place can help you understand the different stages. You will learn more about mitochondria in [Section 5.2](#).

**Anaerobic respiration** occurs in the cytoplasm of cells. In animal cells, the pyruvate produced from glucose by glycolysis is converted to lactate (Figure 2.2.4), which is a waste product and is taken out of the cells. In humans, anaerobic respiration occurs if a person is doing vigorous exercise and their cardiovascular

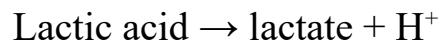
system is unable to supply sufficient oxygen for aerobic respiration to provide ATP at the necessary rate. Although anaerobic respiration releases far less energy per molecule of glucose than aerobic respiration, the extra ATP enables the person to continue exercising for a short period, at a time of great exertion, to maximise power output. One consequence of the build-up of lactate in the muscles that occurs during anaerobic respiration is the sensation of cramp, so this type of respiration cannot be sustained for very long. The word equation for anaerobic respiration in animals is:



**Figure 2.2.4:** Simple diagram to show the products of aerobic and anaerobic respiration.

### EXTENSION

You'll often see both the terms lactate and lactic acid used when you read about anaerobic respiration, so what is the difference? Like other acids, lactic acid is a substance that is able to donate hydrogen ( $H^+$ ) ions. When it loses an electron it is called a base and known as lactate:

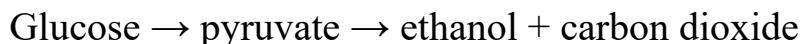


When lactic acid is produced in muscles it will dissociate into lactate and hydrogen ions so the terms are used interchangeably.

Humans can only respire anaerobically for a short period of time. A sprinter running a 100 metre race can run an entire race anaerobically, but a long-distance runner will use only aerobic respiration for maximum efficiency.

Lactate is carried in the blood from muscles to the liver where it is converted back to pyruvate. Pyruvate may either be converted back to glucose, a process which requires energy, or used as a fuel, producing carbon dioxide and water.

In microorganisms, such as yeast, anaerobic respiration is also known as **fermentation**, and produces a different outcome. The pyruvate molecules from glycolysis are converted to ethanol (alcohol) and carbon dioxide (Figure 2.2.4).



No further ATP is produced by the anaerobic respiration of pyruvate, so this type of respiration gives only a small yield of ATP from glucose. Aerobic and anaerobic respiration are compared in Table 2.2.1

|                    | Aerobic  | Anaerobic  |
|--------------------|----------|------------|
| Requires oxygen    | yes      | no         |
| Glycolysis occurs  | yes      | yes        |
| oxidation          | complete | incomplete |
| Glucose completely | yes      | no         |

|                |                           |  |
|----------------|---------------------------|--|
| broken down    |                           |  |
| Waste products | CO <sub>2</sub> and water | Lactic acid (animals) or CO <sub>2</sub> and alcohol (yeast) |
| ATP yield      | 38                        | 2  |

**Table 2.2.1:** Comparing aerobic and anaerobic respiration

---

## 2.2.3 Anaerobic respiration in food production

People have benefitted from the anaerobic respiration of yeast in baking and brewing for thousands of years. Today, many different types of yeast are used in the production of bread, wine and beer. The strains of yeast used for baking and brewing are different and each has been selected for its specific characteristics. Baking yeasts feed on sugar and flour in bread dough and grow more quickly than brewing yeasts, which are slow-growing but able to tolerate higher alcohol concentrations. In bread making, the yeast initially respire aerobically, releasing carbon dioxide gas and water into the dough in a very short period of time. Carbon dioxide in the dough causes it to rise as the gas becomes trapped in pockets between gluten fibres in the flour. When oxygen in the dough has been depleted, the yeast continues to respire anaerobically, producing ethanol which evaporates during baking. The yeast cells are also killed by the high temperature of the oven.

Yoghurt production also relies on anaerobic respiration. Two bacteria that are often used to make yoghurt are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Milk, either raw or pasteurised, is first heated to denature the whey proteins so that the final yoghurt that is made will form a stable gel and not separate if it is stored. Next, it is cooled to about 40 °C. This is the optimum temperature ([Section 2.1](#)) for the bacterial enzymes involved in fermentation. Live bacterial cells are put into the milk and they feed on lactose sugars, converting the sugars into lactic acid as they respire. After about 12 hours, lactic acid causes the milk to thicken and produces the characteristic acidic taste and texture of yoghurt.

## INTERNATIONAL MINDEDNESS

Cow's milk is used to produce yoghurt and kefir in many parts of the world, but milk from yaks, buffalo, goats, sheep and camels is also used where it is available. In Central Asia mare's or donkey's milk is used to produce koumiss, a fermented drink similar to thin yoghurt. The flavour and texture of each product depends on the types of milk and bacteria that are used.

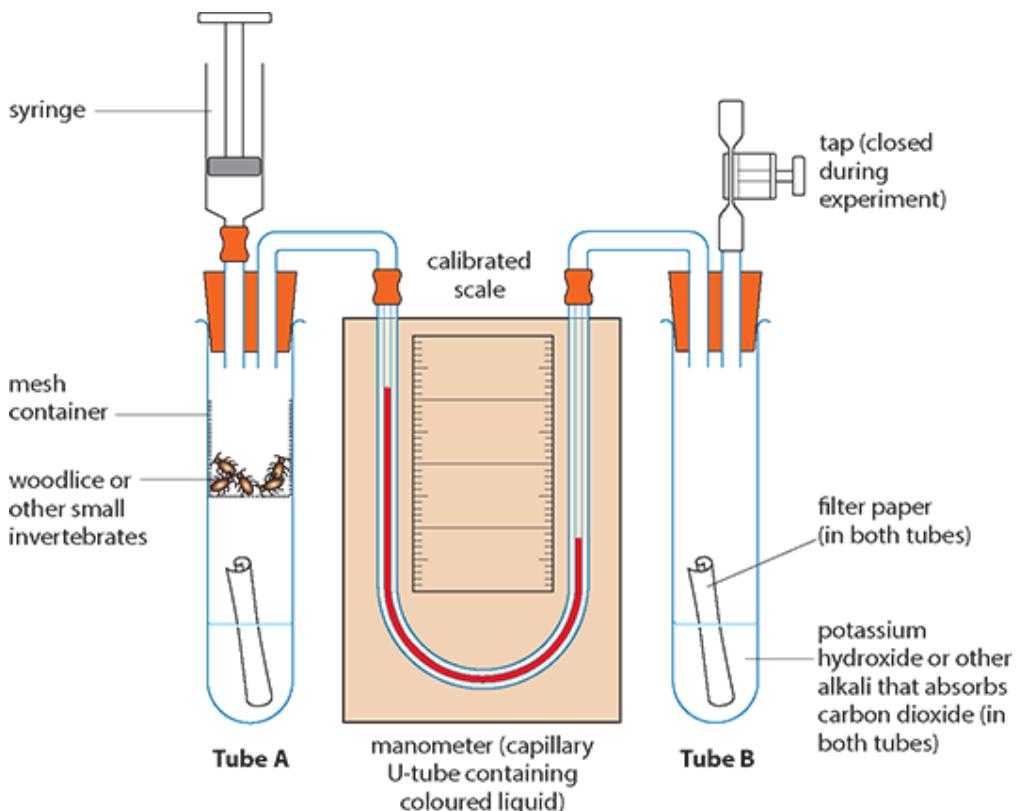
## NATURE OF SCIENCE

### Assessing ethics in science: using invertebrates in a respirometer

A simple respirometer, such as the one shown in Figure 2.2.5, can be used to monitor respiration in small organisms such as woodlice or in germinating seeds. The apparatus can demonstrate that oxygen is used and carbon dioxide is produced during respiration. Test organisms are placed in two large boiling tubes as shown, so that one contains living organisms (tube A) and the other, which acts as a control, contains either dead organisms or is left empty (tube B). Soda lime or another alkali such as potassium hydroxide absorbs carbon dioxide. As oxygen is used by the living things in tube A, the level of liquid rises in the arm of the manometer attached to tube A. If required, measurements of time can be made so that the rate of respiration can be estimated. The temperature in the apparatus is kept constant by immersing the tubes in a water bath. This minimises any change in volume due to temperature change.

#### To consider:

- 1 How can we ensure that the invertebrates used in experiments like this are treated ethically?
- 2 What measures would you use to minimise the distress and disturbance to the organisms and also to the habitat from which they are taken?
- 3 How can we know whether the organisms are experiencing distress?



**Figure 2.2.5:** A simple respirometer.

### TEST YOUR UNDERSTANDING

- 10** Which stage of respiration takes place in both aerobic and anaerobic respiration?

**11** Where does aerobic respiration take place in eukaryotic cells?

**12** Outline the role of anaerobic respiration in baking.

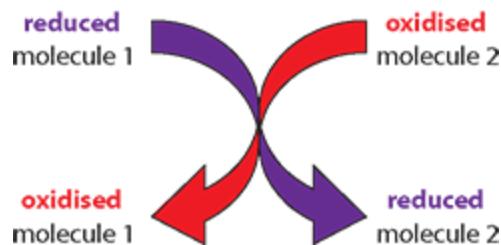
## 2.2.4 Biochemistry of cell respiration

### Oxidation and reduction

#### KEY POINT

**redox reaction** a reaction in which reduction and oxidation occur simultaneously.

Cell respiration involves several oxidation and reduction reactions. Such reactions are common in biochemical pathways. When two molecules react, one of them starts in the oxidised state and becomes reduced, and the other starts in the reduced state and becomes oxidised, as shown in Figure 2.2.6.



**Figure 2.2.6:** Oxidation and reduction are linked processes – as one molecule is reduced another is oxidised in a redox reaction.

There are three different ways in which a molecule can be oxidised or reduced, as outlined in Table 2.2.2. In biological oxidation reactions, addition of oxygen atoms is an alternative to removal of hydrogen atoms. Since a hydrogen atom consists of an electron and a proton, losing hydrogen atoms (oxidation) involves losing one or more electrons.

Oxidation and reduction occur together in biochemical reactions. As one compound loses electrons, another one gains electrons.

In the simple equation for respiration, glucose is oxidised as hydrogen atoms, and therefore electrons, are gradually removed from it and added to hydrogen acceptors (the oxygen atoms on the left side of the equation), which become reduced.



| Oxidation         | Reduction         |
|-------------------|-------------------|
| loss of electrons | gain of electrons |
| loss of hydrogen  | gain of hydrogen  |
| gain of oxygen    | loss of oxygen    |

**Table 2.2.2:** Changes involved in oxidation and reduction.

Chemical reactions like this are referred to as redox reactions. In redox reactions, the reduced molecule always has more potential energy than the oxidised form of the molecule. Electrons passing from one molecule to another carry energy with them.

The electron carriers used during cell respiration are **NAD<sup>+</sup>** and **FAD<sup>+</sup>**.

### KEY POINT

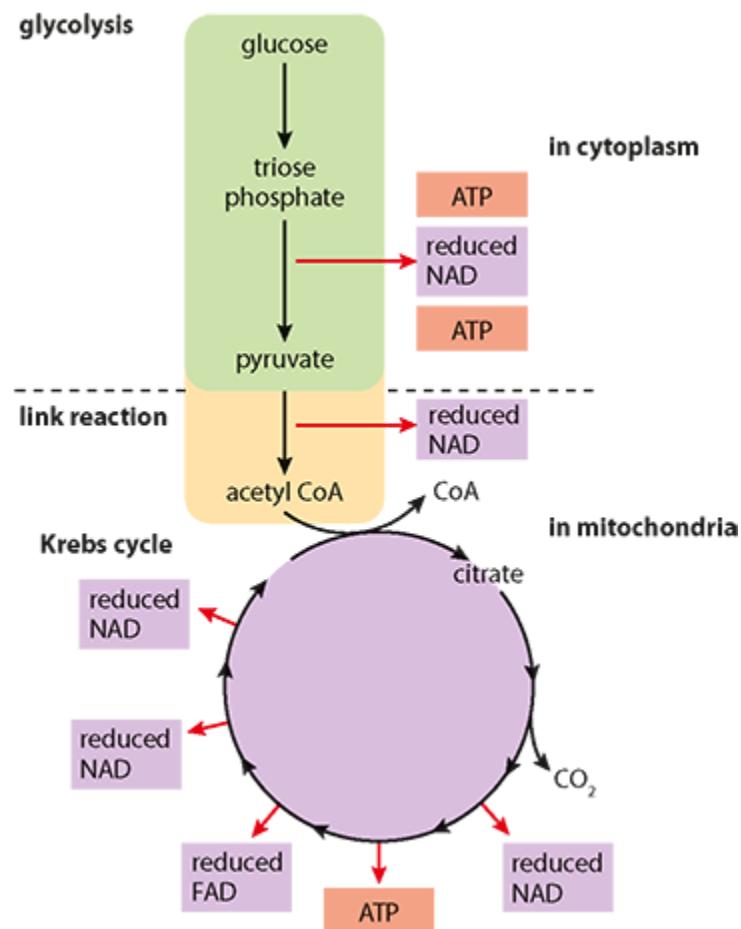
NAD<sup>+</sup> is a hydrogen carrier that accepts hydrogen atoms removed during the reactions of respiration. During glycolysis, two hydrogen atoms are removed and NAD<sup>+</sup> accepts the protons from one of them and the electrons from both of them.



Cell respiration is the controlled breakdown of food molecules such as glucose or fat to release energy, which can be stored for later use. The energy is most commonly stored in the molecule adenosine triphosphate, or ATP. The respiration pathway can be divided into four parts:

- 1 glycolysis
- 2 link reaction
- 3 Krebs cycle
- 4 electron transfer chain and chemiosmosis.

Glycolysis, the link reaction and the Krebs cycle are summarised in Figure 2.2.7, and the electron transfer chain and chemiosmosis are discussed later in this chapter in the section on ‘the electron transport chain, oxidative phosphorylation and chemiosmosis’.

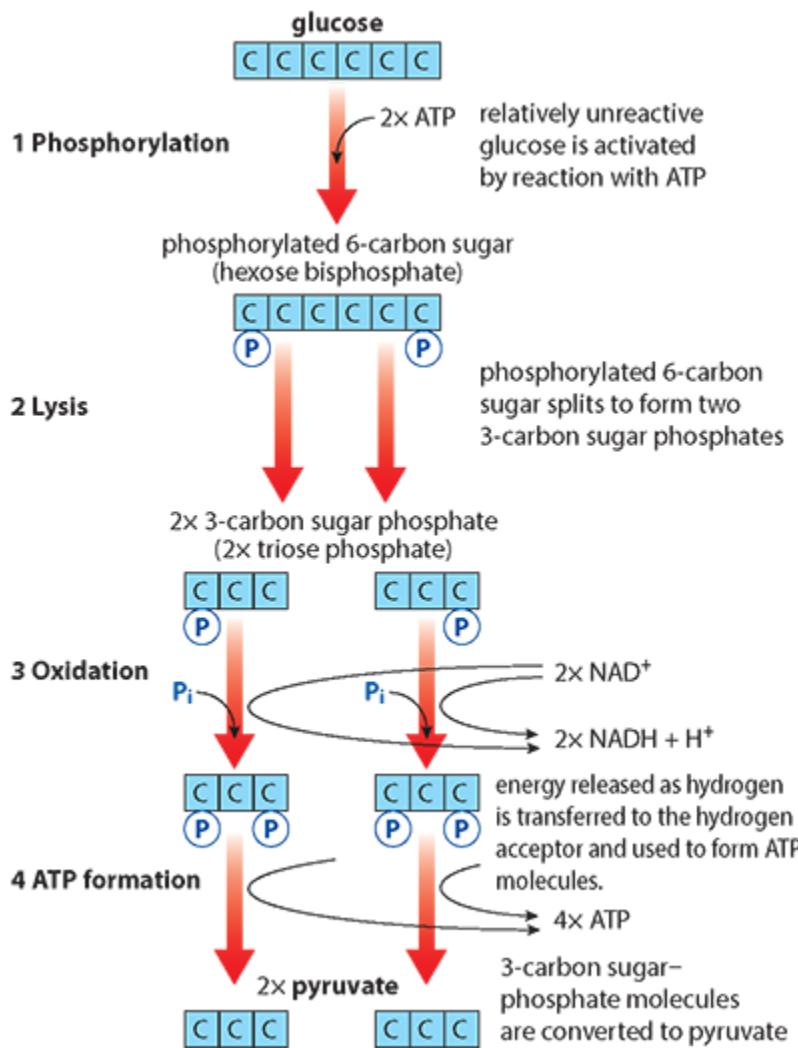


**Figure 2.2.7:** Summary of glycolysis, the link reaction and the Krebs cycle.

## Glycolysis

Glycolysis is the first stage in the series of reactions that make up respiration. It literally means ‘breaking apart glucose’. The glycolysis pathway occurs in the cytoplasm of the cell. It is anaerobic (that is, it can proceed in the absence of oxygen) and produces pyruvate and a small amount of ATP. One molecule of the hexose sugar glucose is converted to two molecules of the three-carbon molecule called pyruvate with the net gain of two molecules of ATP and two molecules of NADH + H<sup>+</sup>. The process is shown in detail in Figure 2.2.8.

- 1 The first steps are to add two phosphate groups from ATP, in a process called phosphorylation, which destabilises the glucose molecule. A hexose bisphosphate molecule is produced. (This appears contrary to the purpose of respiration, which is to *make* ATP, but the two lost ATPs are recovered later.)
- 2 The hexose bisphosphate is now split into two triose phosphates in a reaction called lysis.
- 3 Now, another phosphorylation takes place but this time an inorganic phosphate ion,  $P_i$ , is used and not ATP. Two triose bisphosphates are formed. The energy to add the  $P_i$  comes from an oxidation reaction. The triose bisphosphate is oxidised and at the same time  $NAD^+$  is reduced to  $NADH + H^+$ .



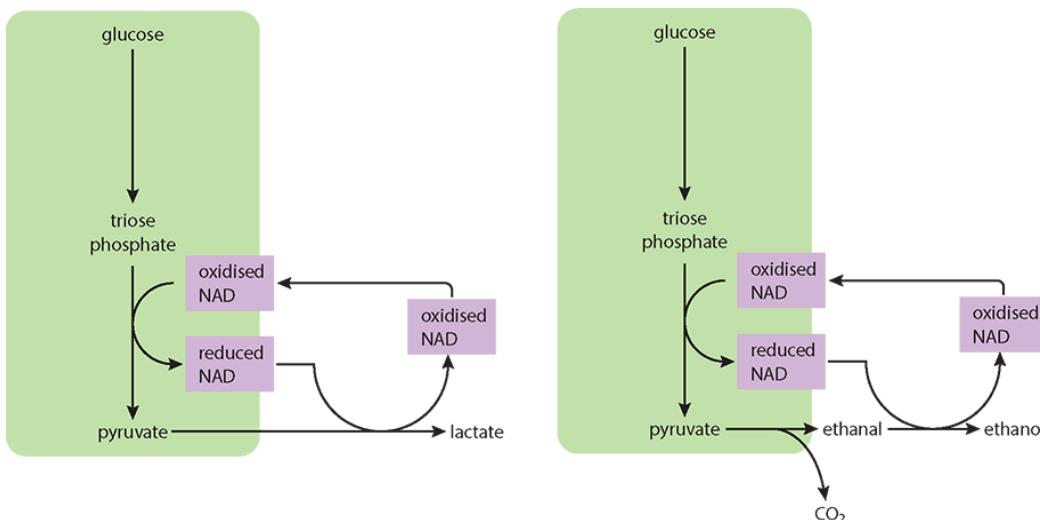
**Figure 2.2.8:** The stages of glycolysis. Note that for each molecule of glucose, two molecules of ATP are used and four are formed, so there is a net gain of two ATPs.

- 4 There now follows a series of reactions in which the two phosphate groups from each triose bisphosphate are transferred onto two molecules of ADP, to form two molecules of ATP: this is ATP formation. A pyruvate molecule is also produced for each triose bisphosphate molecule.

**EXAM TIP**

NADH + H<sup>+</sup> must not be simplified to NADH<sub>2</sub>.

Four molecules of ATP are formed by converting one molecule of glucose to two molecules of pyruvate. However, two molecules of ATP were required to start the pathway and so there is a net gain of two molecules of ATP per glucose. In addition, two NADH + H<sup>+</sup> are formed.



**Figure 2.2.9:** During anaerobic respiration reduced NAD is used to remove pyruvate from glycolysis so that it does not build up. Left: anaerobic respiration in animal cells; right: anaerobic respiration in yeast cells.

To summarise, the net products of glycolysis per glucose molecule are:

- 2 ATP
- 2 NADH + H<sup>+</sup> (reduced NAD)
- 2 molecules of pyruvate.

**EXAM TIP**

Try to think of your own acronym, such as People Love Outdoor Activities, to help you recall the steps in glycolysis.

In anaerobic respiration the reduced NAD ( $\text{NADH} + \text{H}^+$ ) is oxidised (dehydrogenated) and used to reduce pyruvate. This prevents a build-up of harmful concentrations of pyruvate in the cell cytoplasm. In microorganisms this leads to the production of ethanol and carbon dioxide, while in animals the product is lactate. No further ATP is produced by the anaerobic respiration of pyruvate, so this type of respiration gives only a small yield of ATP from glucose (Figure 2.2.9).

## Lipids and Carbohydrates as respiratory substrates

A respiratory substrate is any organic molecule that can be broken down to release energy to synthesise ATP. Glucose, lipids and proteins can all be used as respiratory substrates but they each release different amounts of energy.

### KEY POINTS

**Phosphorylation** of glucose is the first stage in its breakdown and involves the addition of phosphate groups from ATP. This turns glucose into a more unstable phosphorylated compound which can be split to form two three-carbon sugars.

**Energy coupling** involves a sequence of reactions in which energy from an energy-releasing process is used to drive an energy-requiring process. Phosphorylation is an example of energy coupling: the transport of a phosphate group from ATP to a reactant molecule in the coupled reaction supplies energy for that reaction. The reactant molecule becomes a

phosphorylated intermediate, an unstable molecule compared to the unphosphorylated state.

Most of the energy released in respiration comes from the oxidation of hydrogen to water so the more carbon-hydrogen bonds there are in the structure of a molecule, the greater the amount of energy it can provide. Hydrogen atoms are used to generate ATP in the electron transport chain. Fatty acids have more hydrogen per gram than carbohydrates, so lipids have more oxidisable hydrogen and carbon and a greater energy value (Table 2.2.4)

| Respiratory substrate | Energy value/kj g <sup>-1</sup> |
|-----------------------|---------------------------------|
| Carbohydrate          | 16                              |
| Lipid                 | 39                              |
| Protein               | 17                              |

When lipids are respired, they are broken down into fatty acids and glycerol. The glycerol is converted into triose phosphate and enters the glycolysis stage. The fatty acids are broken down into two carbon acetyl groups that enter the Krebs cycle via acetyl co-enzyme A.

Anaerobic respiration can only take place if carbohydrate is the energy substrate.

### TEST YOUR UNDERSTANDING

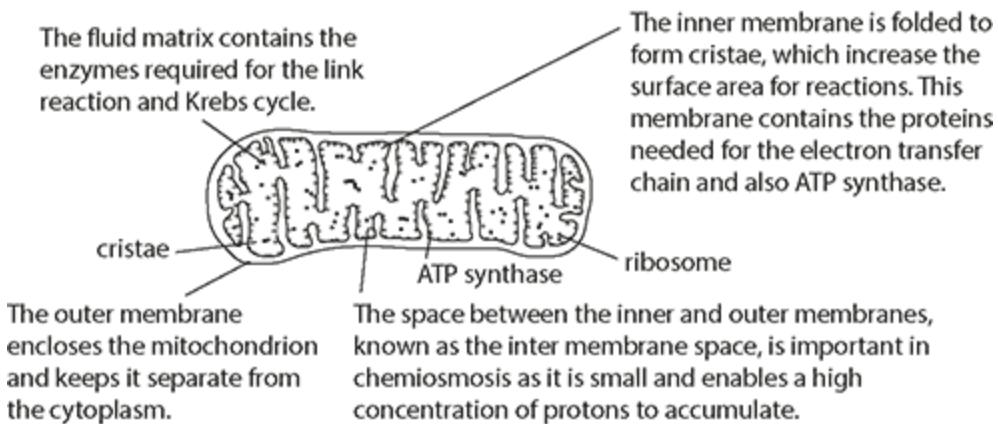
- 13** List three ways in which a substance can be reduced.
- 14** Name the molecule used to phosphorylate glucose at the start of glycolysis.

**15** Outline the importance of phosphorylation in glycolysis.

## 2.2.5 Aerobic respiration

### The link reaction and Krebs cycle

If oxygen is present, pyruvate formed during glycolysis moves into the mitochondrial matrix by facilitated diffusion. The structure of a mitochondrion is shown in Figure 2.2.10.



**Figure 2.2.10:** Diagram of a mitochondrion in longitudinal section.

The link reaction and the Krebs cycle pathways occur in the mitochondrial matrix (Figure 2.2.11).

- 1 The link reaction converts pyruvate to acetyl CoA using co-enzyme A, and a carbon atom is removed as carbon dioxide. This is called a **decarboxylation reaction**. At the same time as the carbon dioxide is removed, pyruvate is oxidised by the removal of hydrogen. The hydrogen atoms are removed by  $\text{NAD}^+$  to form  $\text{NADH} + \text{H}^+$ .
- 2 Acetyl CoA now enters the Krebs cycle to continue the processes of aerobic respiration. Immediately, the co-enzyme A is removed to be recycled. The acetyl component

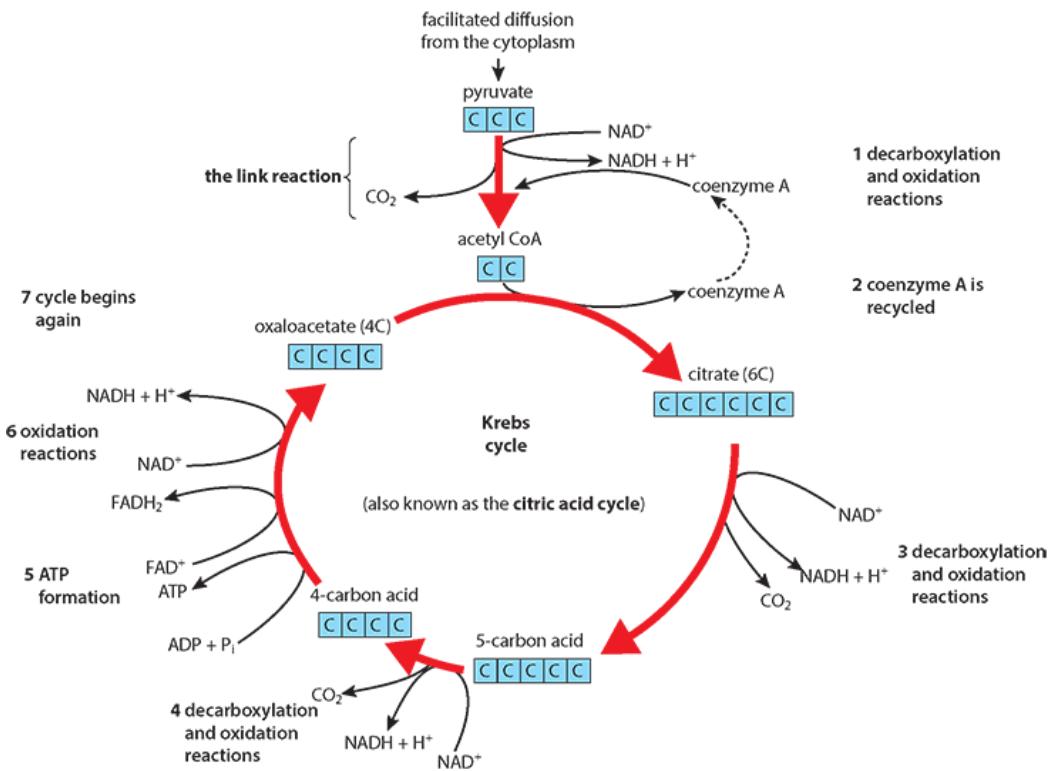
of the acetyl CoA combines with a four-carbon compound to form the six-carbon compound, citrate.

- 3, 4 The acetyl (two-carbon) groups are dehydrogenated to release four pairs of hydrogen atoms and decarboxylated to form two molecules of carbon dioxide so that the two carbons that enter with acetyl CoA leave as carbon dioxide.
- 5 One molecule of ATP is formed.
- 6 Hydrogen is removed during oxidation reactions to the two hydrogen carriers  $\text{NAD}^+$  and  $\text{FAD}^+$ .
- 7 Since the Krebs cycle is a cyclic process, what enters must eventually leave so that the cycle begins and ends with the same substances.

#### EXAM TIP

Reduced FAD is written as  $\text{FADH}_2$ .

Because each molecule of glucose forms two molecules of pyruvate during glycolysis, each glucose molecule requires two link reactions and two rotations of the Krebs cycle. Thus, when working out the products of the cycle we must consider two sets of products. To summarise, the products of the link reaction and Krebs cycle, per glucose molecule, are:



**Figure 2.2.11:** The link reaction and Krebs cycle.

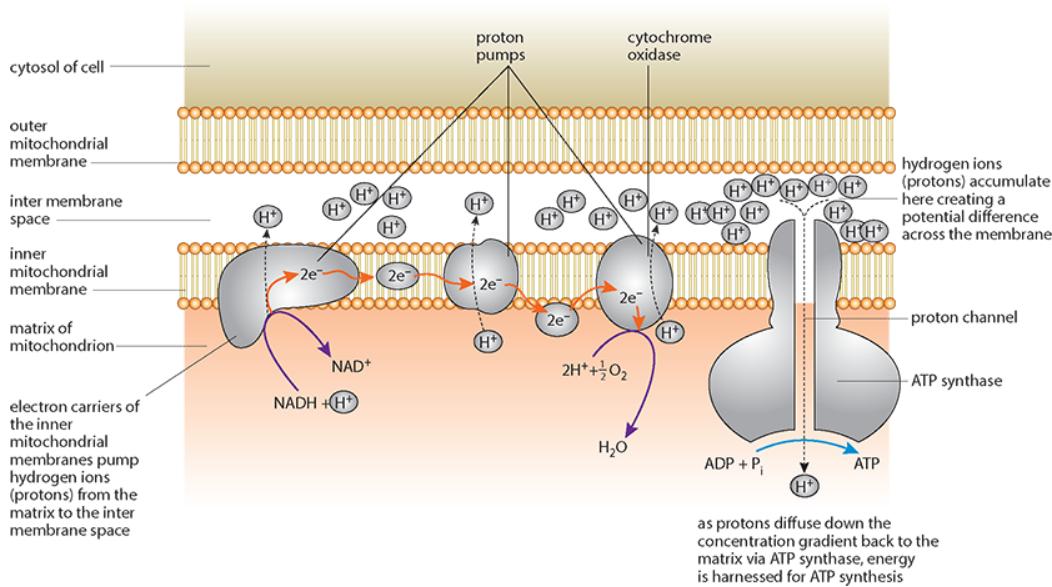
- 8 molecules of NADH + H<sup>+</sup>
- 2 molecules of FADH<sub>2</sub>
- 2 molecules of ATP
- 6 molecules of CO<sub>2</sub>.

## The electron transport chain, oxidative phosphorylation and chemiosmosis

Most of the ATP produced from glucose breakdown occurs in the last phase of respiration at the end of the **electron transport chain (ETC)**. Reactions take place on the inner mitochondrial membrane of the cristae and in the intermembrane space between the inner and outer membranes (Figures 2.2.3 and 2.2.10). The inner membrane holds molecules called **electron**

**carriers**, which pick up electrons and pass them from one to another in a series of oxidations and reduction reactions. The pathway is called the electron transport chain because electrons from hydrogen are moved along it. Just as the inner lining of the small intestine is folded to increase its surface area to absorb food, so the inner mitochondrial membrane is highly folded into cristae to increase its surface area. The cristae provide a large area for the protein molecules used in the electron transport chain. Several protein molecules are electron carriers and the three key ones are shown in Figure 2.2.12.

Electrons from  $\text{NADH} + \text{H}^+$  are transferred onto the first electron carrier. As they pass through the carrier, they lose energy and this is used to pump a proton ( $\text{H}^+$ ) from the matrix to the intermembrane space, lowering the pH of the space. The electrons are then transferred to two further carriers and the process is repeated. As the electrons from one  $\text{NADH} + \text{H}^+$  pass along the chain, a total of nine protons are pumped into the intermembrane space. At the end of the chain, the electrons are combined with protons and oxygen atoms to make water, in the oxidative part of **oxidative phosphorylation**. This completes the release of energy from the oxidation of glucose to produce ATP.



**Figure 2.2.12:** The electron transport chain showing oxidative phosphorylation and chemiosmosis.

The formation of water ensures that the H<sup>+</sup> gradient is maintained.

The space between the membranes is very narrow and allows for a rapid increase in the concentration of the protons that are pumped into it during the electron transfer reactions. The protons in the intermembrane space create a concentration gradient between the space and the matrix. These protons can now flow passively down this concentration gradient back into the matrix, through a very large integral protein. This is called **chemiosmosis**. The large protein contains the enzyme **ATP synthase**, which joins ADP and P<sub>i</sub> to form ATP. Three protons flowing through this enzyme result in one ATP being formed. Since the electrons from one NADH + H<sup>+</sup> pump nine protons into the intermembrane space, each NADH + H<sup>+</sup> results in the formation of three ATP. This is the phosphorylation part of oxidative phosphorylation.

$\text{FADH}_2$  also supplies electrons to the electron transport chain but further down the chain than  $\text{NADH} + \text{H}^+$ , missing the first protein pump.  $\text{FADH}_2$  allows the production of just two ATPs.

### Overall ATP production during aerobic respiration

| Stage                                    |                              | ATP use | ATP yield |
|--|------------------------------|---------|-----------|
| glycolysis                               | 2 ATP used at the start      | -2 ATP  |           |
|  | 2 $\text{NADH} + \text{H}^+$ |         | +4 ATP    |
|  | ATP formation                |         | +4 ATP    |
| link reaction                            | 2 $\text{NADH} + \text{H}^+$ |         | +6 ATP    |
| Krebs cycle and electron transport chain | ATP formation                |         | +2 ATP    |
|  | 6 $\text{NADH} + \text{H}^+$ |         | +18 ATP   |
|  | 2 $\text{FADH}_2$            |         | +4 ATP    |
| net energy yield                         |                              |         | +36 ATP   |

**Table 2.2.3:** Together, glycolysis, the link reaction and the Krebs cycle and the electron transport chain can yield 36 ATP molecules for each molecule of glucose broken down by aerobic respiration.

---



---

## NATURE OF SCIENCE

### Changing views with new discoveries

As Table 2.2.3 shows, the net production of ATP from one molecule of glucose is, in theory, 36. Biochemists have discovered that the actual production is closer to 30 ATPs and propose that this discrepancy occurs because some protons are used to transfer ATP from the matrix to the cytoplasm. There are also losses such as the cost of moving pyruvate, phosphate and ADP (for ATP synthesis) into the mitochondria.

## EXTENSION

The reactions of respiration have the potential to release about 36 molecules of ATP from one molecule of glucose. The process of glycolysis produces 2 ATP and all the rest are produced during the electron transport chain.

But the exact number of ATP molecules generated from glucose is not as precise as the theory suggests. For example, the number of hydrogen ions pumped through the electron transport chain varies between species and ATP yield can also be reduced because the intermediate compounds in the respiration reactions are used for other reactions. The ribose sugars that build nucleic acids and some amino acids are made from intermediates of glycolysis which means fewer molecules proceed to the next stages of respiration. The percentage of potential ATP molecules that are actually produced from the catabolism of glucose can be as low as 40%.

## NATURE OF SCIENCE

### Paradigm shift: the chemiosmosis theory required a significant change of view

A paradigm shift occurs when a new theory radically changes our understanding of key concepts. It can change our view of how the natural world works.

The chemiosmosis hypothesis was proposed in 1961 by Peter Mitchell (1920–1992) to explain how the mitochondria convert ADP to ATP. At the start of the 1960s, scientists did not understand the exact mechanisms by which electron transfer is coupled to ATP synthesis. Various hypotheses at the time proposed a direct chemical relationship between oxidising and phosphorylating enzymes and suggested that a high-energy intermediate compound was formed. Mitchell's theory was completely new and proposed an indirect interaction between these enzymes with no intermediate compound. He suggested that ATP synthesis is driven by a reverse flow of protons down a concentration gradient, the so-called 'chemiosmotic theory'. This theory was first received with scepticism as his work was considered to be radical and outside the popularly held view. Mitchell struggled to persuade his contemporaries to reject the more accepted theories because his theory used a completely different approach. After several years of research, he published detailed evidence to support his theory, both in a pamphlet in 1966 and also in further publications in 1968, which were known as 'the little grey books' because of their bland covers. Eventually in the early 1970s, Mitchell's chemiosmosis theory gained scientific acceptance, and scientists conceded that no high-energy intermediate

compounds were likely to be found. Mitchell was awarded the Nobel Prize for Chemistry in 1978.

### To consider:

- 1 Despite Peter Mitchell's strong evidence for chemiosmosis, which falsified earlier theories, he struggled to have his work accepted.
- 2 Why is it often difficult for a paradigm shift to gain acceptance?

### TEST YOUR UNDERSTANDING

- 16 State the sites of the link reaction and the reactions of the Krebs carboxylic acid cycle.
- 17 Name the molecule that enters the Krebs cycle.
- 18 Where is ATP synthase located?

## Links

- What is the importance of ATP for the movement of substances across cell membranes? (Chapter 6)
- How does the structure and function of a mitochondrion compare with that of a chloroplast? (Chapter 6)
- What is the significance of the inefficiency of respiration and heat losses to the environment? (Chapter 12)

## 2.3 Photosynthesis

### LEARNING OBJECTIVES

In this section you will:

- learn that photosynthesis is a series of metabolic pathways carried out by plants, algae and some prokaryotes, uses light energy to make carbon compounds in cells
- recognise that the inputs for photosynthesis are water, carbon dioxide and light and the outputs are oxygen and glucose
- learn that light from the Sun is made up of a range of wavelengths, visible light has wavelengths between 400 nm (violet) and 700 nm (red)
- understand that chlorophylls are the main photosynthetic pigments and they absorb most red and blue light but reflect green more than the other colours. This phenomenon is shown by absorption spectra
- discover that action spectra demonstrate the wavelengths that are most effective for photosynthesis
- learn that photosynthesis consists of a light-dependent and a light-independent stage
- learn that the light-dependent stages take place in the thylakoids of chloroplasts
- learn that enzymes in the stroma of chloroplasts produce glucose using energy from the light-dependent stages

- understand how temperature, light intensity and carbon dioxide concentration affect the rate of photosynthesis and can be limiting factors
- understand why the net uptake or production of carbon dioxide and oxygen depend on both the rate of photosynthesis and cell respiration
- learn that carbon dioxide enrichment in greenhouses can promote plant growth and may be used as a method of predicting the effect of future rates of photosynthesis

- learn that the light-dependent reactions occur in the intermembrane space of the thylakoids and the intergranal lamellae and involve photosystems located in membranes
- understand how the light-dependent reactions lead to the production of reduced NADP
- discover how absorption of light excites electrons which are transferred between electron carriers
- learn that two photosystems are involved; excited electrons from photosystem I are used to reduce NADP and electrons from both photosystems are used to generate a proton gradient
- understand that the photolysis of water generates electrons to replace excited electrons and produce oxygen as a waste product
- learn that light-independent reactions occur in the stroma and are controlled by enzymes

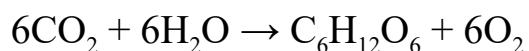
- learn that a carboxylase catalyses the carboxylation of RuBP in the light-independent reactions of the Calvin cycle
- understand that reduced NADP<sup>+</sup> and ATP are used to reduce glyceraldehyde 3-phosphate to triose phosphate
- understand how ATP synthase in thylakoids generates ATP using the proton gradient
- learn that triose phosphate is used to produce carbohydrates and regenerate RuBP and ATP is also needed
- recognise that a wide range of organic molecules are derived from the glucose produced by photosynthesis.

## GUIDING QUESTIONS

- How do plants and algae convert light energy into chemical energy that is stored in organic compounds?
- Why does photosynthesis depend on the presence of pigment molecules?
- Where in cells do the reactions of photosynthesis take place?

### 2.3.1 Photosynthesis and light

The Sun is the source of energy for almost all life on Earth. Plants, algae and some prokaryotes are able to convert light energy into chemical energy in organic compounds by the process of photosynthesis. Photosynthesis is a complex series of metabolic pathways which use carbon dioxide and water to produce glucose, other organic compounds and oxygen. Oxygen is released as a waste product. The series of reactions that occurs during photosynthesis is summarised as:



#### KEY POINT

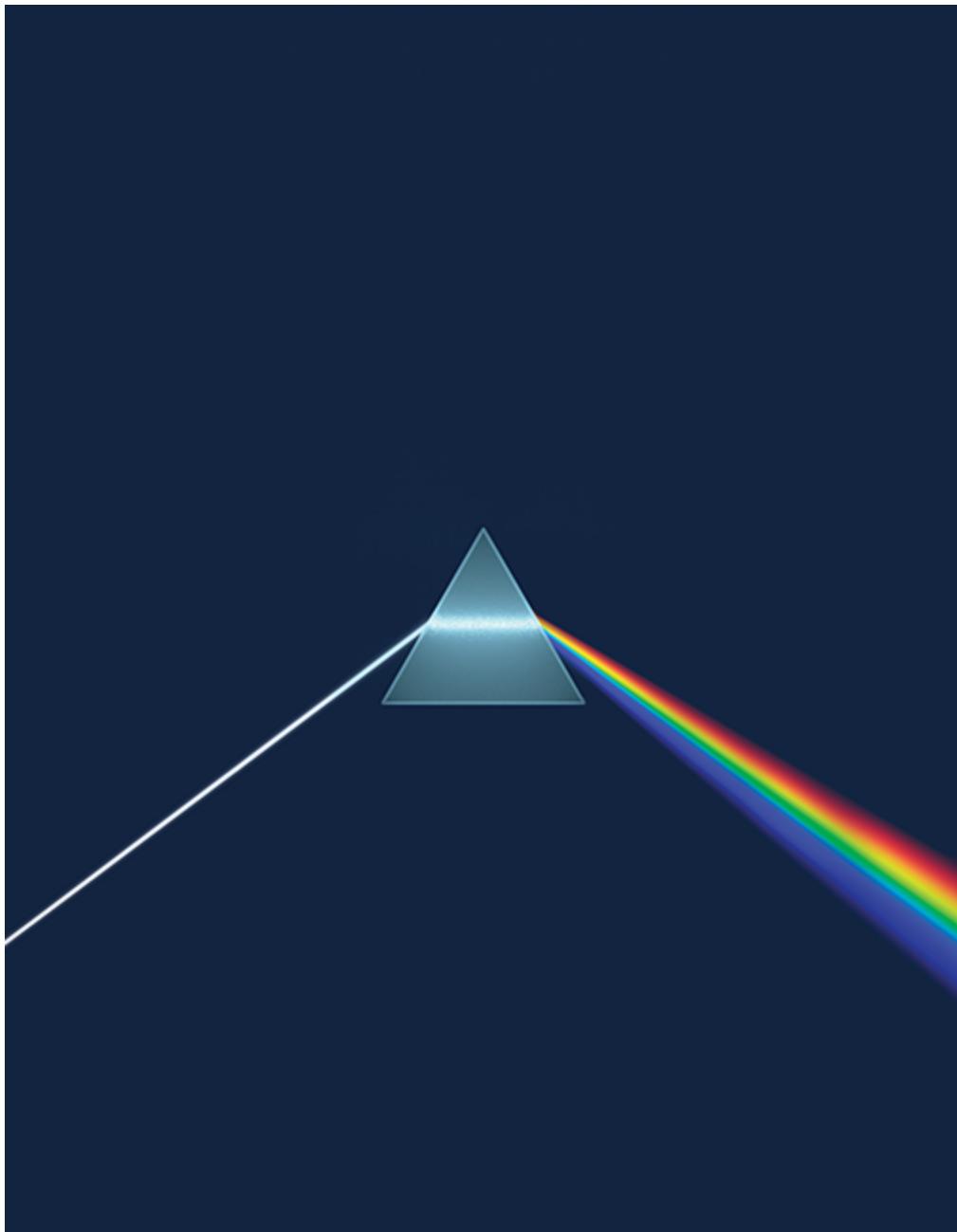
photosynthesis ‘making things with light’. Glucose is the molecule most commonly made.

The energy stored in molecules such as glucose provides a source of food for organisms that cannot use light energy directly and, within the photosynthesising organisms, it can be converted into other organic compounds that are required for life.

Visible light is composed of a spectrum of colours, which can be separated using a prism (Figure 2.3.1). A prism bends rays of light and separates the colours because each one has a slightly different wavelength and is refracted (bent) to a slightly different degree. Visible light has a range of wavelengths that are between 400 and 700 nm. Violet light has the shortest wavelength and red the longest, but the most important regions of the spectrum for photosynthesis are red and blue.

The colour of any object is determined by the wavelength of the light that it reflects back into our eyes. A blue shirt appears blue because it reflects blue light, which our eyes can perceive, but the shirt absorbs other wavelengths that fall on it and we do not see those colours. A black object absorbs all wavelengths of light, while something white reflects them all.

Most plants have green leaves and many other photosynthesising organisms also appear green. This tells us that they do not absorb the green part of the spectrum well; green light is reflected and makes a leaf appear green. The leaves of plants (Figure 8.3.11) have cells which contain chloroplasts. Chloroplasts contain green pigments called chlorophylls which gives them their green colour. Chlorophylls are unable to absorb green light, which is reflected, but do absorb other wavelengths well. Red and blue light are absorbed particularly well and provide the energy needed for photosynthesis. The top graph in Figure 2.3.2 shows that the red and blue ends of the visible spectrum are the wavelengths that the photosynthetic pigments in plants absorb most efficiently. The bottom graph, known as an action spectrum, shows that the rate of photosynthesis is highest when plants absorb these wavelengths.



**Figure 2.3.1:** ‘White light’, such as sunlight, is composed of a range of wavelengths, which become separated as they pass through a glass prism.

#### KEY POINT

chlorophyll the name for the most important group of photosynthetic pigments of green plants, found in the grana of chloroplasts and responsible for trapping light energy (some bacteria have a chemically different form called bacteriochlorophyll).

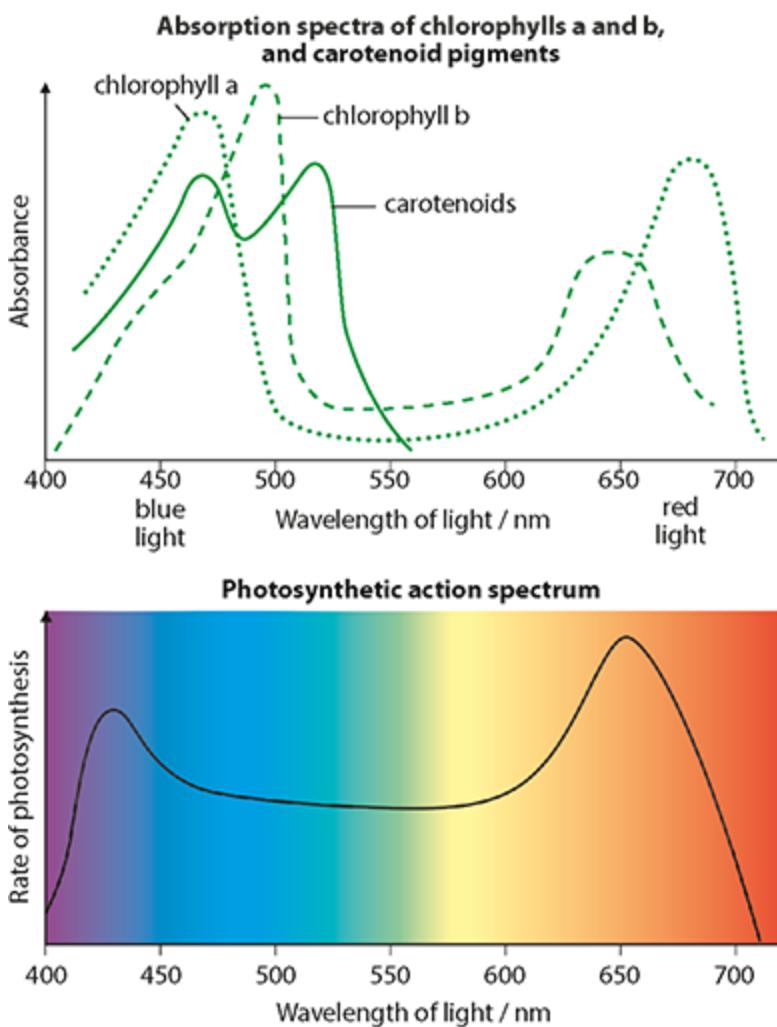
## Photosynthetic pigments

Chloroplasts contain a number of different pigments that are associated with light absorption. Figure 2.3.2 shows **absorption spectra** and **action spectra** for two types of chlorophyll pigment and carotenoid pigments found in green plants. There is a strong correlation between the absorption spectra (the range of wavelengths of light that a pigment is able to absorb) of the pigments and the action spectrum (showing the rate of photosynthesis at each wavelength). In the figure, both have two peaks, one in the blue region and a smaller one in the red region, and both are lower in the green and yellow areas of the spectrum.

### EXAM TIP

Chromatography is a simple technique used to separate different substances in a mixture and it can be used to separate the pigments in extracts from plant leaves.

Two techniques are commonly used: paper chromatography, which uses a special high-grade paper with carefully controlled spaces between the cellulose fibres, and thin-layer chromatography (TLC), which is carried out on a thin plate of glass or plastic coated with a layer of adsorbent material such as silica gel or cellulose (known as the stationary phase).



**Figure 2.3.2:** These graphs show the wavelengths (colours) of light absorbed by plants and the rate of photosynthesis that occurs at each wavelength.

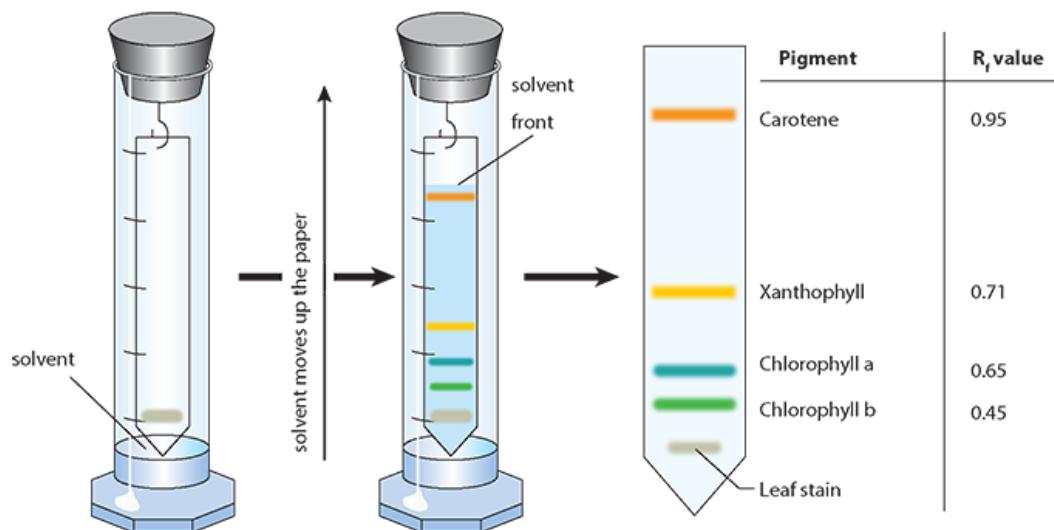
During chromatography a solvent moves up the paper or plate by capillary action and carries pigments with it by mass flow. Smaller molecules are able to move more easily and so can travel further than larger molecules. After a period of time, photosynthetic pigments from chloroplast extracts become separated (Figure 2.3.3) and can be compared and measured.

### EXAM TIP

You should be able use experiments like the one shown in Figure 2.3.3 to work out  $R_f$  values and identify different pigments.

## EXTENSION

The  **$R_f$  value** is a ratio that is used to identify components in a mixture from a chromatogram. It is calculated by dividing of the distance travelled by a component of the mixture by the distance travelled by the solvent front from the origin. The  $R_f$  value can be used to identify each pigment by comparing its  $R_f$  value to that of a known standard at the same temperature using the same type of chromatogram.



**Figure 2.3.3:** A chromatogram can be used to identify different photosynthetic pigments. Different pigments are found in different plants and the pigments may vary with the seasons.

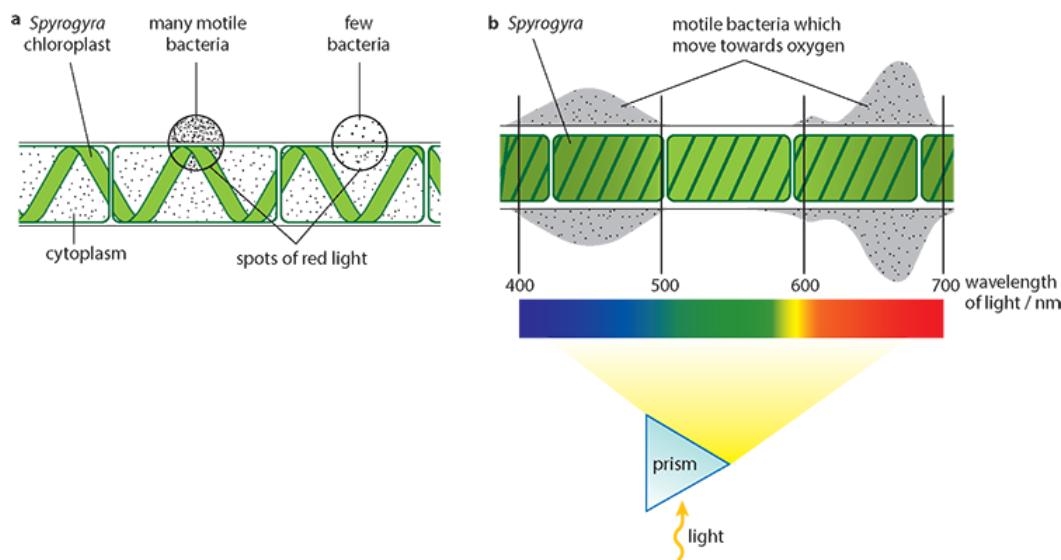
## NATURE OF SCIENCE

**Careful observation: Engelmann's experiment**

Theodor Wilhelm Engelmann (1843–1909) was a German botanist who used the filamentous alga *Spirogyra* to demonstrate not only that oxygen is evolved during photosynthesis but also that different wavelengths of light affect the rate of photosynthesis. *Spirogyra* is an alga that has cylindrical cells containing spiral-shaped chloroplasts. Engelmann mounted a sample of *Spirogyra* under a microscope and, after a period of darkness, illuminated it with different colours of light. He carefully watched the movement of motile bacteria (*Pseudomonas*) that he had added to the water and noticed that, after a period of oxygen deprivation, they moved towards areas where there was a higher concentration of oxygen around the alga's chloroplasts (Figure 2.3.4).

## TEST YOUR UNDERSTANDING

- 19** Use these questions to analyse the results of Engelmann's experiments.
- a** Look at Figure 2.3.4a and explain why the bacteria moved towards certain areas of the *Spirogyra* and not towards others when a spot of red light was used.
  - b** Explain why there are no bacteria between the areas of *Spirogyra* illuminated with light of 500–600 nm (Figure 2.3.4b).



**Figure 2.3.4:** Engelmann used bacteria in two experiments a and b to measure rates of photosynthesis and determine which wavelengths are most effective for photosynthesis.

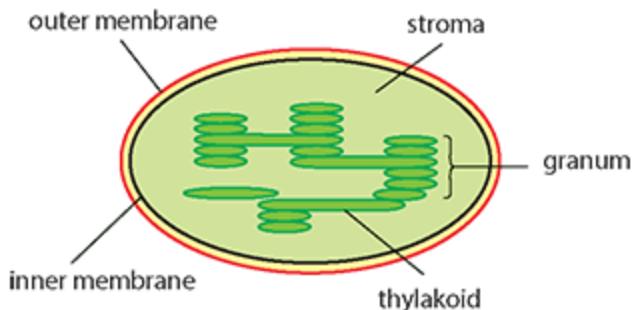
---

## 2.3.2 The chemistry of photosynthesis

Photosynthesis is a complex series of reactions catalysed by a number of different enzymes. The processes take place in chloroplasts (Figure 2.3.5; see also [Section 5.2](#)). To help us understand the reactions, we can consider photosynthesis in two stages: the light-dependent reactions and the light-independent reactions.

### Light-dependent reactions

The first stage of photosynthesis is known as the ‘light-dependent reactions’ because light is essential for them to occur. These take place in the thylakoids of the chloroplast.



**Figure 2.3.5:** Structure of a chloroplast.

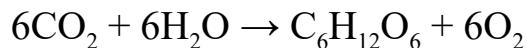
Chlorophyll in the **thylakoids** absorbs light energy and this energy is used to produce ATP. The energy is also used to split water molecules into hydrogen and oxygen in a process called **photolysis**. Hydrogen ions and electrons (from the hydrogen part of water) and oxygen are released. Oxygen is a waste product of photosynthesis but is vital to sustain the lives of aerobic organisms once it has been released into the atmosphere. The ATP, hydrogen ions and electrons are used in the light-independent reactions.

## Light-independent reactions

ATP, hydrogen ions and electrons are used in the second stage of photosynthesis, the ‘light-independent reactions’, which take place in the stroma.

During the ‘light-independent reactions’, enzymes in the stroma use carbon dioxide, taken in from the environment, and combine it with hydrogen using energy from ATP. The reactions form mainly glucose, but also a range of other organic molecules for the plant. The conversion of inorganic carbon dioxide to organic molecules such as glucose is known as **carbon fixation**. ATP provides the energy for the process.

The series of reactions that occurs during photosynthesis is summarised as:



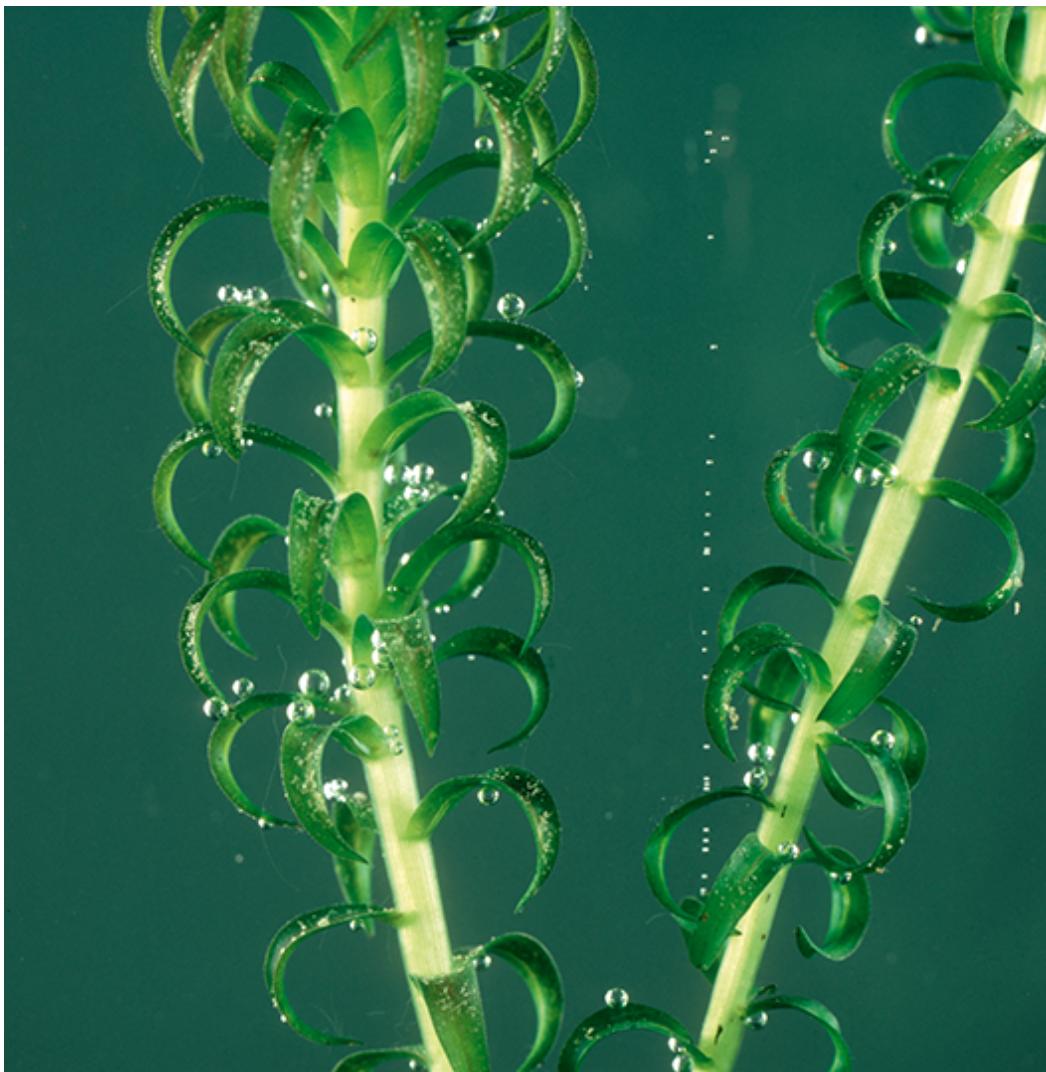
## Measuring the rate of photosynthesis

The equation for carbon dioxide and water shows that when photosynthesis occurs, carbon dioxide is used and oxygen is released. The mass of the plant (its **biomass**) will also increase as glucose is used to produce other plant materials. Any of these three factors can be used to measure how quickly the reactions of photosynthesis are occurring.

Aquatic plants release bubbles of oxygen as they photosynthesise and if the volume of these bubbles is measured for a period of time, the rate of photosynthesis can be determined directly (Figure 2.3.6).

Aquatic plants also remove carbon dioxide from their environment, causing the pH of the water to rise. Carbon dioxide dissolves in water to form a weak acid, so as it is removed the pH will go up. Therefore, another way of determining the rate of photosynthesis experimentally is to monitor the change in pH of the water surrounding an aquatic plant over a period of time.

Terrestrial plants also remove carbon dioxide from their surroundings but this is difficult to measure. It can be done experimentally by supplying a confined plant with radioactive carbon dioxide, which can be measured as it is taken up and released from the plant.



**Figure 2.3.6:** The rate of oxygen production can be used as a direct measure of the rate of photosynthesis.

---

A third method of measuring the rate of photosynthesis in plants is to determine their biomass at different times. This is an indirect method. Samples of the plants can be collected and measured at different times and the rate of increase in their biomass calculated to determine their rate of photosynthesis.

### 2.3.3 Limits to photosynthesis

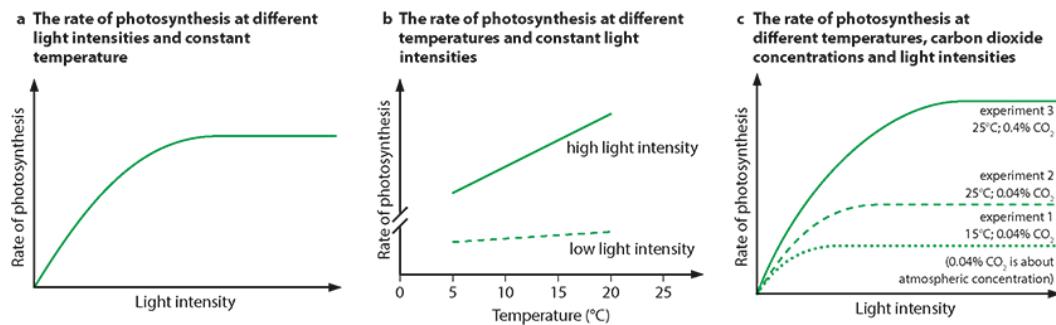
The rate at which a plant can photosynthesise depends on factors in the environment that surrounds it. On a warm, sunny afternoon, photosynthesis will be more rapid than on a cool, shady morning.

More oxygen will be produced and more carbon dioxide used. Temperature, light intensity and carbon dioxide concentration are all possible limiting factors on the rate of photosynthesis. But photosynthesis cannot increase beyond certain limits. The effect of light, temperature and carbon dioxide in the environment can be measured experimentally, varying one factor while keeping the others the same, and graphs such as those in Figure 2.3.7 can be drawn.

An increase in light intensity, when all other variables are unchanging, will produce an increase in the rate of photosynthesis that is directly proportional to the increase in light intensity. However, at a certain light intensity, enzymes will be working at their maximum rate, limited by temperature and the availability of carbon dioxide. At very high light intensities, light absorption (and therefore the rate of photosynthesis) reaches its maximum and cannot increase further. At this point, the graph reaches a plateau (Figure 2.3.7a).

Increasing temperature also increases the rate of photosynthesis as the frequency and energy of molecular collision increases (Figure 2.3.7b). Photosynthesis has an optimum temperature above which the rate will decrease sharply as enzymes are denatured, or the plant wilts and is unable to take in carbon dioxide.

An increase in the concentration of carbon dioxide causes the rate of photosynthesis to increase, as carbon dioxide is a vital raw material for the process. At very high concentrations, the rate will plateau as other factors such as light and temperature limit the rate of reaction (Figure 2.3.7c).



**Figure 2.3.7:** These graphs show the effects on photosynthesis of varying light intensity, temperature and carbon dioxide concentration.

### SCIENCE IN CONTEXT

The effects of temperature, light and carbon dioxide concentration are well known to horticulturalists who grow crops in glasshouses. Commercial producers of cucumbers and tomatoes keep their glasshouses warm and well lit. They may also seal the greenhouse and introduce carbon dioxide to boost photosynthesis to its maximum rate, thereby increasing crop production and profits.

Carbon dioxide enrichment experiments have been used to predict future rates of photosynthesis as the amount of carbon dioxide in the air increases. Some experiments are carried out in sealed greenhouses, others known as free-air carbon dioxide experiments (FACE) are conducted outdoors.

Research these experiments and consider how scientists control the different variables.

## Compensation point

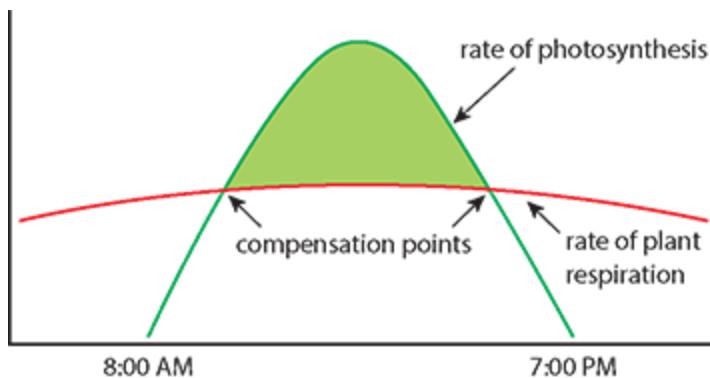
The net production of oxygen (or uptake of carbon dioxide) will depend on both the rate of cellular respiration and the rate of photosynthesis. When the two are in equilibrium, the amount of oxygen being produced by photosynthesis will be equal to that which the plant is using in respiration. This is known as the compensation point (Figure 2.3.8).

### KEY POINT

**compensation point** is when the light intensity at which the amount of carbon dioxide released in respiration equals the amount used in photosynthesis, and at which the amount of oxygen used in respiration equals the amount released in photosynthesis.

### EXAM TIP

Remember that photosynthesising organisms respire throughout the day and night but can only photosynthesise when light is available.



**Figure 2.3.8:** Graph showing the rate of photosynthesis and respiration for a plant over a period of 24 hours.

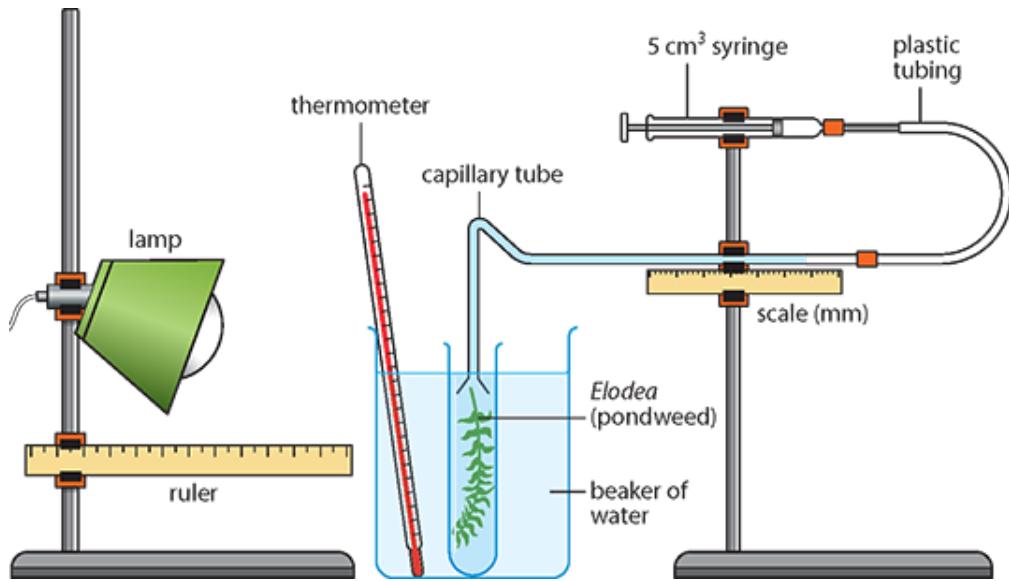
## NATURE OF SCIENCE

### Experimental design: controlling variables

Any investigations involving living organisms must be designed so that all the possible variables are controlled. Consider this diagram of apparatus (Figure 2.3.9) that has been set up in a laboratory to estimate the rate of photosynthesis of a pond plant.

#### To consider:

- 1 Which variable is being controlled by the presence of the beaker of water?
- 2 Why must this variable be controlled?
- 3 Photosynthesis is a metabolic reaction controlled by enzymes. List the factors that affect enzyme action ([Section 3.1](#)).
- 4 How could each one of these be controlled in this experiment?



**Figure 2.3.9:** Diagram of experiment to determine the rate of photosynthesis.

### TEST YOUR UNDERSTANDING

- 20** If you want to make plants grow as efficiently as possible, what colour of light should you shine on them?
- 21** Suggest what would happen to a plant's growth if it was illuminated by green light.
- 22** Where in the chloroplast does the light-dependent reactions take place?
- 23** Outline two ways in which the rate of photosynthesis can be measured.

## 2.3.4 Advanced photosynthesis

Photosynthesis is the process by which light energy is harvested and stored as chemical energy, primarily in sugars but also in other organic molecules such as lipids. It occurs in green plants, algae and some bacteria. All these organisms are known as **autotrophs**, which means they can make their own food.

Photosynthesis can be divided into two parts:

- the light-dependent reactions
- the light-independent reactions.

The light-dependent reactions produce compounds that are used in the light-independent reactions.

### KEY POINTS

light-dependent reactions occur on the thylakoids and produce ATP and NADPH

light-independent reactions series of stages in photosynthesis that take place in the stroma and use the products of the light-dependent reactions to produce carbohydrate.

photosystems arrays of pigment molecules that can generate and emit excited electrons

Both the light-dependent and the light-independent reactions take place in the chloroplasts of plant cells (Figures 2.3.5, 2.3.10 and 2.3.11). The stroma contains the enzymes required for the light-independent reactions and the stacks of thylakoid membranes increase the surface area for the light-dependent reactions.

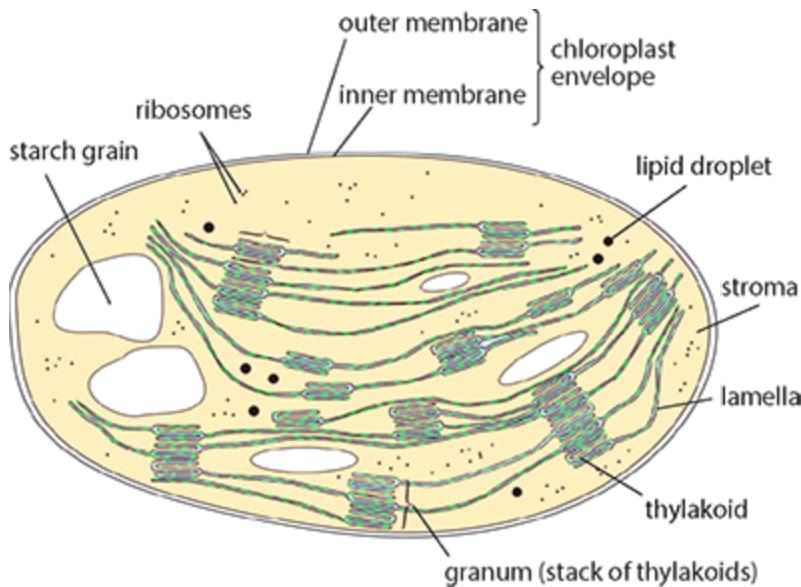
Both these sets of reactions are part of photosynthesis and can only occur when there is sufficient light. Light-dependent reactions can only take place in light and although light-independent reactions do not require light directly – and can take place when it is dark – they do require the products of the light-dependent reactions.

## The light-dependent reactions

The **light-dependent reactions** are a series of stages in photosynthesis that occur on the grana of the chloroplasts. Light is used to split water, and ATP and NADPH + H<sup>+</sup> are produced.



**Figure 2.3.10:** Coloured electron micrograph of a chloroplast ( $\times 20\,000$ ).



**Figure 2.3.11:** Diagram of a chloroplast.

The reactions take place on the thylakoid membranes that make up the grana of the chloroplast and are powered by light energy from the Sun. Each thylakoid is a flattened sac so the space in the middle is narrow. The thylakoid membranes form the stacks called **grana**, which may be joined together by intergranal lamellae (membranes). Light is absorbed by photosynthetic pigments, including the chlorophylls, which are found on the granal membranes. There are several pigments found in plants and each one absorbs light of a slightly different wavelength.

The photosynthetic pigments are combined into two complex groups called **photosystems** I and II (PS I and PS II), which absorb the light energy and use this to boost electrons to a higher energy level so that they become ‘excited’. The pigments are associated with proteins that are involved in electron transport, proton pumping and chemiosmosis. The arrangement of photosystems in membranes and of different pigment molecules in the photosystems means that the passage of electrons is structured effectively to enable photosynthesis to take place.

Both PS I and PS II have a chlorophyll a molecule at their centre together with accessory pigments, such as chlorophyll b and carotenoids, around them.

At the centre of each chlorophyll a molecule is a magnesium ( $Mg^{2+}$ ) ion which is essential to its structure and functioning ([Section 1.1](#)). Fluctuations in magnesium levels in the chloroplast regulate the activity of key photosynthetic enzymes.

In the light-dependent reactions, electrons are removed from water and passed through PS II and PS I before ending up in NADPH. This process needs light to be absorbed by both photosystems and it also produces ATP. The process is called **photophosphorylation** because it uses light energy to produce ATP from ADP.

The key stages of the light-dependent reactions are as follows:

## 1 Photoactivation of PS II

Light is absorbed by pigments in PS II and passed to the reaction centre. Here energy boosts an electron in chlorophyll a to a higher energy level. The electron is passed to an acceptor molecule at the start of the electron transport chain.

Lost electrons must be replaced and this is done by taking them from water. Water is split into electrons, protons (hydrogen ions) and an oxygen atom. Since the splitting is brought about by light energy, it is called photolysis. The oxygen is released as a waste product and is the oxygen we breathe.

## 2 ATP synthesis

Excited, high-energy electrons travel down the electron transport chain into PS I. As they do this, they lose energy which is used to pump protons into the thylakoid interior (in a similar way as occurs in the electron transport chain in the mitochondrion). The thylakoid interior is small and so a proton concentration gradient builds up quickly. The protons then flow out through a large channel protein, almost identical to the one in mitochondria, which contains the enzyme ATP synthase. Ions flow down their gradient and into the stroma, driving ATP production in a process known as chemiosmosis. This time though, the formation of ATP is called photophosphorylation and it occurs between PS II and I (Figure 2.3.12).

### **3 Light absorption in PS I**

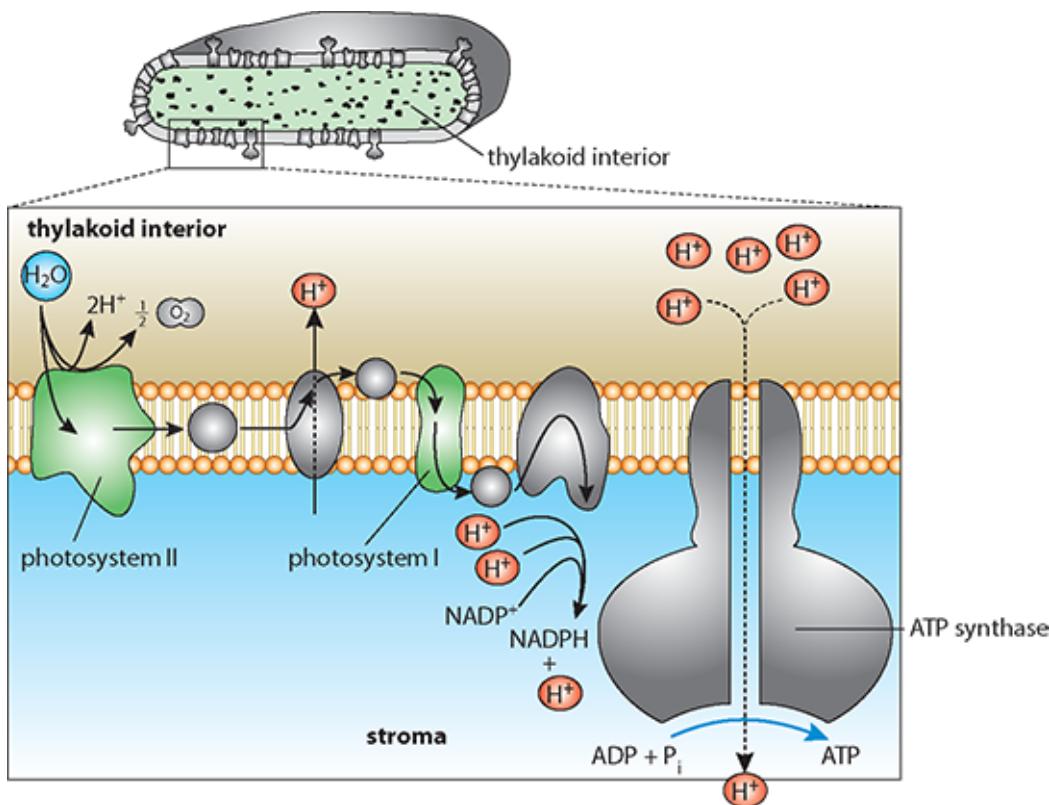
Absorption of light energy causes photoactivation in PS I, boosting more electrons to an even higher energy level. The electrons that arrive from PS II replace those that are displaced.

### **4 NADPH formation**

The electrons at the higher energy level continue down the electron transport chain and are combined with protons in the hydrogen carrier  $\text{NADP}^+$  to form  $\text{NADPH} + \text{H}^+$ .

The two products of the light-dependent reaction, ATP and  $\text{NADPH} + \text{H}^+$ , are used to drive the light-independent reaction.

The net effect of these steps is to convert light energy into chemical energy in the form of ATP and NADPH. The ATP and NADPH from the light-dependent reactions are used to make sugars in the next stage of photosynthesis, the Calvin cycle.



**Figure 2.3.12:** Chemiosmosis in photosynthesis.

### The light-independent reactions

The light-independent reactions occur in the stroma of the chloroplast. These reactions are catalysed by enzymes and therefore are temperature dependent.

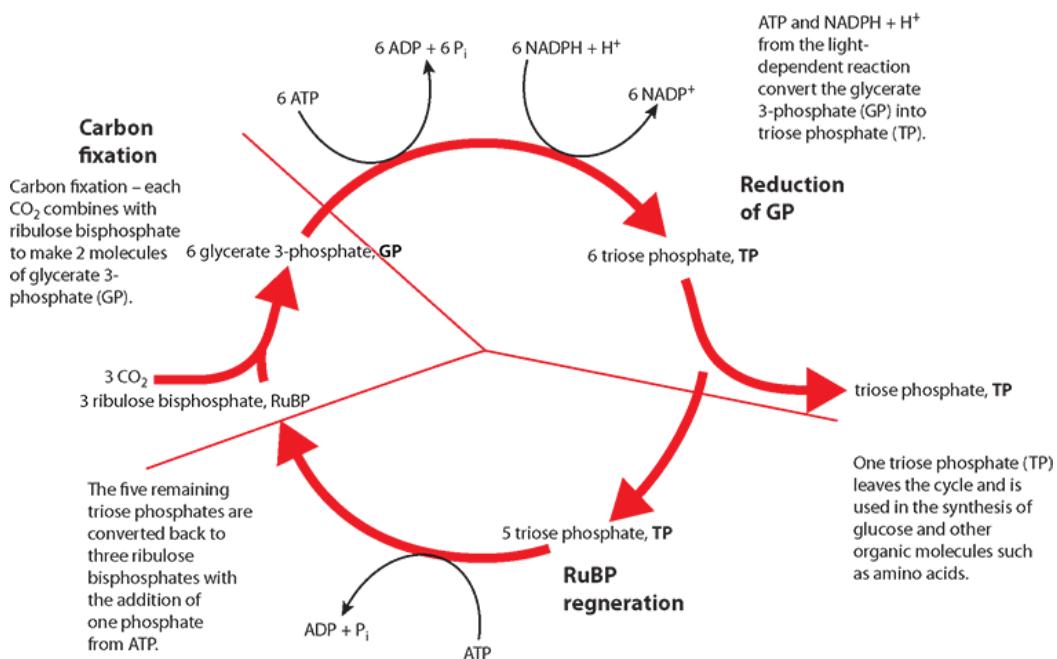
The reactions follow a cyclic pathway called the **Calvin cycle** (shown in Figure 2.3.13). ATP and NADPH + H<sup>+</sup> formed during the light-dependent stage supply energy and reducing power for the Calvin cycle. The final product of the cycle is carbohydrate.

#### KEY POINT

Calvin cycle of light-independent reactions in the stroma of the chloroplast in which carbon dioxide reacts with ribulose

bisphosphate (RuBP), producing glycerate 3-phosphate, triose phosphate and regenerating RuBP.

During each turn of the Calvin cycle, one molecule of carbon dioxide is used, so Figure 2.3.13 shows three cycles combined together. As this is a cycle, what goes in must leave, so three carbons enter in three molecules of carbon dioxide and three carbons leave in one molecule of triose phosphate, which can be used to form glucose or other organic compounds. There are three stages in the cycle, as follows.



**Figure 2.3.13:** The light-independent pathway of photosynthesis – the Calvin cycle.

## 1 Carbon fixation

At the start of the cycle, the acceptor molecule ribulose bisphosphate (RuBP) combines with incoming carbon dioxide from the air to form glycerate 3-phosphate (GP). This reaction is called carbon fixation because it ‘locks’ the

carbon into the cycle. It is catalysed by RuBP carboxylase (this enzyme is sometimes called Rubisco).

## 2 Reduction of glycerate 3-phosphate

The ATP and NADPH + H<sup>+</sup> from the light-dependent reaction convert the glycerate 3-phosphate into triose phosphate (TP). Glycerate 3-phosphate is reduced to triose phosphate. No more phosphate is added so the only input from ATP is energy.

Six molecules of triose phosphate are produced but only five are needed to reform the ribulose bisphosphate to keep the cycle going. The extra triose phosphate leaves the cycle and is used to synthesise organic molecules such as glucose or amino acids.

## 3 RuBP regeneration

The triose phosphate that leaves the cycle takes a phosphate with it, so this is replaced in the cycle with a phosphate from ATP, as the five remaining triose phosphates are converted back to three ribulose bisphosphate molecules, and the cycle begins again. This is an example of a cyclic metabolic pathway.

Six ‘turns’ of the Calvin cycle produce two triose phosphate molecules, which can be combined to form the final product, glucose. Many triose phosphate molecules will be converted immediately to starch. Other triose phosphate molecules are exported to the cytoplasm to be converted to sucrose and transported around the plant. The products of the Calvin cycle are used to make all the other different organic molecules the plant needs, such as cellulose, amino acids, fatty acids and vitamins.

## KEY POINT

**RuBP carboxylase (Rubisco)** is the enzyme that catalyses carbon fixation in the Calvin cycle in the light-independent reactions of photosynthesis; in carbon fixation, the five-carbon acceptor molecule ribulose bisphosphate (RuBP) combines with carbon dioxide to form glyceralate 3-phosphate.

## TEST YOUR UNDERSTANDING

- 24** Where do the light-dependent reactions take place?
- 25** State the colour of light that is not absorbed by green plants and algae.
- 26** Where in a chloroplast do you find magnesium ions?
- 27** State the names of the two products of the light-dependent reactions that are needed for the light-independent reactions.
- 28** State the name of the acceptor molecule that reacts with carbon dioxide in the Calvin cycle.

## Links

- How does the structure of chlorophyll compare with that of hemoglobin? ([Chapter 1](#))
- How does the structure of a chloroplast compare with that of a mitochondrion?
- What is the origin of chloroplasts in eukaryotic cells? ([Chapter 5](#))

- What are the similarities and differences between chemiosmosis in respiration and photosynthesis?

## SELF-ASSESSMENT CHECKLIST

Think about the topics covered in this chapter. Which parts are you most confident with? Which topics require some extra practice?

| I can...  | Subsection | Needs more work | Nearly there | Confident to move on |
|---|------------|-----------------|--------------|----------------------|
| define metabolism and state that reactions may take place inside or outside cells | 2.1.1      |                 |              |                      |
| recall that metabolic pathways may be linear or cyclical                          | 2.1.1      |                 |              |                      |
| recall that metabolic processes may be anabolic or catabolic                      | 2.1.1      |                 |              |                      |
| explain the importance of enzymes and their properties in metabolism              | 2.1.2      |                 |              |                      |
|   |            |                 |              |                      |

|  |       |  |  |  |
|--|-------|--|--|--|
| outline the induced-fit hypothesis of enzyme action  | 2.1.2 |  |  |  |
| summarise the importance of temperature, pH and substrate concentration on enzyme action and interpret graphs which show these | 2.1.2 |  |  |  |
| outline how enzymes lower activation energy  | 2.1.3 |  |  |  |
| summarise how enzymes can be affected by other molecules that bind to them at their active or allosteric sites                 | 2.1.4 |  |  |  |
| describe the effects of competitive and non-competitive inhibitors on rates of reaction  | 2.1.4 |  |  |  |
| outline end-product inhibition   | 2.1.5 |  |  |  |

|  |              |  |  |  |
|--|--------------|--|--|--|
| define co-enzymes and co-factors and give an example of each                                       | 2.1.6        |  |  |  |
| explain the inputs and products of cellular respiration  | 2.2.1        |  |  |  |
| recall that respiration occurs as a series of metabolic pathways catalysed by enzymes              | 2.2.1        |  |  |  |
| outline the uses of energy-requiring actions and recall that energy is lost from organisms as heat | 2.2.1        |  |  |  |
| outline the stages of anaerobic respiration and explain its lower energy yield                     | 2.2.2, 2.2.3 |  |  |  |
| recall that aerobic respiration has a higher yield of energy, takes                                | 2.2.2        |  |  |  |

|  |              |  |  |  |
|--|--------------|--|--|--|
| place in mitochondria and requires oxygen to produce carbon dioxide and water            |              |  |  |  |
| outline the importance of redox reactions involving NAD and FAD                          | 2.2.4        |  |  |  |
| define phosphorylation, decarboxylation and explain their importance in cell respiration | 2.2.4, 2.2.5 |  |  |  |
| list the four steps involved in aerobic respiration                                      | 2.2.4        |  |  |  |
| compare the products and energy output of aerobic and anaerobic respiration              | 2.2.5        |  |  |  |
| draw a mitochondrion and indicate where reactions of                                     | 2.2.5        |  |  |  |

|   |       |  |  |  |
|---|-------|--|--|--|
| respiration take place  |       |  |  |  |
| summarise the reactions that occur in the ETC   | 2.2.5 |  |  |  |
| define chemiosmosis and explain its importance in generating ATP                                      | 2.2.5 |  |  |  |
| explain the inputs and products of photosynthesis and write an equation that summarises the reactions | 2.3.1 |  |  |  |
| recall the colours of light that are used for photosynthesis  | 2.3.1 |  |  |  |
| explain the difference between an absorption spectrum and an action spectrum                          | 2.3.1 |  |  |  |
| name the two stages of photosynthesis   | 2.3.2 |  |  |  |

|   |       |  |  |  |
|---|-------|--|--|--|
| and state where they take place   |       |  |  |  |
| name three limiting factors on the rate of photosynthesis and sketch graphs to show their effects     | 2.3.3 |  |  |  |
| explain how photosynthesis and respiration are related in algae and plants                            | 2.3.3 |  |  |  |
| outline the location and structure of the two photosystems  | 2.3.4 |  |  |  |
| state the precise locations of the light-dependent and light-independent reactions in the chloroplast | 2.3.4 |  |  |  |
| summarise the differences between the light-dependent and light-independent reactions                 | 2.3.4 |  |  |  |

|   |       |  |  |  |
|---|-------|--|--|--|
| name the products of the light-dependent reactions that enter the light-independent reactions | 2.3.4 |  |  |  |
| summarise the three stages of the Calvin cycle  | 2.3.4 |  |  |  |
| outline the importance of carboxylase, RuBP and glyceralate 3-phosphate in the Calvin cycle.  | 2.3.4 |  |  |  |

## REFLECTION

How well is your understanding of respiration developing?  
Could you explain the various stages to a fellow student?

## EXAM-STYLE QUESTIONS

You can find questions in the style of IB exams in the digital coursebook.



## › Chapter 3

# DNA and protein synthesis

D1.1, D1.2, D1.3

## INTRODUCTION

Nucleic acids are very large macromolecules composed of a backbone of sugar and phosphate molecules each with a nitrogenous base attached. In [Chapter 2](#) the basic structure of these molecules was considered. Here we will look at the vital role of nucleic acids in producing the proteins we need for life and how the genetic information contained in DNA is passed from one generation to the next.

## 3.1 DNA replication

### LEARNING OBJECTIVES

In this section you will:

- understand that DNA replication is a semi-conservative process that produces two identical new molecules
- learn that the enzyme helicase unwinds the double helix and separates the strands
- understand that the polymerase chain reaction is a laboratory process that amplifies small quantities of DNA
- recognise that gel electrophoresis separates DNA fragments by their charge and size and is useful in paternity and forensic investigations

- recognise that DNA strands are antiparallel and are orientated in opposite directions
- learn that DNA replication is regulated by a series of enzymes: primase, polymerase and ligase
- understand that DNA polymerase can only work in a 5' to 3' direction
- discover that DNA polymerases proofread new DNA strands.

## GUIDING QUESTIONS

- How is inherited material copied?
- How do laboratory techniques enable us to analyse DNA?

### 3.1.1 DNA replication

An essential feature of DNA is that it must be able to replicate itself accurately, so that when a cell divides, the genetic code it carries can be passed on to the daughter cells. DNA replication copies DNA precisely so that new molecules are produced with exactly the same sequence of bases as the original strands. DNA replication takes place in the nucleus during interphase of the cell cycle when DNA is not tightly coiled ([Section 6.5](#)).

#### KEY POINTS

DNA replication is copying DNA so that two identical new molecules are produced.

replication fork is the point where the DNA double helix is being separated to expose the two strands as templates for replication.

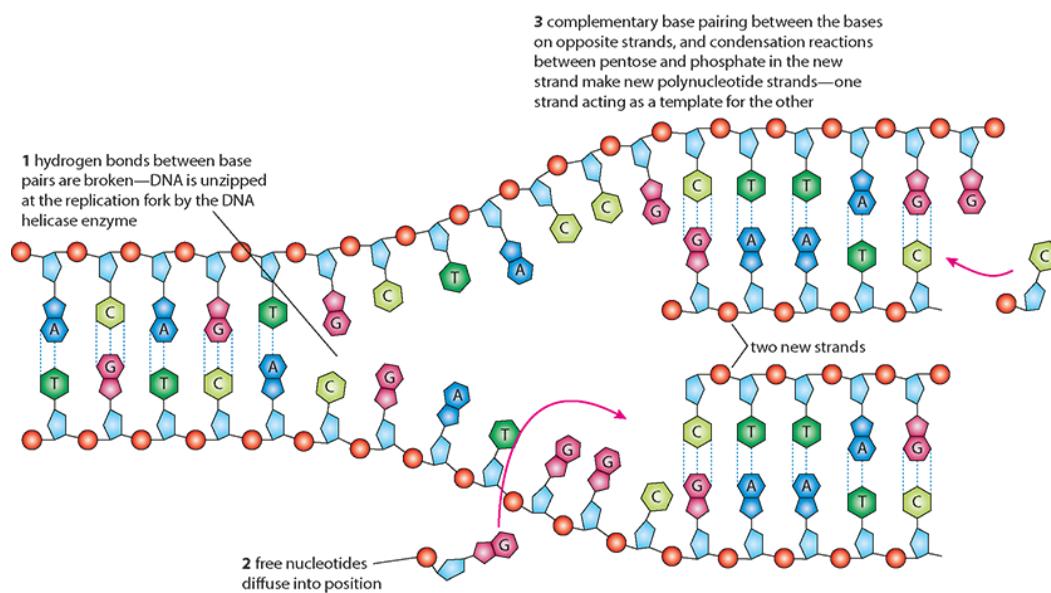
semi-conservative replication happens when both strands of a DNA double helix are used as templates for replication so that new DNA molecules contain one original and one new strand.

As Figure 3.1.1 shows, DNA replication does not occur in a haphazard manner. An enzyme called DNA helicase unzips one region of the DNA molecule and nucleotides are added in a step-by-step process that links them to one another and to their complementary bases in an area known as the replication fork.

- 1 The first step in the process is the ‘unzipping’ of the two strands. **DNA helicase** moves along the double helix, unwinding the two strands, which separate from one another as the relatively weak hydrogen bonds between the bases are broken.

- 2 The unpaired nucleotides are exposed and each single strand now acts as a template for the formation of a new complementary strand. Free nucleotides move into place: C pairs with G and A pairs with T.
- 3 The free nucleotide bases form complementary pairs with the bases on the single DNA strands. **DNA polymerase** is the enzyme involved in linking the new nucleotides into place. Finally, the two new DNA molecules are rewound, each one forming a new double helix.

The two new DNA strands that are produced are absolutely identical to the original strands. Complementary base pairing between the template strand and the new strand ensures that an accurate copy of the original DNA is made every time replication occurs. DNA replication is said to be semi-conservative replication because no DNA molecule is ever completely new. Every double helix contains one ‘original’ and one ‘new’ strand.



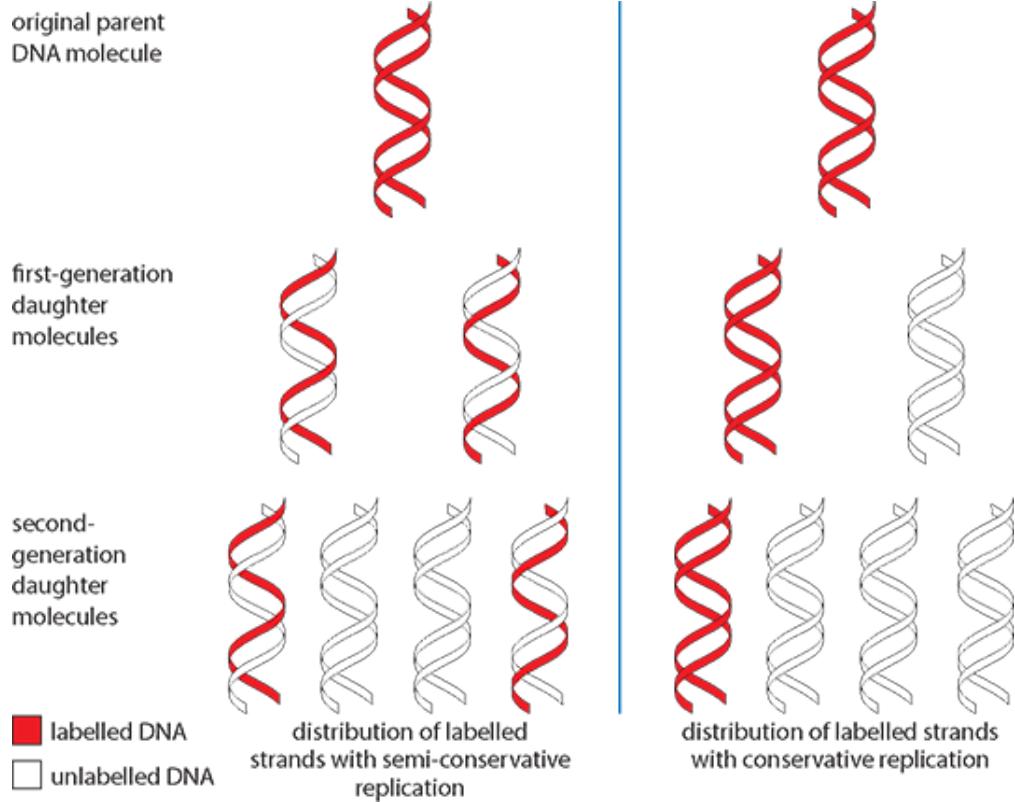
**Figure 3.1.1:** DNA replication.

## NATURE OF SCIENCE

### Obtaining evidence: Meselson and Stahl's experiment and semi-conservative replication of DNA

The research of Meselson and Stahl demonstrates the importance of making and testing a hypothesis in science. They investigated the two hypotheses about DNA replication that were current in the 1950s. The first hypothesis proposed that when DNA is replicated the original helix is conserved unchanged and the newly produced helix contains all new material. This conservative hypothesis was in contrast to the semi-conservative hypothesis, which proposed that one of the original DNA strands from a helix would always be found as one-half of the new double helix produced after replication.

Meselson and Stahl designed their experiments using *Escherichia coli*. The bacteria were grown on a medium containing nitrogen  $^{15}\text{N}$ , which is a heavy **isotope** of the normal  $^{14}\text{N}$ . These isotopes were essential to Meselson and Stahl's experiments. After many generations the bacteria have incorporated  $^{15}\text{N}$  into their cells, so that their DNA became 'labelled' with the heavy isotope and could be identified easily. The bacteria were then transferred to a new medium containing the lighter isotope  $^{14}\text{N}$ , and allowed to grow for a period of time that corresponded to the length of a generation. Figure 3.1.2 shows how the labelled DNA would be distributed among the daughter molecules after one and two replications, according to the semi-conservative theory and the conservative theory. Meselson and Stahl's careful measurements of the amounts of  $^{15}\text{N}$  in the daughter molecules after one replication showed that all the helices contained one strand of labelled DNA and one strand of normal DNA. Their results therefore supported the theory of semi-conservative replication.



**Figure 3.1.2:** The distribution of labelled DNA in daughter molecules after replication, according to the semi-conservative theory and the conservative theory of replication.

### 3.1.2 DNA sequencing

DNA sequencing is a technique that analyses sequences of DNA bases to work out the sequence of individual genes, groups of genes or even entire chromosomes and genomes. Since the advances in technology made during the Human Genome Project at the turn of the century many new and automated methods of sequencing have been developed. Geneticists and forensic scientists use these techniques to analyse and compare sequences in DNA, some of which are common to different species and others that are repeated many times. The results are used in criminal investigations, for establishing family relationships and in medical diagnoses.

#### KEY POINTS

chromosome in eukaryotes, a structure consisting of a long thread of DNA and protein that carries the genetic information of the cell; in bacteria, the DNA molecule that contains the genetic information of the cell.

genome refers to the whole of the genetic information of an organism.

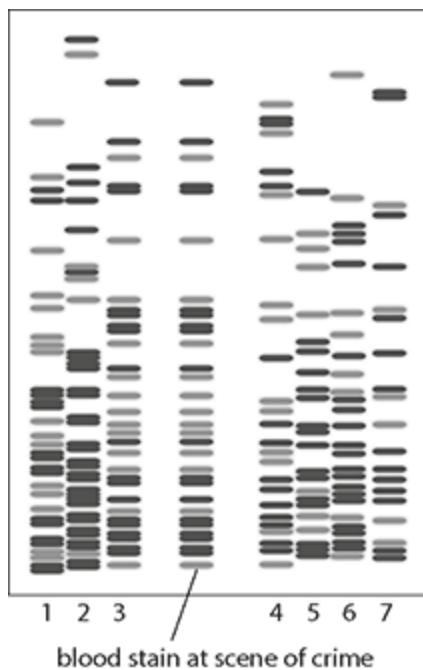
### DNA profiling

At a crime scene, forensic scientists check for fingerprints because a person's fingerprint is unique and can be used to identify them. Forensic scientists also collect samples of hair, skin, blood and other body fluids left at a crime scene because they all contain a person's DNA and that too is a unique record of their presence.

Matching the DNA from a sample to a known individual is called **DNA profiling**. In forensic science, DNA profiles from crime scenes can be used to establish the possibility of guilt or to prove a suspect innocent (Figure 3.1.3). DNA profiling can also be used to determine paternity. For example, a woman might believe that a particular man is the father of her child. By comparing DNA samples from all three individuals – the woman, the man and the child – paternity can be established.

### KEY POINT

DNA profiling is the process of producing a specific DNA pattern, called a profile, from a sample of DNA.



**Figure 3.1.3:** DNA profile of a blood stain found at the scene of a crime compared with profiles from seven suspects. Which suspect was at the scene of the crime? What is the evidence to support your answer?

## The polymerase chain reaction

The polymerase chain reaction (PCR) is an automated method used to amplify (copy) segments of DNA. To study DNA in forensic or genetic analysis large amounts of a sample of DNA are needed. In most cases only small amounts are available, so PCR is a vital tool.

### KEY POINT

polymerase chain reaction (PCR) a process in which small quantities of DNA are artificially amplified for research and diagnosis.

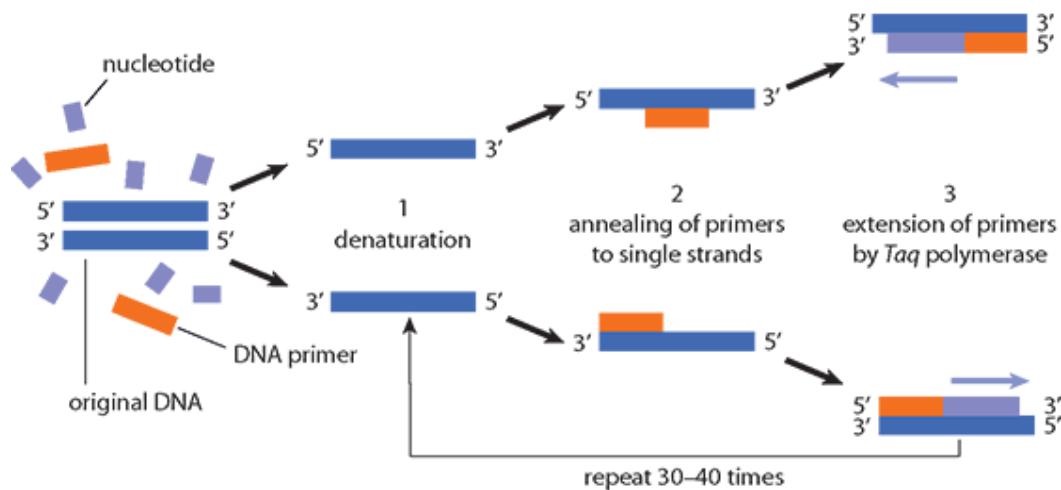
Sometimes, at a crime scene or when a body is found after a very long time, only a minute amount can be collected. The PCR can make millions of copies of tiny amounts of DNA so there is a sufficient amount to produce a profile or study a gene of interest. Technicians must take great care when handling the original sample so that it is not contaminated with their own or other DNA. Only the DNA region that is of interest will be amplified. A geneticist might be studying gene function or a forensic scientist could want to match crime scene DNA with that of a suspect. In medicine, the DNA of bacteria or viruses can be used in diagnosis. DNA amplified by PCR may be used for sequencing or to produce DNA profiles using gel electrophoresis (see the following stages).

The stages in the process are:

- 1 Denaturation – Heat the DNA sample to 95 °C so that the double strands of DNA separate into two single strands.
- 2 Annealing and extension – Cool to 68 °C and add the enzyme *Taq* polymerase, DNA primers and DNA

nucleotides that are needed to build duplicate copies of the original DNA using the two strands of DNA as templates.

- 3 Repeat the cycle of separation and synthesis of new DNA 30–40 times so that eventually more than a billion exact copies of the original DNA segment are produced (Figure 3.1.4).



**Figure 3.1.4:** Stages in the polymerase chain reaction.

PCR is automatically controlled in a machine called a thermocycler that alters the temperature of the reaction every few minutes, firstly to cause DNA separation and then for synthesis of new strands.

### ***Taq* polymerase**

The PCR needs the enzyme DNA polymerase to build new strands of DNA from the existing template strands, just as a cell does when it copies its DNA. The DNA polymerase used in PCR is *Taq* polymerase. It has been obtained from the heat-tolerant bacterium *Thermus aquaticus*, which is found in thermal vents and in hot springs. *Taq* polymerase is unaffected by high

temperature and is most active around 70 °C, a temperature at which a human DNA polymerase would not work.

### KEY POINT

**Taq** polymerase a heat-stable DNA polymerase named after the microorganism *Thermus aquaticus* used to amplify DNA in the polymerase chain reaction.

## PCR primers

Primers are short sequences of nucleotides that provide a starting point for DNA synthesis by *Taq* polymerase. The technician using PCR will add primers to select the region of DNA that is to be copied, or amplified. PCR primers are pieces of single-stranded DNA, usually around 20 nucleotides in length.

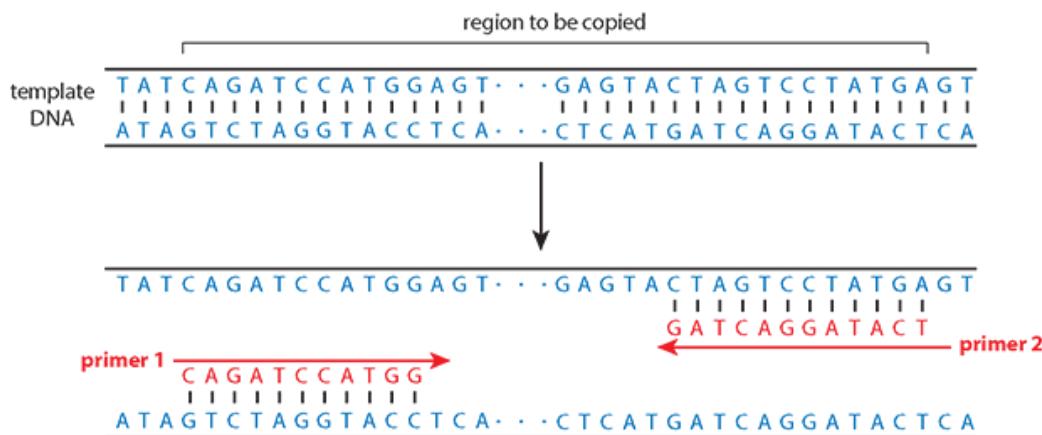
Two primers are used in each PCR reaction. The sequences bind by complementary base pairing to opposite strands of DNA at the ends of the region to be copied. The *Taq* polymerase will add nucleotides to the primers so that the region between them is copied (Figure 3.1.5).

## PCR in diagnosis

PCR can be used in medical diagnosis to detect genetic sequences of bacteria and viruses and thus identify active infections. Using specific primers, PCR can be used to amplify known sequences that only exist in certain viruses or bacteria. If that sequence is not found and there is no infection, then no amplification will take place and no DNA will be produced. Pathogens that are difficult, or take a long time, to culture can be identified quickly. The detection of the presence of bacteria in clinical specimens such as spinal fluid, blood and urine can

enable doctors to make speedy diagnoses and give the correct treatments.

Genetic disorders are caused by mutations (changes in DNA) that can be just a few base sequences, or be changes in large sequences of DNA or, sometimes, whole chromosomes. PCR enables geneticists to study just a small segment of DNA at a specific region of chromosome. The sequences of bases in a gene can be amplified and disorders detected, diagnosed and monitored. In some cases, **gene therapy** (Section 3.3) is available to rectify these disorders, and PCR can be used to monitor the functioning of the relevant genes and gene segments.



**Figure 3.1.5:** Positions of primers on single strands of DNA that are to be copied.

## SCIENCE IN CONTEXT

### Hunting for coronaviruses

There are two main types of coronavirus test: those that can detect the presence of the virus that is active in the body and those that detect a previous response to the virus by the immune system.

The PCR test is used in the first type of test, and looks for evidence that the COVID-19 virus, SARS-CoV-2, is present in a person's body by detecting the presence of its RNA in a swab sample from their nose or throat. The PCR test detects the genetic material from the virus by amplifying tiny amounts that may be present. PCR can only tell us if the virus is currently present in a person's body.

PCR tests involve several stages so errors are possible between sampling and analysis. False negatives (that is, a result that is negative when in fact the patient has the virus) do occur but estimates are that 80–85% of the results are correct.

## Gel electrophoresis

Gel electrophoresis is a method used to separate fragments of DNA on the basis of size and the electric charge they carry. It can identify natural variations found in every individual's DNA.

Any DNA sample usually contains long molecules that are too large to be used for profiling so DNA profiling often examines repetitive sequences of so-called 'satellite' DNA that vary in their degree of repetitiveness from person to person. These are called variable number tandem repeats (VNTRs) and short tandem repeats (STRs). These regions have repeated sequences of DNA that are very similar in close relatives but so variable in unrelated people that non-relatives are extremely unlikely to have the same repeated sequences.

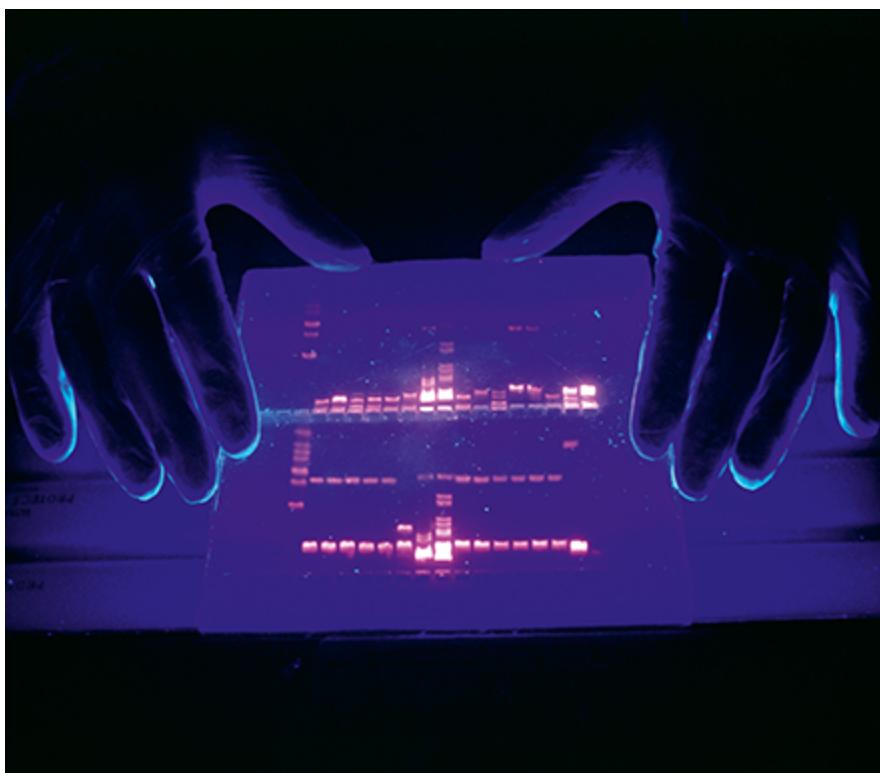
The DNA fragments are placed in a well in a plate of gel (a jelly-like material) and one well is reserved for a reference fragment known as a DNA ladder, this is a DNA molecule of a known length, used as a reference to estimate the size of the unknown DNA molecules in the sample.

## KEY POINTS

DNA ladder DNA molecules of different lengths used in gel electrophoresis, used as a reference to estimate the size of unknown DNA molecules.

gel electrophoresis a technique which separates DNA fragments according to their size and charge.

An electric field is applied and because each DNA fragment has a small negative charge, it will move in the electric field, through the gel. The distance a fragment can move depends on its size; smaller fragments move most easily through the gel matrix and travel further, while larger fragments are left behind close to their starting point. After the fragments have been separated in the gel, they are stained and produce a unique pattern of bands called a DNA profile (Figures 3.1.3 and 3.1.6).



**Figure 3.1.6:** Scientist examining an agarose electrophoresis gel used to prepare a DNA profile. The sample of DNA is marked with a radioactive substance, so the DNA banding pattern appears pink under ultraviolet light. The pattern is preserved by applying radiographic film to the gel.

## SCIENCE IN CONTEXT

### Short tandem repeats and tracing our ancestors

Any one STR will be shared by between 5 and 20% of people who are not related. But in forensic science many STRs are examined at the same time; the more STR regions that are examined, the more accurate the test becomes. The pattern of repeats can identify an individual with a high degree of accuracy. In the world of genealogy (tracing family history), DNA profiling and STRs are also used as vital tools. Today, if you want to prove that you are descended from a certain line then you may be able to use genetics to prove it. Genealogists research the ancestry of families, looking for groups of people who share the same STRs and who can be identified as being related to each other over hundreds or even thousands of years.

## THEORY OF KNOWLEDGE

### DNA profile databases

In the USA, the Federal Bureau of Investigation (FBI) has a national database of DNA profiles from convicted criminals, suspects, missing persons and crime scenes. The data that are held may be used in current investigations and to solve unsolved crimes. There are many commercial laboratories that carry out DNA profiling analysis on behalf of the law

enforcement agencies. Many of them check 13 key STR sequences in DNA samples, which vary considerably between individuals. The FBI has recommended that these should be used because they provide odds of one in one thousand million that two people will have the same results.

CODIS is the acronym for the Combined DNA Index System, a computer software program that operates the national database of DNA profiles. Every US state has a statutory right to establish a DNA database that holds DNA profiles from offenders convicted of particular crimes. CODIS software enables laboratories to compare DNA profiles electronically, linking serial crimes to each other and identifying suspects from profiles of convicted offenders. CODIS has contributed to thousands of cases that have been solved by matching crime scene evidence to known convicted offenders.

### To consider:

- 1** DNA profiles do not show individual base sequences but only identify repeated sequences. How much confidence should be placed on DNA evidence?
- 2** How secure is DNA profiling?
- 3** What are the implications for society if the authorities were to hold a DNA profile for every person?
- 4** What safeguards should be in place to protect the rights of individuals whose DNA profiles have been placed on a database but who have not been convicted of a crime?
- 5** Is it right to convict a person on DNA evidence alone?

### TEST YOUR UNDERSTANDING

- 1** Outline what is meant by the term semi-conservative replication.
- 2** State the role of the enzyme helicase.
- 3** Give two examples of the use of DNA profiles.