

Molecules (Interaction and interdependence)

revision notes

How molecules interact to drive life. Enzymes catalyse and control the network of metabolism, cell respiration transfers energy from carbon compounds to ATP, and photosynthesis transforms light energy into chemical energy in carbon compounds. HL adds enzyme inhibition, the detailed pathways of respiration and the light-dependent and light-independent reactions.

For IB Diploma Biology, Standard Level and Higher Level (first assessment 2025). **HL only** is additional higher level content, for HL students only.

1 Enzymes and metabolism **Spec C1.1**

Enzymes as catalysts and their role in metabolism **Spec C1.1.1, C1.1.2, C1.1.3**

An **enzyme** is a protein that acts as a **biological catalyst**: it increases the rate of a chemical reaction and is not changed or used up by the reaction, so one enzyme molecule can be used again and again.

Without enzymes, most reactions in cells would happen far too slowly at body temperature to keep the organism alive. Raising the temperature enough to speed them up would damage cell structures.

Enzymes give fast rates at the moderate temperatures found in cells, and the cell can switch reactions on or off by making, activating or inhibiting particular enzymes.

Metabolism

Metabolism is the complex network of interdependent and interacting chemical reactions occurring in living organisms. The product of one reaction is often the substrate of the next, so reactions are linked into pathways, and pathways share molecules with each other.

Enzymes are **specific**: each one catalyses one reaction or one type of reaction. Living organisms therefore need many different enzymes, often thousands. Because every step depends on its own enzyme, control over metabolism can be exerted through these enzymes, for example by changing how much of an enzyme is made or how active it is.

Anabolic and catabolic reactions

The two types of metabolic reaction		
	Anabolism	Catabolism
What happens	Larger molecules built from smaller ones	Larger molecules broken down into smaller ones
Typical reaction	Condensation: monomers joined, water released	Hydrolysis (water used to split bonds) or oxidation
Energy	Needs an energy input (usually from ATP)	Often releases energy
Examples	Protein synthesis (amino acids joined by peptide bonds); glycogen formation from glucose; photosynthesis (carbon compounds made from CO ₂)	Hydrolysis of macromolecules into monomers in digestion; oxidation of substrates such as glucose in respiration

Anabolism	Reactions that build macromolecules from monomers, for example by condensation.
Catabolism	Reactions that break down macromolecules into monomers, for example by hydrolysis, or oxidise substrates as in respiration.

Common mistake: Photosynthesis is anabolic and respiration is catabolic. Do not describe digestion as anabolic just because it happens before new molecules are built.

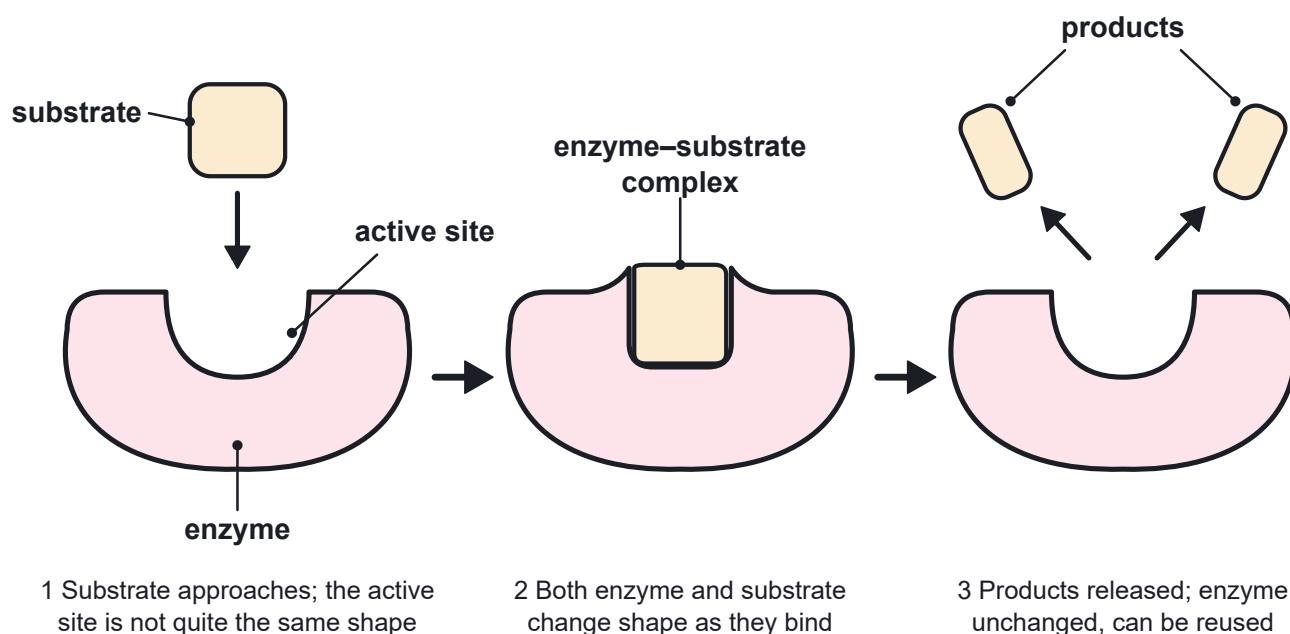
Active sites, induced fit, collisions and denaturation Spec C1.1.4, C1.1.5, C1.1.6, C1.1.7

Enzymes are **globular proteins**. The polypeptide is folded into a compact, roughly spherical shape that is usually soluble in water. Part of the surface forms the **active site**, the region where the substrate binds and the reaction is catalysed.

The active site is made of only a **few amino acids**. Their R groups give it a particular shape and chemical properties (charge, polarity, hydrophobic areas). But these few amino acids are held in exactly the right positions by interactions between amino acids throughout the rest of the protein (hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions). The whole three-dimensional structure is needed for the active site to work.

Induced-fit binding

The substrate binds to the active site by chemical attraction between them. In the **induced-fit** model, both the substrate and the enzyme change shape when binding occurs. The active site moulds more tightly around the substrate, which stresses bonds in the substrate and makes them easier to break or form. The products are then released and the active site returns to its original shape.



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Induced fit: **both the enzyme and the substrate change shape** as the substrate binds to the active site.

Molecular motion and collisions

For a reaction to be catalysed, a substrate molecule and an active site must **collide**, in a suitable orientation. Enzymes and substrates dissolved in water move randomly, and these collisions happen by chance. Sometimes only one of them moves: large substrate molecules (such as starch in a starch grain

or cellulose in a cell wall) may be immobilised while enzymes move to them, and some enzymes are immobilised by being embedded in membranes while their substrates move to them.

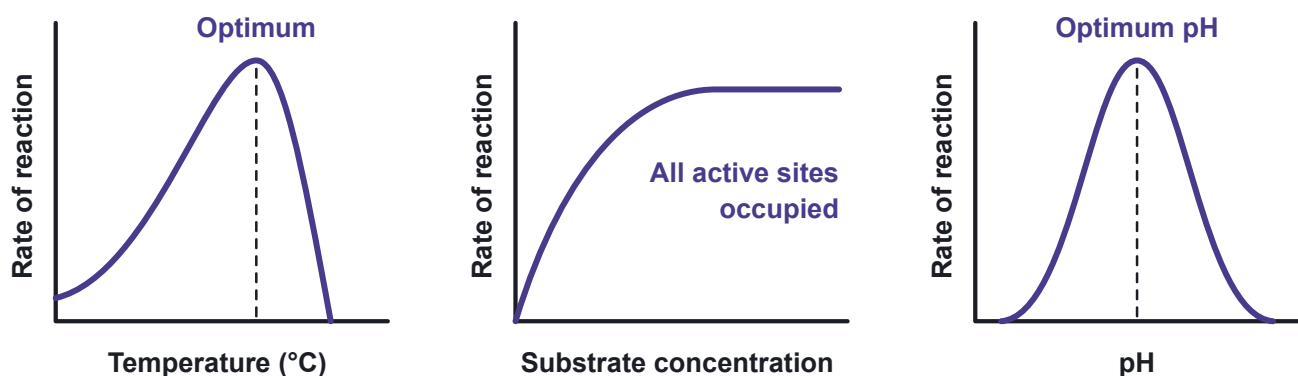
Specificity and denaturation

Enzyme-substrate **specificity** exists because only a substrate with a shape and chemical properties that match the active site can bind to it. Change the structure of the active site and binding fails.

Denaturation is a change to the three-dimensional structure of the protein, caused by high temperature or extreme pH. Bonds holding the tertiary structure break, so the active site changes shape and its chemical properties change. The substrate can no longer bind, so the enzyme cannot catalyse the reaction. Denaturation is usually permanent.

Exam tip: When explaining denaturation, link the chain: bonds in the tertiary structure break, the active site changes shape, the substrate no longer fits or binds, so no enzyme-substrate complexes form.

Effects of temperature, pH and substrate concentration Spec C1.1.8, C1.1.9



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Generalised sketch graphs for the effects of temperature, substrate concentration and pH on the rate of an enzyme-catalysed reaction.

Temperature

As temperature rises, enzyme and substrate molecules have more kinetic energy and move faster. Collisions between substrate and active site become more frequent and more of them have enough energy to react, so the rate increases. Above the **optimum temperature**, the extra vibration breaks bonds holding the enzyme's structure; more and more enzyme molecules denature, so the rate falls steeply. The curve is not symmetrical: the fall is much steeper than the rise.

pH

Each enzyme has an **optimum pH**. A change in pH alters the concentration of hydrogen ions, which changes the charges on R groups in the active site and elsewhere. Ionic and hydrogen bonds are disrupted, so the substrate binds less well and the rate falls on either side of the optimum. Far from the optimum the enzyme is denatured. For example, pepsin in the stomach works best in acid conditions, while many enzymes in the small intestine work best near neutral pH.

Substrate concentration

At low substrate concentrations, raising the concentration increases the frequency of substrate-active site collisions, so the rate rises steeply. As the concentration increases further, more active sites are occupied at any moment, so the rise slows. Eventually **all active sites are occupied** nearly all the time and the rate levels off at a maximum. Enzyme concentration is then the limiting factor.

Exam tip: Sketch graphs like these are models. They can be evaluated by comparing them with real results from enzyme experiments. When describing a graph, give the trend, the optimum or the plateau, and quote values with units.

Measurements in enzyme-catalysed reactions

The rate of an enzyme-catalysed reaction is measured as the amount of **product formed** or **substrate used** per unit time. Common methods include measuring the volume of oxygen released when catalase breaks down hydrogen peroxide (gas syringe or displacement of water), and timing how long starch takes to disappear when amylase acts on it (samples tested with iodine). Rates can also be found from secondary data.

- Mean rate = change in amount $\div$ time taken.
- Initial rate: draw a tangent to the curve at time zero and find its gradient. This is the fairest comparison because the substrate concentration has not yet fallen.
- If only a time is measured (for example, time for starch to disappear), rate can be expressed as $1 \div$ time.

Calculating rates from enzyme data

Catalase was added to hydrogen peroxide. 18 cm³ of oxygen was collected in the first 30 s and 30 cm³ by 90 s. Calculate the mean rate over the first 30 s and over the whole 90 s.

1. First 30 s: rate = $18 \text{ cm}^3 \div 30 \text{ s} = 0.60 \text{ cm}^3 \text{ s}^{-1}$.
2. Whole 90 s: rate = $30 \text{ cm}^3 \div 90 \text{ s} = 0.33 \text{ cm}^3 \text{ s}^{-1}$.
3. The rate falls over time because substrate is used up, so there are fewer collisions with active sites.

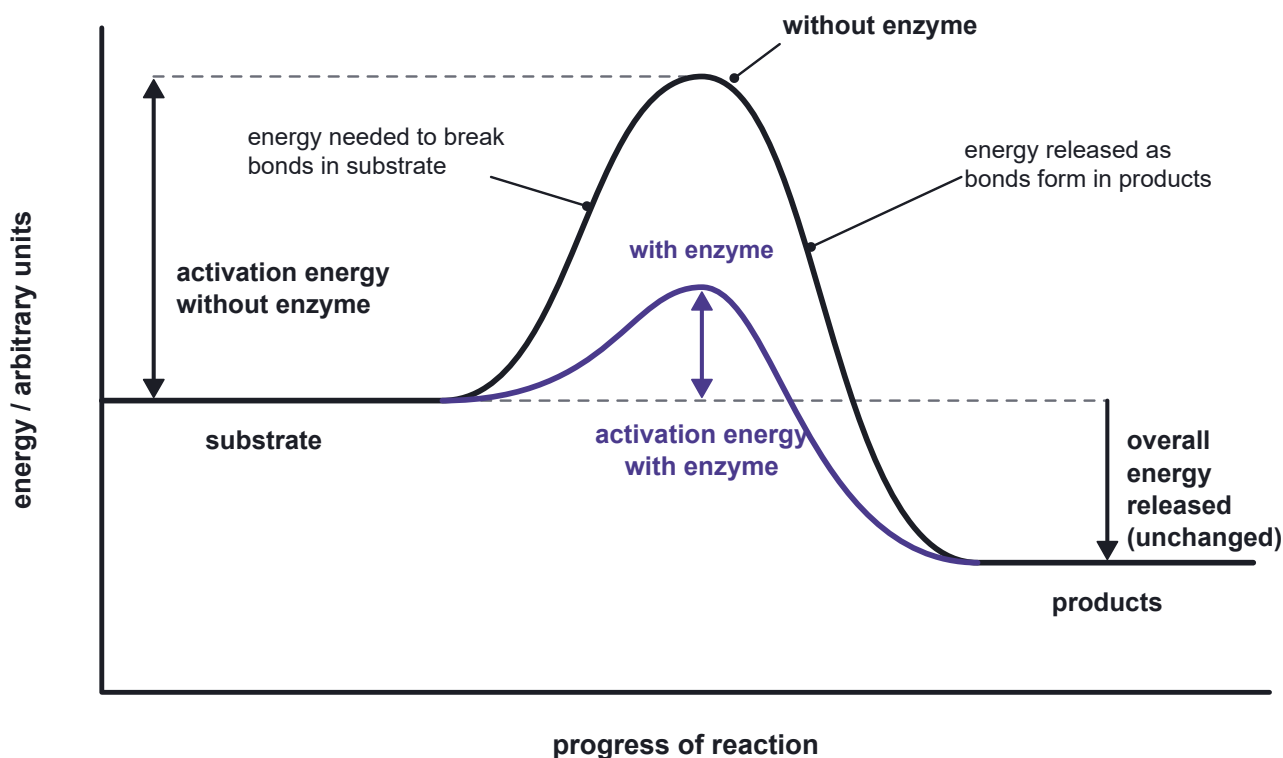
Answer: 0.60 cm³ s⁻¹ over the first 30 s; 0.33 cm³ s⁻¹ over 90 s.

Practical skill: Control temperature with a thermostatically controlled water bath, pH with buffer solutions, and keep enzyme concentration and volumes constant. Repeat each condition at least three times and calculate a mean.

Effect of enzymes on activation energy Spec C1.1.10

Before a reaction can happen, bonds within the substrate must be broken or weakened. This needs an input of energy called the **activation energy**. When new bonds form to make the products, energy is released (the energy yield). In many metabolic reactions the products have less energy than the substrates, so there is an overall release of energy.

Enzymes **lower the activation energy**. Binding to the active site stresses and weakens bonds in the substrate, so less energy is needed to reach the transition state. A larger proportion of substrate molecules then have enough energy to react, and the reaction is faster at the same temperature. The enzyme does **not** change the energy of the substrates or the products, so the overall energy change is unchanged.



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Enzymes **lower the activation energy**, measured from the substrate level to the peak; the overall energy change is unchanged.

Exam tip: On an energy graph, the activation energy is measured from the substrate level to the peak, not from the products or from zero.

Exam tip: Linking question: enzymes are a clear structure–function relationship in a macromolecule. The folded polypeptide creates an active site that lowers activation energy for one specific reaction.

Intracellular and extracellular reactions, heat and metabolic pathways

Spec C1.1.11, C1.1.12, C1.1.13

HL only

Intracellular and extracellular enzymes

Where enzyme-catalysed reactions happen		
	Intracellular	Extracellular
Where the enzyme acts	Inside the cell that made it	Outside the cell that made it, after secretion by exocytosis
Examples	Glycolysis (cytoplasm); Krebs cycle (mitochondrial matrix)	Chemical digestion in the gut lumen, e.g. amylase, proteases and lipase
Why	Products are used within the cell	Food macromolecules are too large to enter cells, so they must be hydrolysed first

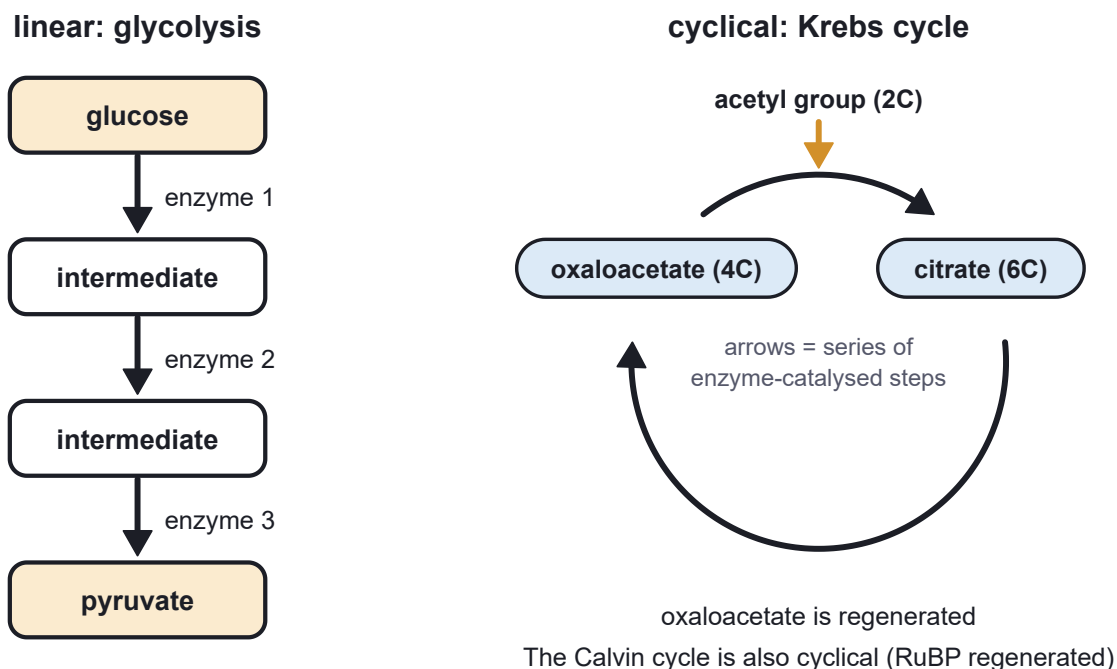
Heat from metabolism

No metabolic reaction is 100% efficient in energy transfer. Some energy is always transferred to the surroundings as **heat**, so heat generation is inevitable. Mammals and birds (and some other animals, such as some large fish and insects) depend on this heat to maintain a constant body temperature. They can increase heat output by increasing the rate of metabolic reactions, for example by shivering.

Linear and cyclical pathways

In a **linear** pathway, the substrate is converted through a chain of intermediates to an end product, each step catalysed by a different enzyme. **Glycolysis** is linear: glucose is converted step by step to pyruvate.

In a **cyclical** pathway, the last step regenerates the molecule that started the cycle, so intermediates are not used up. In the **Krebs cycle** an acetyl group joins oxaloacetate and oxaloacetate is regenerated. In the **Calvin cycle** CO_2 is fixed by RuBP and RuBP is regenerated.



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Linear pathways end in a product; in a **cyclical** pathway the starting molecule is **regenerated**.

Exam tip: To classify a pathway, ask whether the starting molecule is regenerated. If it is, the pathway is cyclical; if the substrate is used up to form an end product, it is linear.

Non-competitive and competitive inhibition Spec C1.1.14, C1.1.15 HL only

Allosteric sites and non-competitive inhibition

An **allosteric site** is a binding site on an enzyme other than the active site. Only specific substances can bind to it. When an inhibitor binds there, it causes interactions within the enzyme that lead to a **conformational change**: the shape of the protein changes, altering the active site enough to prevent catalysis. The substrate may still bind, but the reaction does not happen. Binding is reversible.

This is **non-competitive inhibition**: the inhibitor does not compete with the substrate for the active site. Adding more substrate does not remove the inhibitor, so the maximum rate is lowered at every substrate concentration.

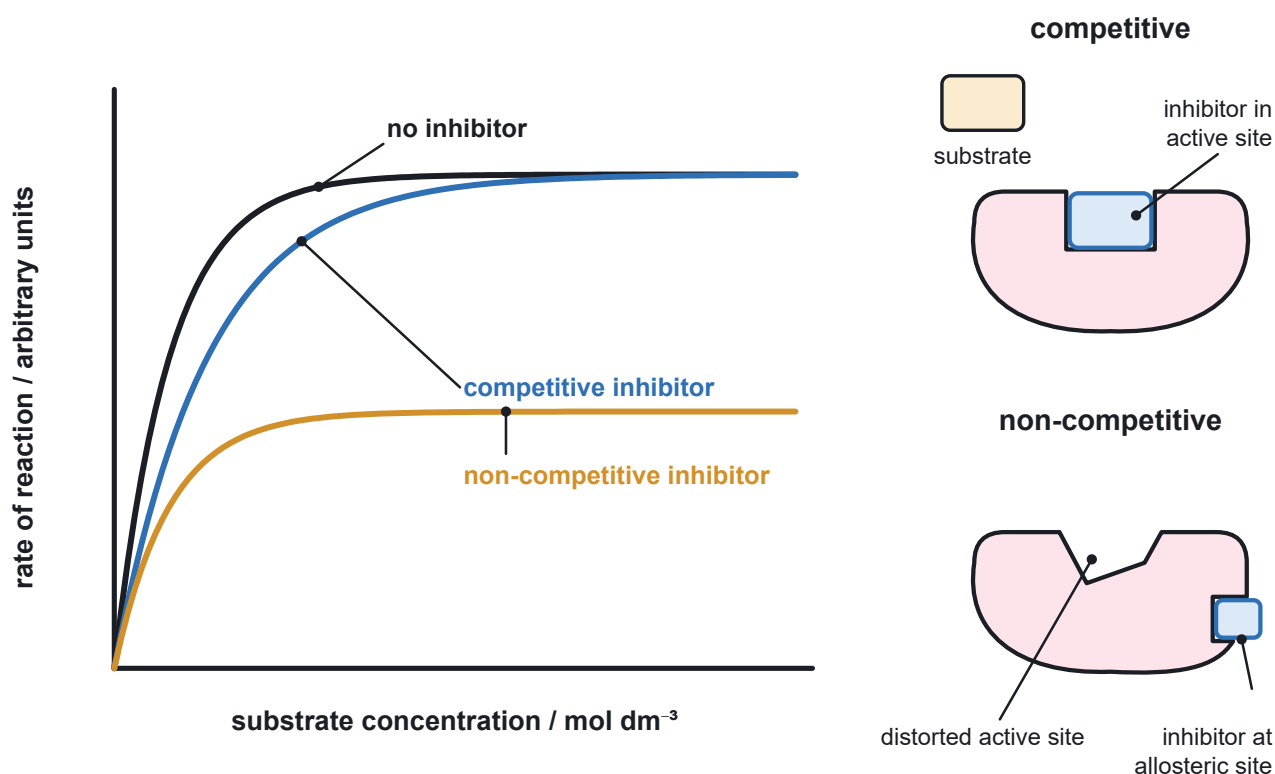
Competitive inhibition

A **competitive inhibitor** has a shape and chemistry similar to the substrate. It binds reversibly to the active site, blocking it, but is not converted into products. Substrate and inhibitor compete for the same site, so the proportion of active sites occupied by substrate depends on their relative concentrations. At high substrate concentrations the substrate wins most collisions, so the rate approaches the same maximum as without inhibitor.

Statins are competitive inhibitors used as drugs to lower blood cholesterol. They inhibit an enzyme in the liver (HMG-CoA reductase) that catalyses a step in the pathway that synthesises cholesterol.

Comparing the two types of reversible inhibition

	Competitive	Non-competitive
Where the inhibitor binds	Active site	Allosteric site
Shape of inhibitor	Similar to the substrate	Not similar to the substrate
Interaction with substrate	Inhibitor and substrate compete for the active site	No competition; both can bind at once
Effect of raising substrate concentration	Overcomes inhibition; maximum rate eventually reached	Does not overcome inhibition; maximum rate lowered
Example	Statins	End-product inhibitors acting at allosteric sites



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At high substrate concentration a **competitive** inhibitor is outcompeted; a **non-competitive** inhibitor lowers the maximum rate.

Exam tip: To tell the types apart from a graph, look at high substrate concentrations: if the inhibited curve reaches the uninhibited maximum, the inhibitor is competitive.

Feedback inhibition and mechanism-based inhibition Spec C1.1.16, C1.1.17 HL only

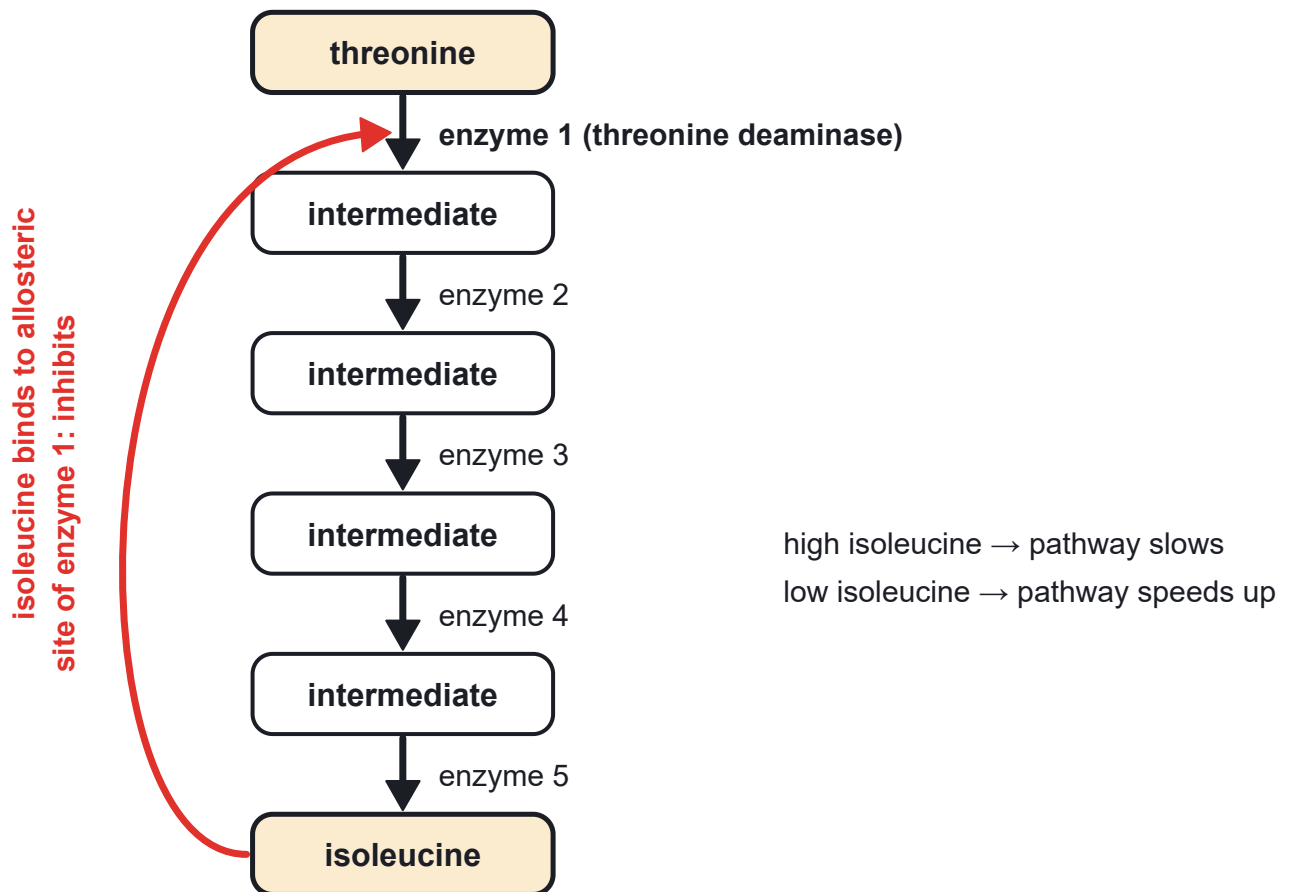
Feedback inhibition

In **feedback inhibition** the end product of a metabolic pathway inhibits an enzyme that catalyses an early step, usually the first. The end product binds to an allosteric site, so this is a form of non-competitive inhibition, and binding is reversible.

The pathway that produces the amino acid **isoleucine** from threonine has five steps, each catalysed by a different enzyme. Isoleucine binds to the allosteric site of the first enzyme, threonine deaminase (also called threonine dehydratase).

1. When isoleucine concentration is high, more isoleucine molecules bind to threonine deaminase.
2. The enzyme is inhibited, so the whole pathway slows and less isoleucine is made.
3. As isoleucine is used up (for example in protein synthesis), its concentration falls and it leaves the allosteric sites.
4. The enzyme becomes active again and production increases.

The concentration of the end product is therefore kept within a narrow range, and threonine and energy are not wasted making isoleucine that is not needed.



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Feedback inhibition: isoleucine binds to the **allosteric site of threonine deaminase**, so production slows when isoleucine is high.

Mechanism-based inhibition

In **mechanism-based inhibition** an inhibitor binds irreversibly to the active site and causes a chemical change to it, so the enzyme is permanently inactivated. Raising the substrate concentration has no effect.

Penicillin inhibits **transpeptidases**, bacterial enzymes that form the cross-links between peptidoglycan chains in the cell wall. Penicillin enters the active site and forms a permanent covalent bond with an amino acid there. Without cross-links, the walls of growing bacteria are weak and the cells burst due to osmotic pressure.

Some bacteria are **resistant to penicillin** because they have a changed transpeptidase. Its active site has a different structure, so penicillin binds poorly or not at all, but the enzyme can still make cross-links. The gene for the altered transpeptidase can spread through a population by natural selection when penicillin is used.

Common mistake: Mechanism-based inhibition is not competitive inhibition. Competitive inhibitors bind reversibly; penicillin binds irreversibly and changes the active site chemically.

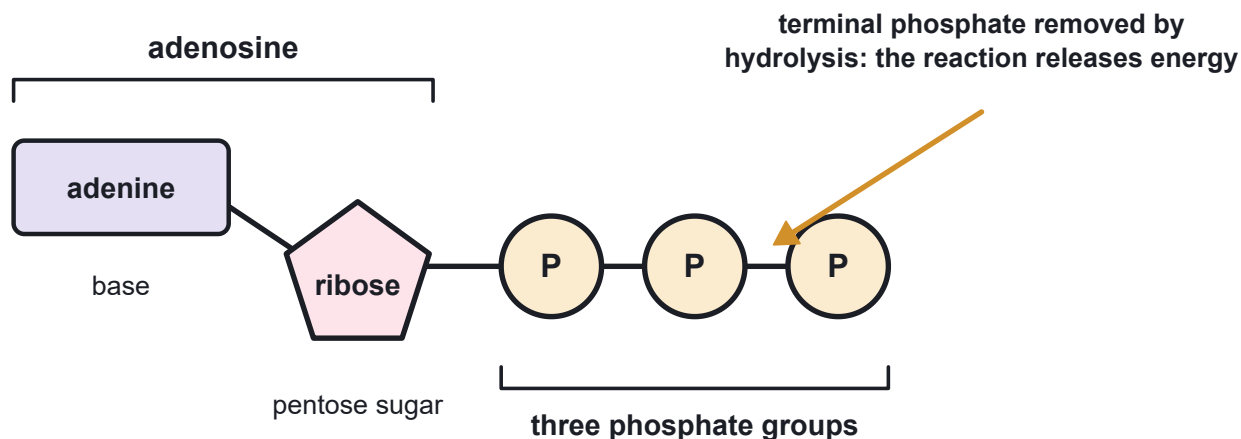
Quick check

1. What is metabolism?
2. Give one example of an anabolic and one of a catabolic reaction.
3. In the induced-fit model, what changes shape when the substrate binds?
4. What happens to the activation energy and the overall energy change when an enzyme catalyses a reaction?
5. **HL only** Which enzyme does isoleucine inhibit in feedback inhibition?

2 Cell respiration Spec C1.2

ATP: the molecule that distributes energy Spec C1.2.1, C1.2.2, C1.2.3

ATP (adenosine triphosphate) is the molecule that distributes energy within cells. It is a **nucleotide**, made of the base adenine, the pentose sugar ribose and a chain of three phosphate groups.



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ATP is a nucleotide: adenine, ribose and three phosphates. Hydrolysis of the **terminal phosphate** releases energy.

Why ATP suits its role as energy currency

- It is **soluble** in water, so it moves easily to wherever energy is needed in the cell.
- It is **stable** at the pH of a cell and does not break down unless an enzyme catalyses it, so energy is not wasted.
- It **cannot pass freely through membranes**, so it stays inside the cell where it was made.
- Hydrolysis of one phosphate group releases an amount of energy that is **sufficient for many tasks** in the cell, without releasing so much that most is wasted as heat.
- It is **rapidly regenerated** from ADP and phosphate, so a small pool of ATP can be recycled many times.

Life processes that ATP supplies with energy

- **Active transport** across membranes, for example pumping ions against their concentration gradient.
- **Synthesis of macromolecules** (anabolism), such as proteins, DNA and glycogen.
- **Movement**: of the whole cell (for example muscle contraction, cilia and flagella) or of cell components such as chromosomes during cell division.

Interconversion of ATP and ADP



Energy is released by **hydrolysis of ATP** to ADP (adenosine diphosphate) and phosphate. Energy is **required to synthesise ATP** from ADP and phosphate. In cells this energy comes from cell respiration (and in photosynthetic cells also from light). Often the phosphate removed from ATP is transferred to another molecule, making it more reactive.

Common mistake: ATP does not store large amounts of energy long term; it distributes energy. Fats and carbohydrates are the energy stores.

Cell respiration: aerobic and anaerobic Spec C1.2.4, C1.2.5

Cell respiration is a system for producing ATP within the cell using energy released from carbon compounds. Glucose and fatty acids are the principal substrates, but a wide range of carbon (organic) compounds can be used, including amino acids.

Do not confuse it with **gas exchange**. Gas exchange is the diffusion of oxygen and carbon dioxide between an organism and its environment, for example across the alveoli. Cell respiration is a set of enzyme-catalysed reactions inside every living cell. Gas exchange supplies the oxygen that aerobic respiration uses and removes the carbon dioxide it produces.

Word equations (glucose as substrate)

Aerobic: glucose + oxygen → carbon dioxide + water

Anaerobic (humans): glucose → lactate

Differences between anaerobic and aerobic cell respiration in humans

	Aerobic	Anaerobic
Respiratory substrates	Carbohydrates, lipids (fatty acids) and amino acids	Glucose (carbohydrate) only
Oxygen required	Yes	No
Relative ATP yield per glucose	Large (about 30 ATP; older textbooks give 36–38)	Small (2 ATP)
Waste products	Carbon dioxide and water	Lactate
Where in the cell	Starts in the cytoplasm, mostly in mitochondria	Cytoplasm only
Mitochondria needed	Yes	No

Humans use anaerobic respiration when oxygen cannot be supplied fast enough, for example in muscles during very intense exercise. It gives ATP quickly but in small amounts, and the lactate must be removed later.

Exam tip: In 'compare' questions, write paired statements: 'aerobic respiration needs oxygen whereas anaerobic does not'. Lactate is the IB term; do not write lactic acid unless the question does.

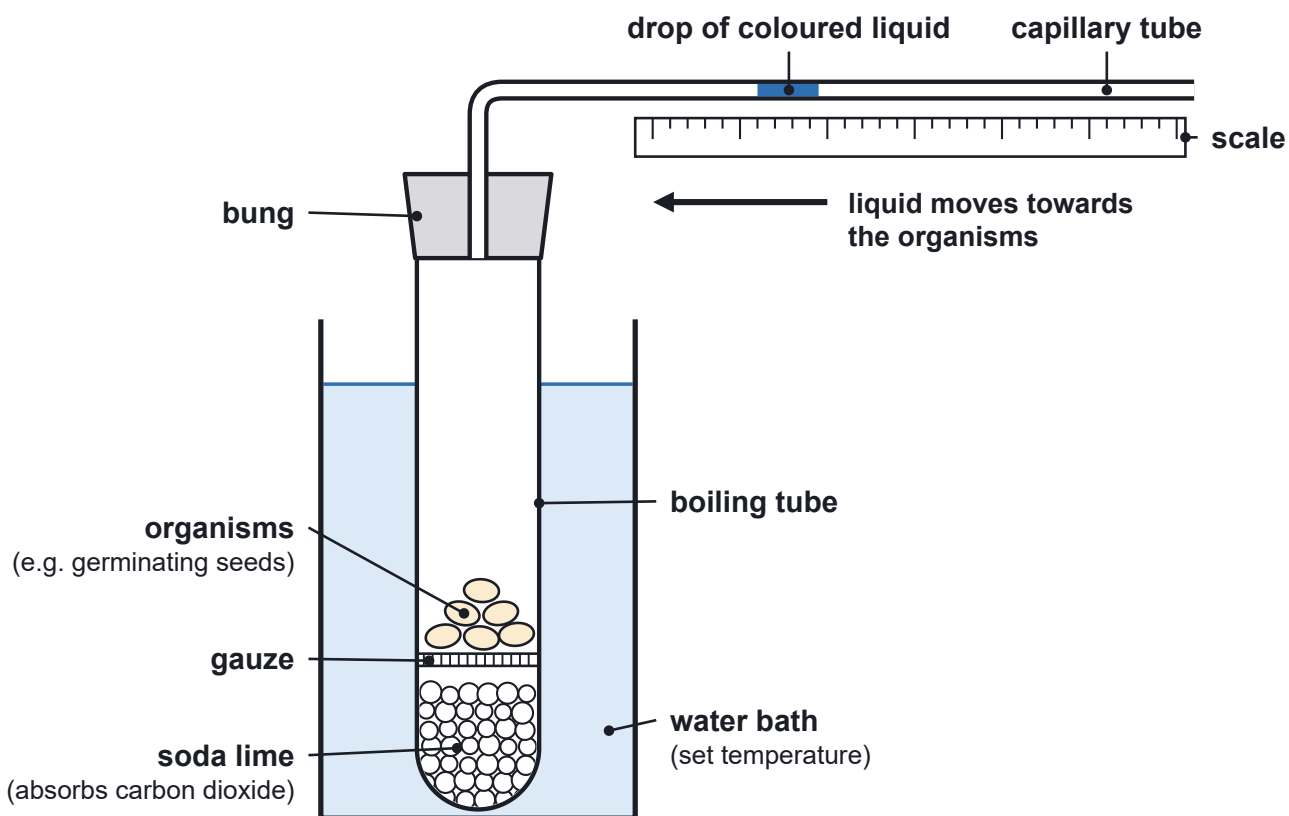
Variables affecting the rate of cell respiration Spec C1.2.6

The rate of cell respiration is the amount of oxygen used, carbon dioxide produced or substrate used **per unit time**. To compare organisms of different sizes, divide by mass, for example mm³ of oxygen per gram per minute.

Variables that affect the rate

- **Temperature:** respiration is enzyme-catalysed, so rate rises to an optimum, then falls as enzymes denature.
- **Oxygen concentration:** low oxygen limits aerobic respiration.
- **Substrate availability:** for example glucose concentration for yeast, or stored food in germinating seeds.

- Others: type and activity of the organism, pH, and stage of development (germinating seeds respire faster than dry seeds).



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A simple respirometer. Soda lime absorbs carbon dioxide, so the **drop moves towards the organisms** as they use oxygen.

Measuring the rate

1. Place a known mass of organisms (for example germinating seeds or invertebrates) in a sealed tube with an alkali such as soda lime to absorb carbon dioxide.
2. Keep the tube in a water bath at a set temperature and let it equilibrate.
3. Record the distance the liquid drop moves in a fixed time.
4. Set up a control tube with glass beads of the same volume instead of organisms, to correct for temperature and pressure changes.
5. Repeat at different values of the independent variable (for example temperature) and calculate rates.

Rates can also be measured with oxygen or carbon dioxide sensors and a data logger, or for yeast by measuring the volume of carbon dioxide released.

Rate of respiration from a respirometer

2.5 g of germinating seeds were placed in a respirometer. The drop in the capillary tube (radius 0.50 mm) moved 30 mm in 10 minutes. Calculate the rate of oxygen uptake per gram.

1. Volume of oxygen = $\pi r^2 h = 3.14 \times 0.50^2 \times 30 = 23.6 \text{ mm}^3$.
2. Rate = $23.6 \text{ mm}^3 \div 10 \text{ min} = 2.36 \text{ mm}^3 \text{ min}^{-1}$.
3. Per gram: $2.36 \div 2.5 = 0.94 \text{ mm}^3 \text{ g}^{-1} \text{ min}^{-1}$.

Answer: $0.94 \text{ mm}^3 \text{ g}^{-1} \text{ min}^{-1}$ (to 2 significant figures).

Maths skill: *Rate = amount ÷ time. Always state units, and divide by mass when organisms or samples differ in size.*

Practical skill: Use living organisms ethically: handle invertebrates gently, use gauze to keep them away from the corrosive soda lime, and return them to their habitat afterwards.

NAD, oxidation and glycolysis Spec C1.2.7, C1.2.8 HL only

Oxidation, reduction and NAD

Oxidation is a process of electron loss; **reduction** is electron gain. When hydrogen (with its electron) is removed from a substrate, a reaction called **dehydrogenation**, the substrate has been oxidised. Redox reactions involve both: whenever one molecule is oxidised, another is reduced.

NAD (nicotinamide adenine dinucleotide) is the main carrier of hydrogen in cell respiration. It accepts hydrogen removed from substrates and is reduced, forming reduced NAD. Reduced NAD carries the hydrogen (and its energy) to the electron transport chain.



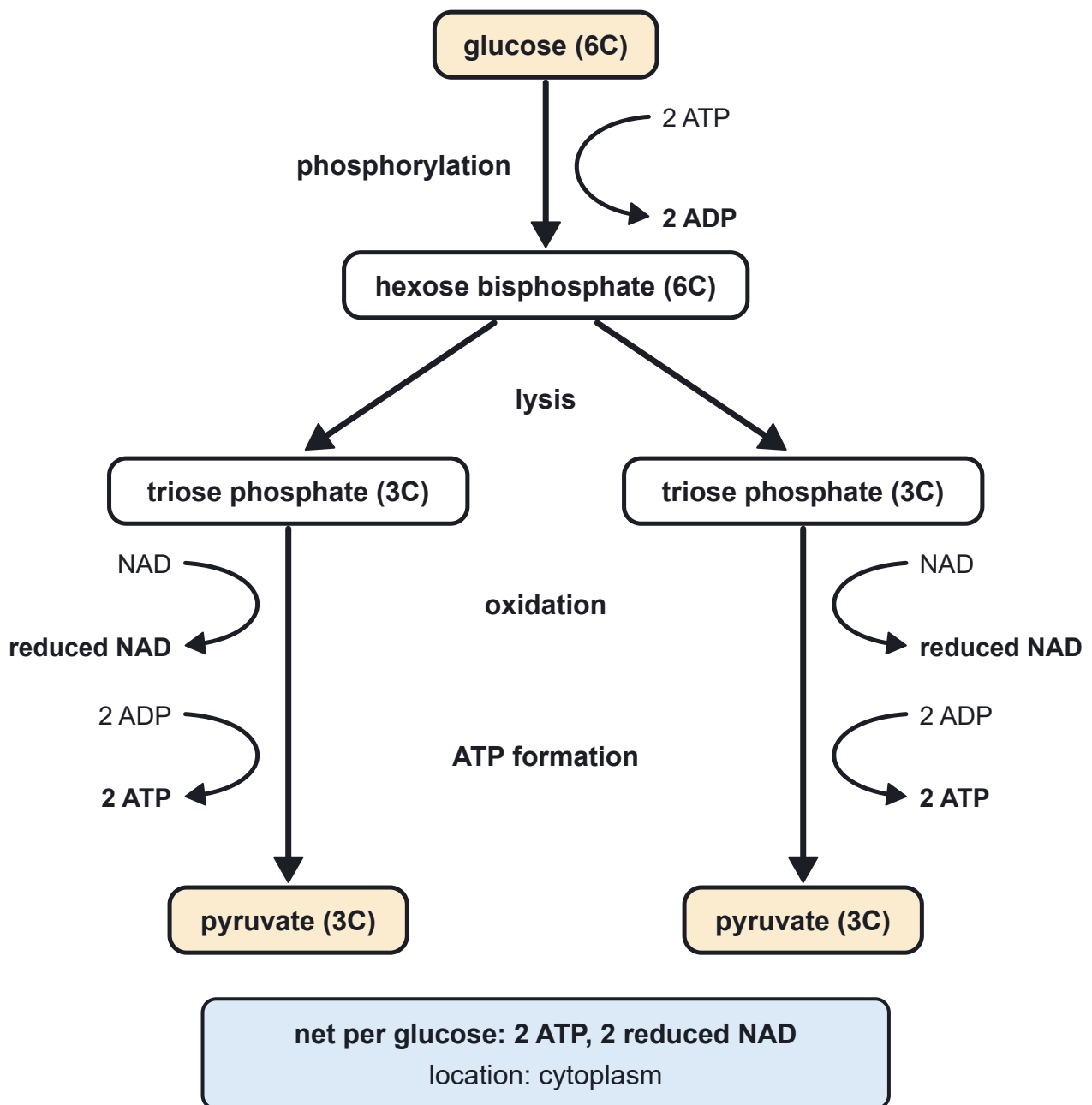
Glycolysis

Glycolysis is the conversion of glucose (6C) to two molecules of pyruvate (3C). It takes place in the cytoplasm, does not need oxygen and is a linear pathway in which each step is catalysed by a different enzyme. It has four key stages:

1. **Phosphorylation:** two phosphate groups are added to glucose, using 2 ATP. This makes the sugar less stable and ready to split.
2. **Lysis:** the 6C sugar bisphosphate splits into two 3C triose phosphate molecules.
3. **Oxidation:** hydrogen is removed from each triose phosphate and accepted by NAD, giving 2 reduced NAD per glucose.
4. **ATP formation:** phosphate groups are transferred to ADP, forming 4 ATP per glucose, and two pyruvate are produced.

Yield of glycolysis per molecule of glucose

Product	Number
ATP used	2
ATP produced	4
Net ATP	2
Reduced NAD	2
Pyruvate	2



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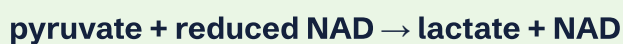
Glycolysis: phosphorylation, lysis, oxidation and ATP formation give a net yield of **2 ATP and 2 reduced NAD** per glucose.

Exam tip: You do not need the names of the intermediates, but you must say that each step is catalysed by a different enzyme and give the net yield: 2 ATP and 2 reduced NAD.

Anaerobic respiration: lactate and yeast Spec C1.2.9, C1.2.10 HL only

Glycolysis needs a supply of NAD to accept hydrogen. Without oxygen, reduced NAD cannot be oxidised by the electron transport chain, so the cell's NAD would soon all be reduced and glycolysis would stop. Anaerobic respiration solves this by using pyruvate as the hydrogen acceptor.

Humans: pyruvate to lactate



Regeneration of NAD allows glycolysis to continue, with a **net yield of two ATP** molecules per molecule of glucose. No carbon dioxide is produced. The reaction happens in the cytoplasm.

Yeast

The pathways of anaerobic respiration are the **same in humans and yeasts** apart from the regeneration of NAD using pyruvate, and therefore the final products. In yeast, pyruvate is decarboxylated (CO₂ removed) to ethanal, which is then reduced by reduced NAD to **ethanol**, regenerating NAD.

Yeast: glucose → ethanol + carbon dioxide

Uses of anaerobic respiration in yeast

Process	Useful product	What happens to the other product
Brewing (beer, wine)	Ethanol	CO ₂ escapes, or is kept to make the drink fizzy
Baking (bread)	Carbon dioxide, which makes the dough rise	Ethanol evaporates during baking

Common mistake: Lactate formation does not produce extra ATP. Its purpose is to regenerate NAD so glycolysis can keep making its 2 ATP.

The link reaction and the Krebs cycle Spec C1.2.11, C1.2.12 HL only

The link reaction

In aerobic respiration, pyruvate from glycolysis is transported into the **mitochondrial matrix**. There it undergoes **oxidation** (hydrogen removed, NAD reduced) and **decarboxylation** (carbon removed as CO₂). The remaining 2C acetyl group is attached to coenzyme A, forming **acetyl CoA**, which transfers the acetyl group to the Krebs cycle.

pyruvate (3C) + NAD + CoA → acetyl CoA (2C) + CO₂ + reduced NAD

Lipids and carbohydrates are both metabolised to form acetyl groups (2C), so this is where the pathways for different respiratory substrates join. Per glucose the link reaction happens twice.

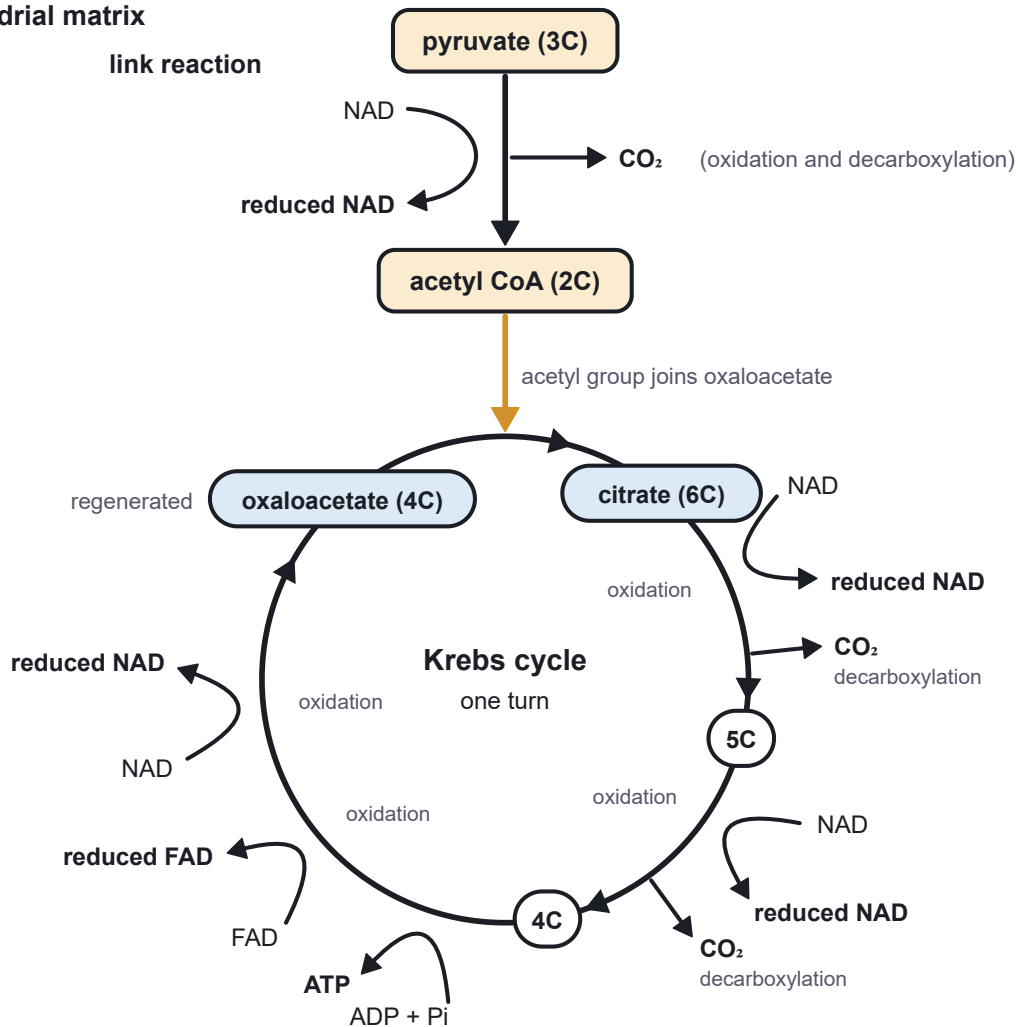
The Krebs cycle

1. The acetyl group (2C) is transferred from coenzyme A to **oxaloacetate (4C)**, forming **citrate (6C)**. Coenzyme A is released to collect another acetyl group.
2. Citrate is converted back to oxaloacetate by a series of reactions that include **two decarboxylations**, releasing 2 CO₂.
3. The cycle includes **four oxidations**, which are dehydrogenation reactions. Three of them reduce NAD; the fourth reduces another hydrogen carrier, FAD. Reduced FAD also passes its electrons to the electron transport chain, but IB questions focus on NAD.
4. One ATP is produced directly in each turn.
5. Oxaloacetate is regenerated, so the cycle can accept another acetyl group.

Products per turn of the Krebs cycle (two turns per glucose)

Product	Per turn	Per glucose
CO ₂	2	4
Reduced NAD	3	6
Reduced FAD	1	2
ATP	1	2

mitochondrial matrix



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Each turn of the Krebs cycle: **2 CO₂, 3 reduced NAD, 1 reduced FAD and 1 ATP**; oxaloacetate is regenerated.

Exam tip: Name only citrate and oxaloacetate. Remember the counts: two decarboxylations and four oxidations per turn, and two turns per glucose.

Electron transport chain, chemiosmosis and oxygen

Spec C1.2.13, C1.2.14, C1.2.15, C1.2.16

HL only

Reduced NAD delivers energy to the chain

Reduced NAD comes from **glycolysis**, the **link reaction** and the **Krebs cycle**. Energy is transferred when reduced NAD passes a pair of electrons to the first carrier in the **electron transport chain**, a series of carriers embedded in the inner mitochondrial membrane (on the cristae). This converts reduced NAD back to NAD, which can return to accept more hydrogen.

A proton gradient

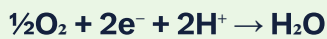
The electrons pass from carrier to carrier. At each step they lose energy, and some carriers use this energy to **pump protons** (H^+) from the matrix across the inner membrane into the **intermembrane space**. Because the inner membrane is impermeable to protons, a **proton gradient** builds up: a higher concentration of protons in the intermembrane space than in the matrix.

Chemiosmosis and ATP synthase

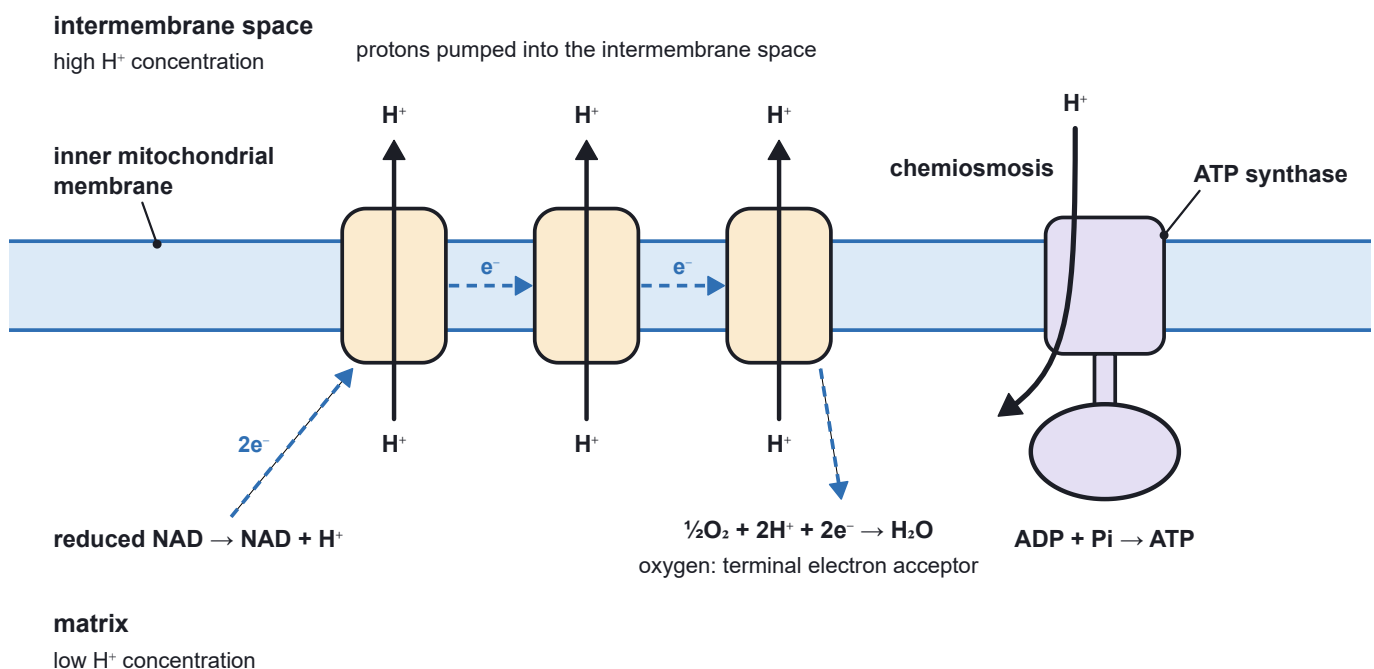
Chemiosmosis is the movement of protons down their concentration gradient through ATP synthase. Protons can only diffuse back into the matrix through channels in **ATP synthase**. As they flow through, part of the enzyme rotates, and ATP synthase couples the release of energy from the proton gradient with the **phosphorylation of ADP**, forming ATP. Most of the ATP from aerobic respiration is made this way.

Oxygen as terminal electron acceptor

At the end of the chain, **oxygen** accepts electrons from the last carrier and protons from the matrix, producing **metabolic water**.



Removing electrons at the end allows continued flow of electrons along the chain. Without oxygen the carriers stay reduced, reduced NAD cannot be oxidised, the proton gradient is not maintained and no NAD is available for the link reaction and Krebs cycle, so aerobic respiration stops.



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Electron flow pumps protons into the intermembrane space; they return through **ATP synthase** (chemiosmosis). Oxygen is the terminal electron acceptor.

Exam tip: Give the direction precisely: protons are pumped into the intermembrane space and flow back into the matrix through ATP synthase. You do not need the names of the protein complexes.

Lipids and carbohydrates as respiratory substrates Spec C1.2.17 HL only

Lipids compared with carbohydrates as respiratory substrates		
	Carbohydrate (e.g. glucose)	Lipid (fatty acids)
Energy yield per gram	Lower	Higher, roughly twice as much
Why	More oxygen in the molecule; less oxidisable hydrogen and carbon	Less oxygen; more oxidisable hydrogen and carbon
Entry into respiration	Glycolysis → pyruvate → link reaction	Broken into 2C acetyl groups, entering as acetyl CoA
Anaerobic respiration possible	Yes	No

Energy in respiration comes from oxidising carbon and hydrogen in the substrate. A fatty acid is mostly long hydrocarbon chain with very little oxygen, so more of its atoms can be oxidised than in glucose, where many carbons already carry oxygen. Each gram of lipid therefore yields more reduced NAD and more ATP.

Glycolysis and anaerobic respiration occur only if carbohydrate is the substrate. Fatty acids are broken down into 2C acetyl groups, which enter the pathway via acetyl CoA and are then oxidised in the Krebs cycle. As this needs oxygen to regenerate NAD through the electron transport chain, fatty acids cannot be used in anaerobic respiration. (Beyond the syllabus: the small glycerol part of a triglyceride can be converted to triose phosphate and enter glycolysis, but fatty acids supply nearly all the energy in a lipid.)

Exam tip: Linking question: in what forms is energy stored in living organisms? Fat stores more energy per gram than glycogen, but only glycogen can supply glucose for anaerobic respiration.

Quick check

1. What is the full name of ATP and what type of molecule is it?
2. Write the word equation for anaerobic respiration in humans.
3. Why is soda lime used in a respirometer?
4. HL only Name the two Krebs cycle intermediates you need to know and their number of carbons.
5. HL only What is the role of oxygen in aerobic respiration?

3 Photosynthesis Spec C1.3

Photosynthesis: energy, carbon dioxide, water and oxygen Spec C1.3.1, C1.3.2, C1.3.3

Photosynthesis involves the **transformation of light energy to chemical energy**. Light energy absorbed by pigments is used to make carbon compounds, and the energy is then held in the chemical bonds of those compounds. This energy transformation supplies most of the chemical energy needed for life processes in ecosystems: producers use it directly, and consumers and decomposers obtain it by feeding.

Carbon dioxide, water and glucose

Carbon dioxide is converted to glucose using **hydrogen obtained by splitting water**. Carbon dioxide provides the carbon and oxygen atoms of glucose, but glucose also contains hydrogen, which must come from somewhere: it comes from water molecules, split using energy from light.



Oxygen as a by-product

When water is split, the hydrogen is used and the oxygen is released as a **by-product**. So **the oxygen produced by photosynthesis comes from the splitting of water**, not from carbon dioxide. Oxygen-producing photosynthesis happens in **plants, algae and cyanobacteria**.

Common mistake: Do not write that the oxygen released comes from carbon dioxide. It comes from water; the oxygen atoms of CO₂ end up in glucose and water.

Exam tip: Linking question: the consequences of photosynthesis for ecosystems include supplying chemical energy to food chains and removing carbon dioxide from the atmosphere.

Separating photosynthetic pigments by chromatography Spec C1.3.4

Leaves contain several photosynthetic pigments. They can be separated by **chromatography**, using **paper** or a **thin-layer chromatography (TLC)** plate. Each pigment travels a different distance because it differs in how soluble it is in the solvent and how strongly it is attracted to the stationary phase.

1. Grind leaf tissue with a little sand and propanone (or another suitable solvent) to extract the pigments.
2. Draw a pencil origin line about 1 cm from the bottom of the paper or plate.
3. Apply a small, concentrated spot of extract to the origin, letting it dry between applications.
4. Stand the paper or plate in a small depth of running solvent, below the origin, in a covered container.
5. Remove it when the solvent is near the top and immediately mark the solvent front with pencil.
6. Mark the centre of each pigment spot, measure distances from the origin and calculate R_f values.

$$R_f = \text{distance moved by pigment} \div \text{distance moved by solvent front}$$

R_f values are always between 0 and 1 and have no units. Pigments are identified by their **colour** and by comparing their **R_f values** with reference values for the same solvent and stationary phase.

Common photosynthetic pigments and their colours

Pigment	Colour on the chromatogram
Carotene	Orange-yellow (usually travels furthest)
Xanthophylls	Yellow
Chlorophyll a	Blue-green
Chlorophyll b	Yellow-green

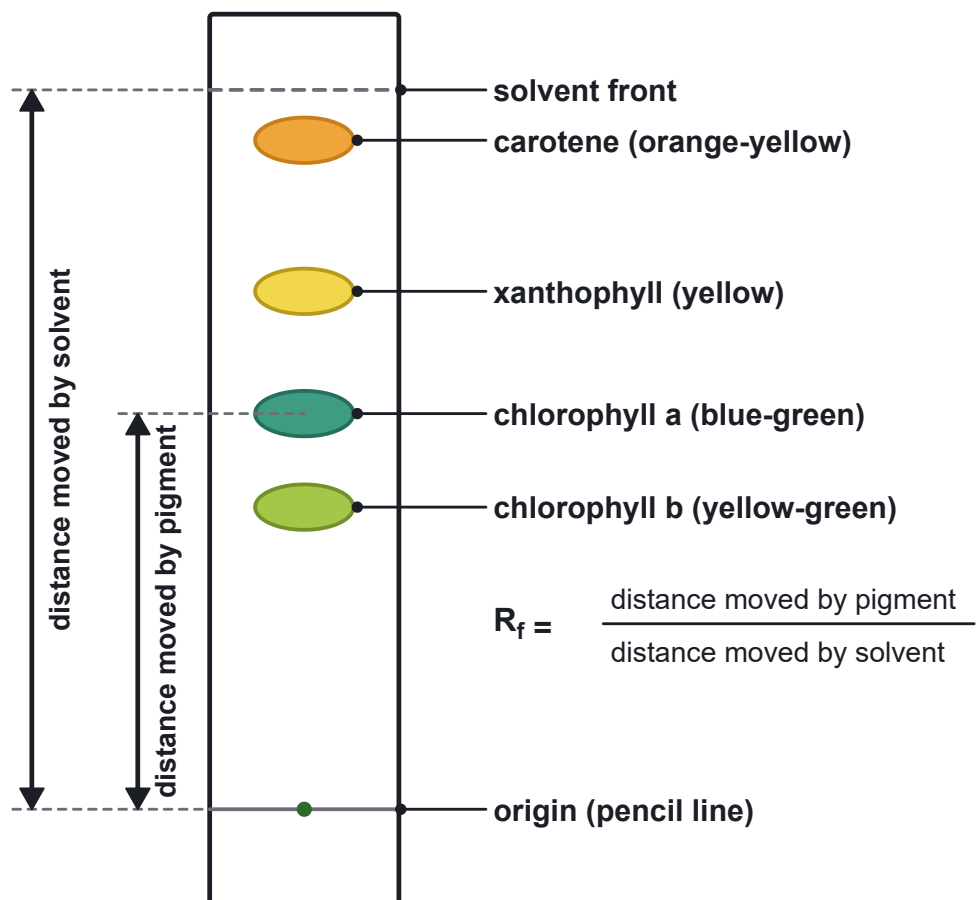
Calculating an R_f value

On a chromatogram the solvent front moved 8.0 cm from the origin. A blue-green spot moved 4.4 cm. Calculate its R_f value.

1. $R_f = \text{distance moved by pigment} \div \text{distance moved by solvent front}.$
2. $R_f = 4.4 \div 8.0 = 0.55.$

Answer: $R_f = 0.55$ (no units). With its blue-green colour this is likely chlorophyll a; confirm with reference values for the same method.

Practical skill: Use a pencil for the origin (ink would separate too), keep the origin above the solvent, and measure to the centre of each spot. R_f values depend on the solvent and medium, so only compare with references from the same method.



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A typical chromatogram of leaf pigments (illustrative positions; order and R_f values depend on the solvent and medium). $R_f = \text{pigment distance} \div \text{solvent distance}$.

Absorption of light, absorption spectra and action spectra Spec C1.3.5, C1.3.6

Absorption of specific wavelengths

When a pigment molecule absorbs light, the energy **excites an electron** within the molecule, raising it to a higher energy level. This is the first step in transforming light energy to chemical energy, because the excited electron can be passed on to other molecules.

Only some wavelengths are absorbed. Electrons in a pigment can only occupy certain energy levels, so only photons whose energy matches the gap between levels can be absorbed. Other wavelengths are reflected or transmitted. **Chlorophyll** absorbs red and blue light most strongly and reflects green light, which is why leaves look green. Accessory pigments such as carotenoids absorb other wavelengths, mainly in the blue-green region.

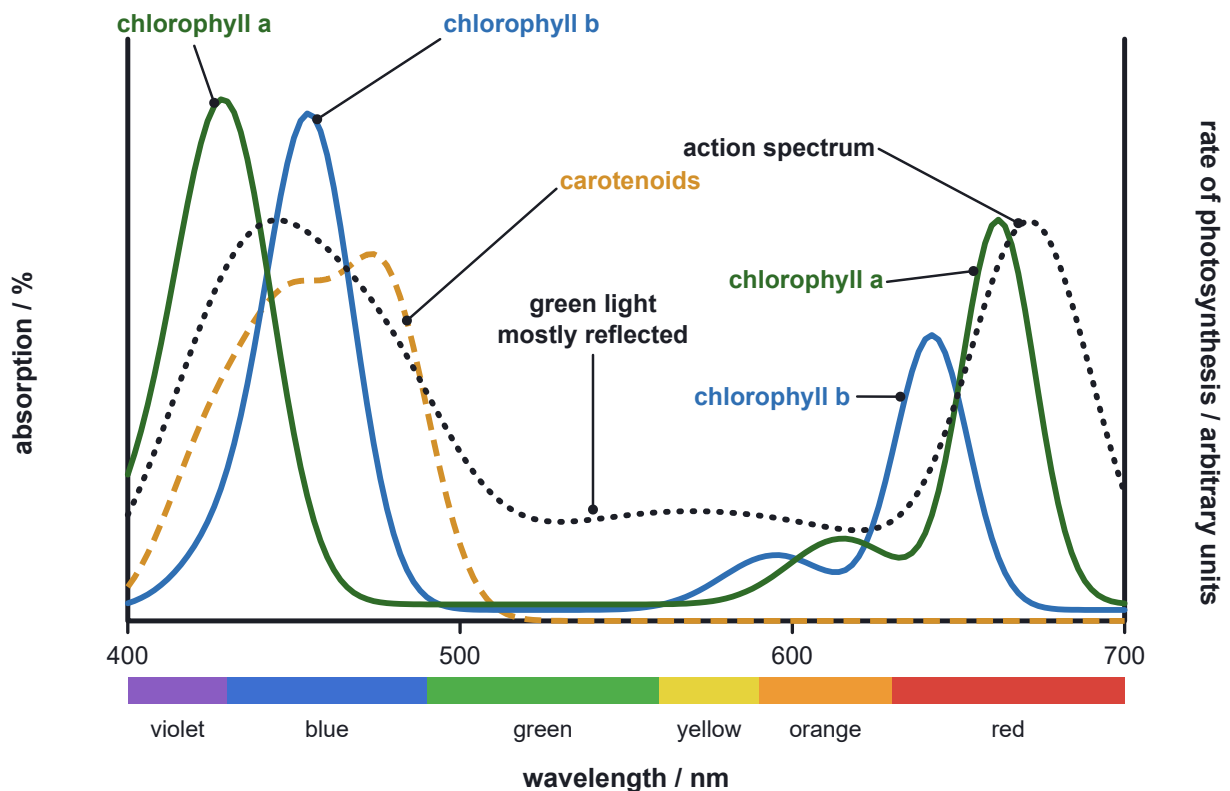
Absorption spectra and action spectra

Absorption spectrum	A graph showing the percentage of light absorbed by a pigment (or pigments) at each wavelength.
Action spectrum	A graph showing the rate of photosynthesis at each wavelength of light.

Both graphs have **wavelength** (in nm, from about 400 to 700 nm) on the horizontal axis, which should also show the **colours** of light: violet and blue at the short-wavelength end, then green, yellow, orange and red.

Similarities and differences of absorption and action spectra		
	Absorption spectrum	Action spectrum
Vertical axis	% of light absorbed	Rate of photosynthesis
Peaks	Blue and red for chlorophyll	Also blue and red
Green region	Low absorption by chlorophyll	Rate low but not zero, because accessory pigments absorb some light and some green light is absorbed as it passes through the leaf
How obtained	Shine each wavelength through a pigment extract and measure absorbance	Measure rate of oxygen production or carbon dioxide consumption under each wavelength

The **similar shape** of the two graphs is evidence that the light absorbed by these pigments is the light used in photosynthesis.



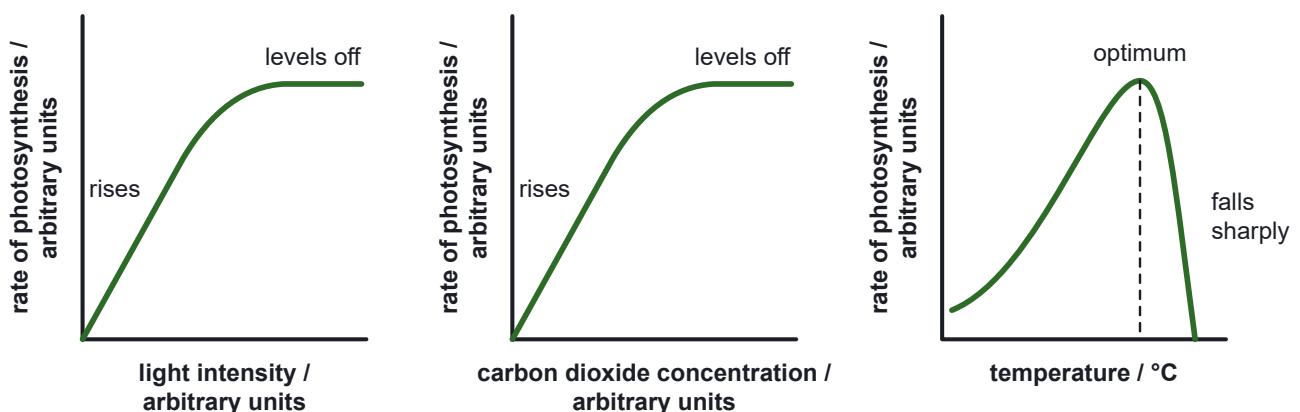
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Illustrative curves: the action spectrum **matches the absorption spectra**, with peaks in blue and red light; green light is mostly reflected.

Maths skill: To make an action spectrum, calculate the rate at each wavelength (for example volume of oxygen $\div$ time, or CO_2 uptake $\div$ time), then plot rate against wavelength with points joined by a smooth curve or straight lines.

Investigating limiting factors on photosynthesis Spec C1.3.7

The rate of photosynthesis depends on several factors. At any moment, the **limiting factor** is the one that is furthest from its optimum: increasing it raises the rate, while increasing the others does not.



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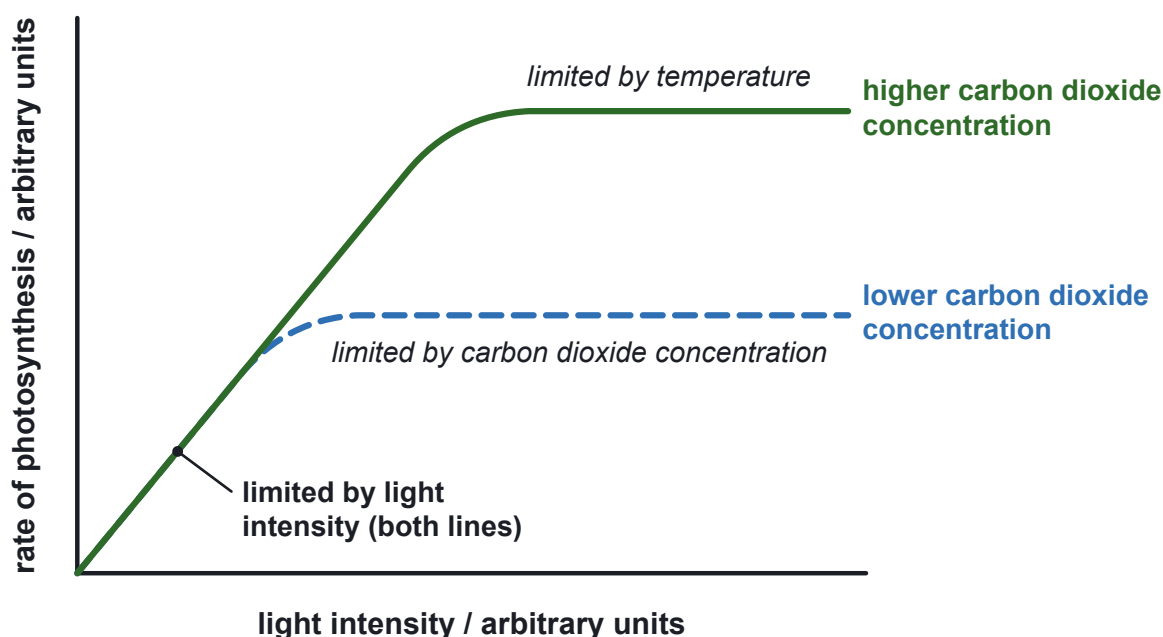
Light intensity and carbon dioxide concentration: the rate **rises, then levels off** when another factor limits. Temperature: the rate rises to an optimum, then falls as enzymes denature.

Techniques for varying each factor

How to vary each limiting factor		
Independent variable	How to vary it	Notes
Light intensity	Change the distance of a lamp from the plant, or use filters or a dimmable LED	Light intensity falls with distance (roughly proportional to $1 \div \text{distance}^2$); use an LED or a heat shield so temperature does not change
Carbon dioxide concentration	Use different concentrations of sodium hydrogencarbonate solution around aquatic plants or leaf discs	Hydrogencarbonate releases CO_2 in water
Temperature	Place the plant in water baths at different temperatures	Allow time to equilibrate; check with a thermometer

Measuring the rate

- Count bubbles or collect the volume of oxygen released by an aquatic plant such as *Elodea* or *Cabomba* per minute.
- Time how long leaf discs (with air removed so they sink) take to float as oxygen builds up.
- Measure carbon dioxide uptake with a sensor and data logger, or by the colour change of a hydrogencarbonate indicator.



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Where each line levels off, light is no longer limiting: the **factor in shortest supply** sets the plateau.

Practical skill: Identify the independent variable (the factor you change) and the dependent variable (the rate, for example oxygen per minute). Keep the other two factors constant and repeat each condition.

Exam tip: A hypothesis is a provisional explanation that needs repeated testing. You can suggest one from theory before the experiment (for example, 'rate rises with light intensity because more light energy is absorbed by chlorophyll') or from results after it.

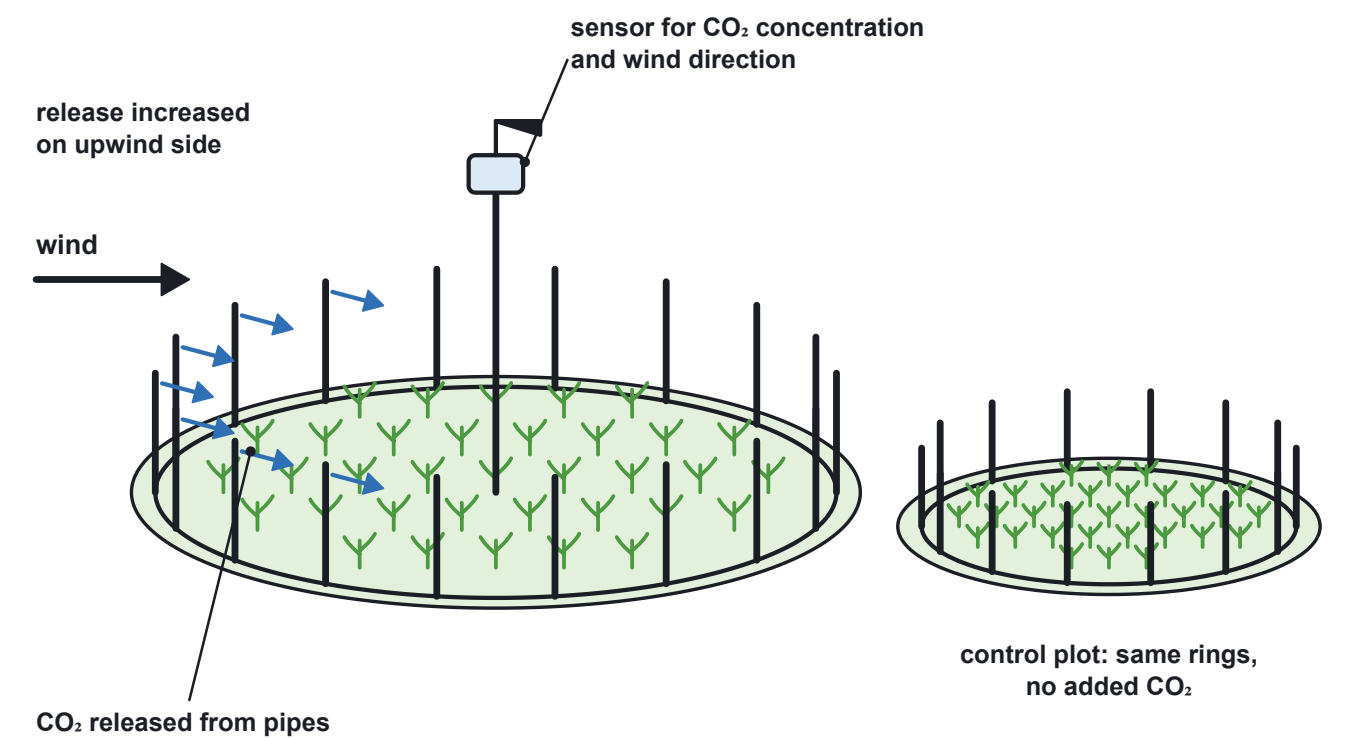
Carbon dioxide enrichment experiments Spec C1.3.8

Atmospheric carbon dioxide concentration is rising. Carbon dioxide enrichment experiments grow plants at concentrations predicted for the future to find out how rates of photosynthesis and plant growth may change.

Two types of CO ₂ enrichment experiment		
	Enclosed greenhouse experiments	Free-air CO ₂ enrichment (FACE)
Set-up	Plants grown in closed glasshouses or chambers with CO ₂ added to the air	Rings of pipes release CO ₂ around plots in a field or natural ecosystem; sensors adjust the release to hold a target concentration
Control of variables	Easier: temperature, light, water and minerals can be controlled	Harder: weather, soil and pests vary, but affect treated and control plots similarly
How realistic	Less natural: enclosure changes temperature, humidity and wind	More realistic: plants grow in field conditions with other species
Control	Identical greenhouse at current CO ₂	Plots with the same ring structure but no added CO ₂

Higher carbon dioxide generally increases the rate of photosynthesis and growth, at least for a time. However, several FACE studies of crops found smaller increases in growth and yield than enclosed experiments had predicted (the size of the gap is still debated). In the field, other factors such as water, temperature or soil nitrogen can become limiting, and the effect of extra CO₂ can shrink over the years.

Practical skill: Be able to identify a controlled variable, such as plant species, light intensity in a greenhouse, or plot size in a FACE experiment. Field experiments are less controlled than laboratory ones, but some questions can only be answered in natural ecosystems.



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A FACE experiment: pipes release CO₂ around a field plot to hold a target concentration; control plots have the same rings but no added CO₂.

Photosystems and the photolysis of water Spec C1.3.9, C1.3.10, C1.3.11 HL only

Photosystems

A **photosystem** is a molecular array of chlorophyll and accessory pigments, held in position by proteins, with a **special chlorophyll** at the **reaction centre**. Photosystems are always located in **membranes**: in the thylakoids of chloroplasts in photosynthetic eukaryotes, and in membranes inside cyanobacteria.

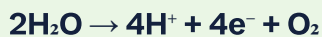
Pigment molecules around the reaction centre absorb light and pass the energy from molecule to molecule until it reaches the reaction centre chlorophyll. There an electron is excited so strongly that it is **emitted** and passed to an electron acceptor. There are two types: photosystem II and photosystem I.

Advantages of a structured array

- Different pigments absorb different wavelengths, so the array captures energy from a wider range of light.
- Many pigment molecules capture far more photons than one, and all their energy is funnelled to one reaction centre.
- The reaction centre is positioned next to an electron acceptor, so the excited electron is passed on instead of falling back and losing its energy as heat or light.
- A single molecule of chlorophyll or any other pigment would not be able to perform any part of photosynthesis on its own.

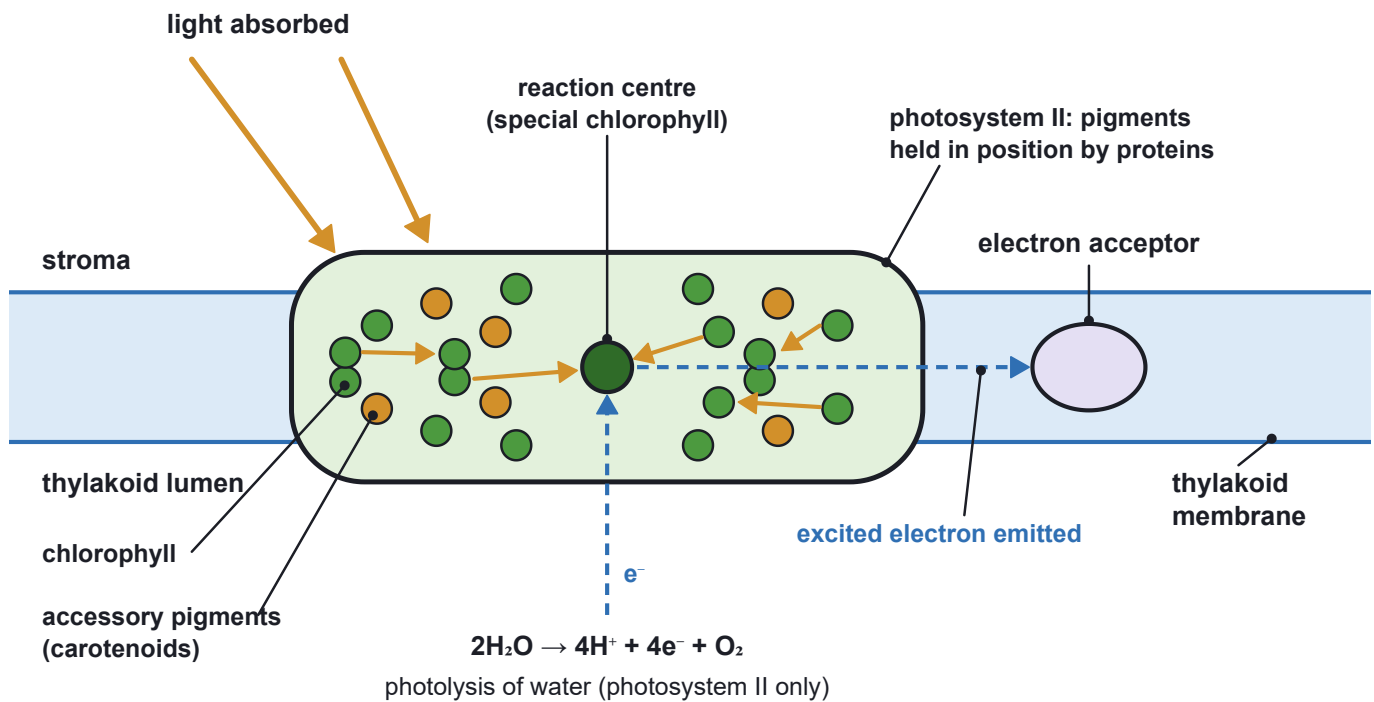
Photolysis of water in photosystem II

Photosystem II loses electrons from its reaction centre. They are replaced by **photolysis**: the splitting of water, catalysed by an enzyme complex in photosystem II on the thylakoid lumen side of the membrane.



The **electrons** replace those emitted by photosystem II, and the **protons**, released into the thylakoid lumen, add to the proton gradient used to make ATP by chemiosmosis. Both are used in photosynthesis, but **oxygen is a waste product** and diffuses out.

The advent of oxygen generation by photolysis, in early cyanobacteria, had immense consequences for life and geological processes on Earth. Oxygen accumulated in the oceans and atmosphere, dissolved iron was oxidised and precipitated (forming banded iron rock deposits), an ozone layer formed, and aerobic respiration became possible, while many anaerobic organisms were poisoned.



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A photosystem funnels light energy from many pigments to the **reaction centre**, which emits an excited electron. In photosystem II, photolysis replaces it.

Chemiosmosis, reduction of NADP and thylakoids

Spec C1.3.12, C1.3.13, C1.3.14

HL only

ATP production by chemiosmosis

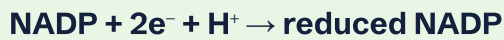
1. Excited electrons emitted by a photosystem pass along a **chain of electron carriers** in the thylakoid membrane.
2. Energy released as the electrons flow is used to **pump protons** from the stroma into the thylakoid lumen.
3. Protons (also released into the lumen by photolysis) build up a **proton gradient**: high concentration in the lumen, low in the stroma.
4. Protons diffuse back into the stroma through **ATP synthase**, which uses the energy to phosphorylate ADP, releasing ATP into the stroma. This is **chemiosmosis**.

Two ways of supplying electrons for ATP production

	Non-cyclic photophosphorylation	Cyclic photophosphorylation
Source of electrons	Photosystem II	Photosystem I
Path of electrons	PSII → carrier chain → PSI → NADP	PSI → carrier chain → back to PSI
Products	ATP, reduced NADP and oxygen	ATP only
Photolysis of water	Yes	No

Reduction of NADP by photosystem I

Photosystem I absorbs light and emits excited electrons. NADP is reduced by **accepting two electrons** that have come from photosystem I. It also **accepts a hydrogen ion from the stroma**, forming **reduced NADP**. Electrons lost from photosystem I are replaced by electrons arriving along the carrier chain from photosystem II.

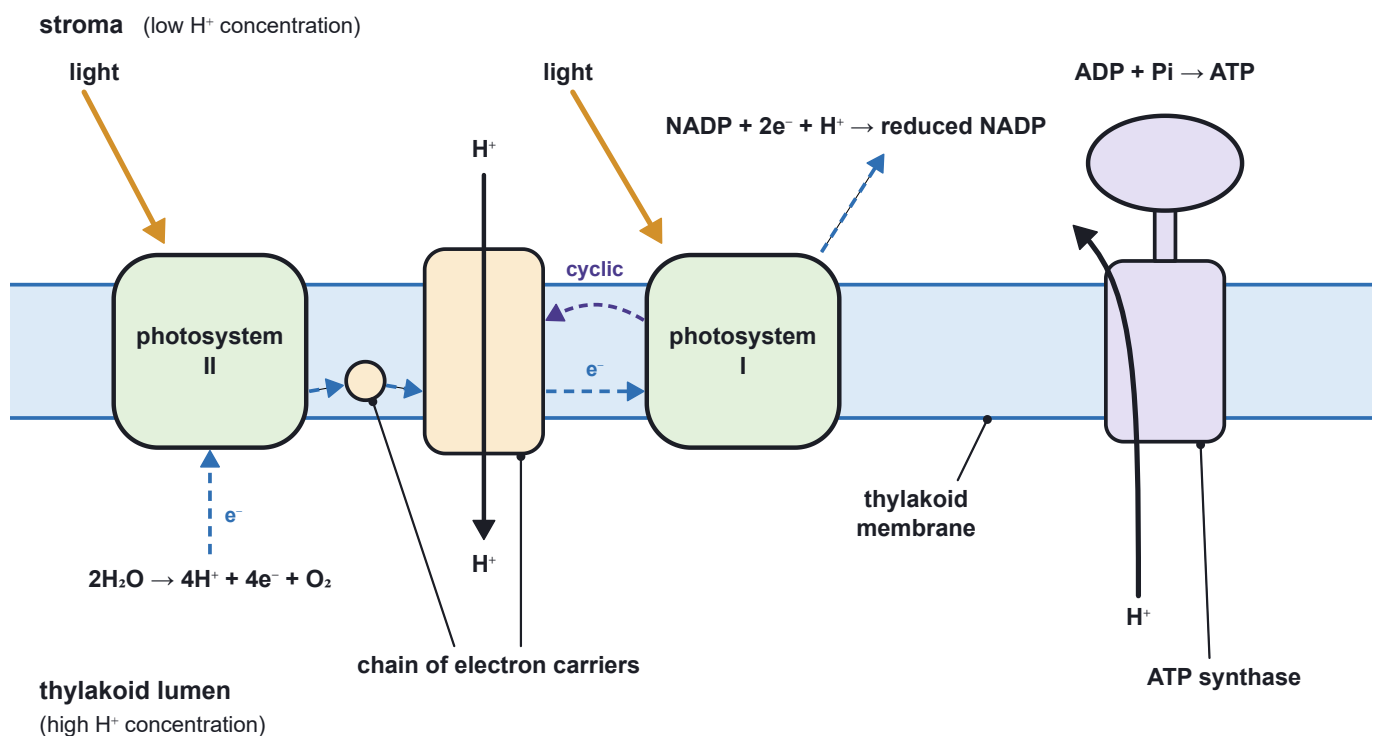


Exam tip: Pair the terms consistently: write 'NADP and reduced NADP' or 'NADP⁺ and NADPH', never a mixture such as 'NADP and NADPH'.

Thylakoids as systems for the light-dependent reactions

Where each light-dependent process happens in a thylakoid	
Process	Location
Light absorption by photosystems	Thylakoid membrane
Photolysis of water	Photosystem II, on the lumen side of the membrane; protons released into the lumen
Proton pumping	Electron carriers in the membrane, from stroma into lumen
ATP synthesis by chemiosmosis	ATP synthase in the membrane; ATP released into the stroma
Reduction of NADP	Stroma side of photosystem I

The small volume of the thylakoid lumen means a steep proton gradient builds up quickly, and stacking thylakoids into grana gives a large membrane area for photosystems. ATP and reduced NADP are both released into the **stroma**, where the Calvin cycle uses them.



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The light-dependent reactions: electrons flow from **photosystem II to photosystem I to NADP**, building a proton gradient that drives ATP synthase.

The Calvin cycle: carbon fixation, triose phosphate and RuBP Spec C1.3.15, C1.3.16, C1.3.17

Carbon fixation by Rubisco

In the stroma, the enzyme **Rubisco** catalyses the combination of **carbon dioxide** with **RuBP** (ribulose biphosphate, 5C). The product splits immediately into two molecules of **glycerate 3-phosphate (GP)**, a 3C compound. This is carbon fixation: inorganic carbon becomes part of an organic molecule.



Rubisco is the **most abundant enzyme on Earth**. High concentrations of it are needed in the stroma because it works relatively slowly and is not effective in low carbon dioxide concentrations.

Synthesis of triose phosphate

Glycerate 3-phosphate is converted into **triose phosphate (TP)** using reduced NADP and ATP from the light-dependent reactions. Reduced NADP supplies the hydrogen (GP is reduced) and ATP supplies energy.

Regeneration of RuBP

Most triose phosphate is used to regenerate RuBP, using more ATP. You do not need the individual reactions, but **five molecules of triose phosphate are converted to three molecules of RuBP** ($5 \times 3\text{C} = 15\text{C} = 3 \times 5\text{C}$), allowing the Calvin cycle to continue.

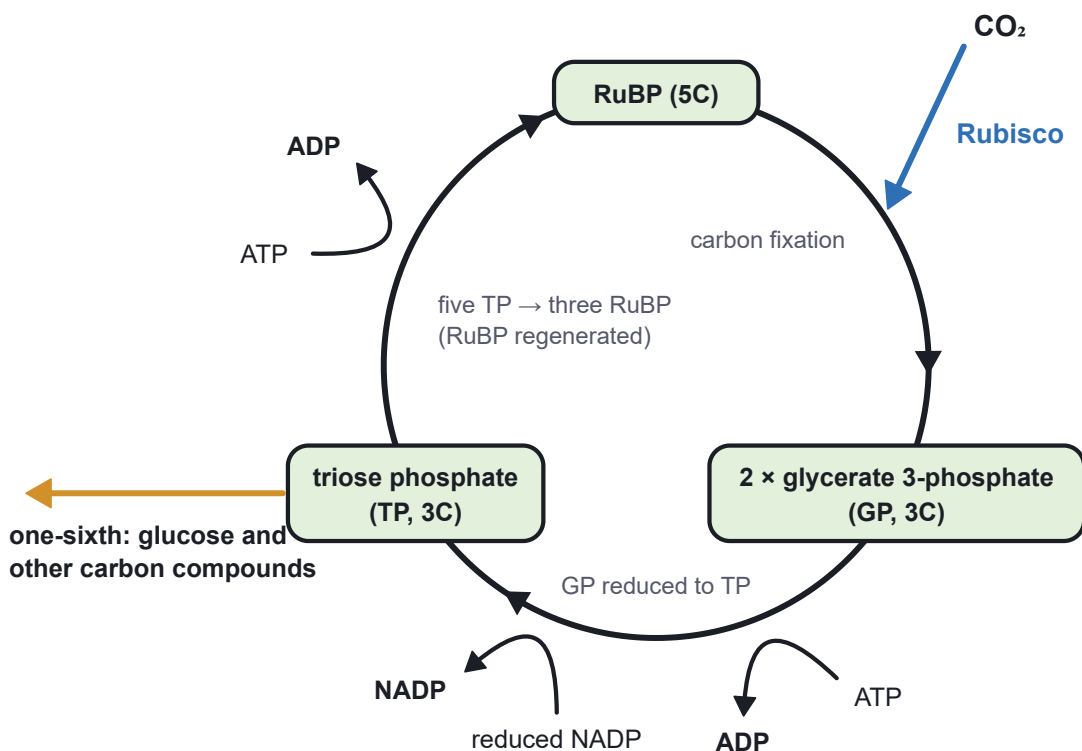
Why five-sixths of the triose phosphate is recycled

Show that if glucose is the product, five-sixths of the triose phosphate must be converted back to RuBP.

1. One glucose (6C) needs six carbon atoms, so 6 CO₂ must be fixed by 6 RuBP.
2. $6 \text{ RuBP} + 6 \text{ CO}_2 \rightarrow 12 \text{ GP} \rightarrow 12 \text{ TP}$ (36 carbon atoms).
3. To regenerate the 6 RuBP (30 carbon atoms), 10 TP are needed ($10 \times 3\text{C} = 30\text{C}$).
4. That leaves 2 TP (6C) to make one glucose.
5. 10 of 12 TP are recycled: $10 \div 12 = 5/6$.

Answer: Five-sixths of the TP is used to regenerate RuBP and one-sixth forms glucose.

stroma



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The Calvin cycle: **carbon fixation by Rubisco**, reduction of GP to TP, and regeneration of RuBP.

Exam tip: Use the IB names: RuBP, glycerate 3-phosphate (GP) and triose phosphate (TP). The intermediate between RuBP and GP is not required.

Making other carbon compounds and interdependence of the reactions

Spec C1.3.18, C1.3.19

HL only

Synthesis of other carbon compounds

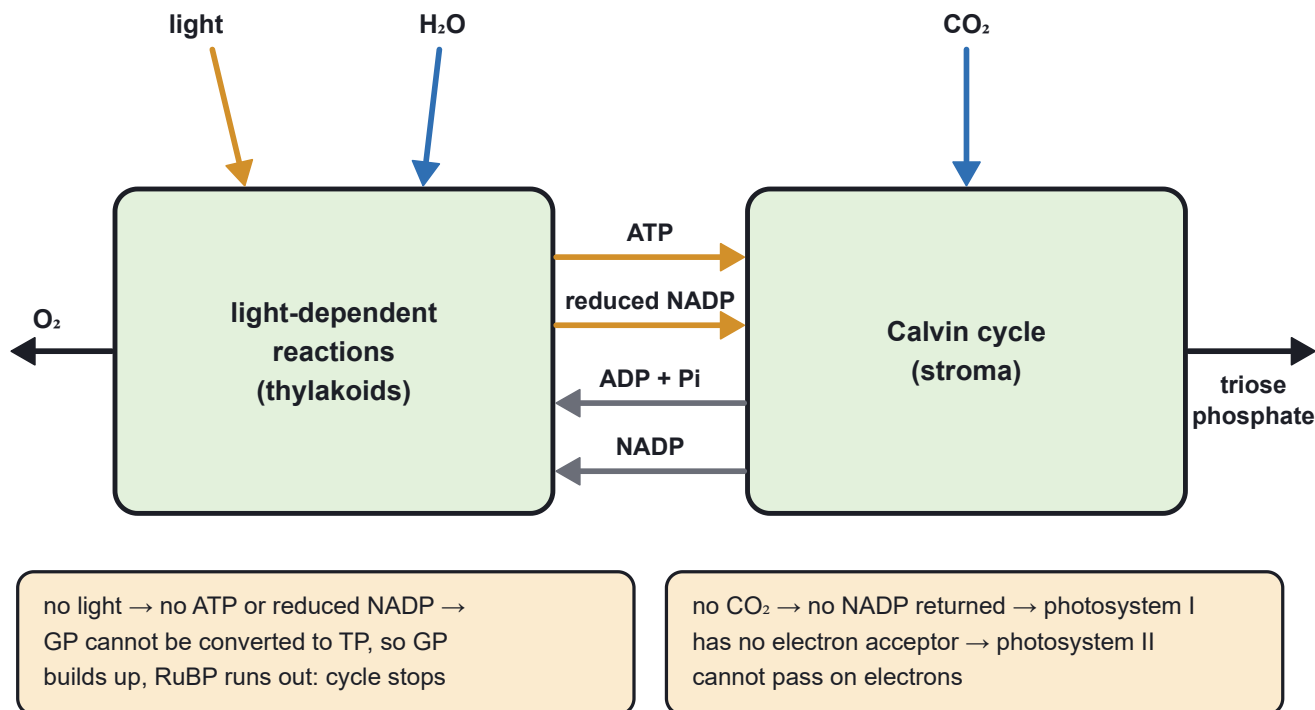
All of the carbon in compounds in photosynthesising organisms is fixed in the Calvin cycle. Glucose is only one product. Other carbon compounds are made by metabolic pathways that can be traced back to an intermediate in the cycle, usually triose phosphate. You do not need the details of these pathways.

Carbon compounds made from Calvin cycle products		
Product	Made from	Mineral nutrients also needed
Glucose, sucrose, starch, cellulose	Triose phosphate	None
Fatty acids and glycerol (lipids)	Triose phosphate and other intermediates	None (phosphate for phospholipids)
Amino acids (proteins)	Calvin cycle intermediates	Nitrogen (nitrate or ammonium); sulfur for some
Nucleotides (DNA, RNA, ATP)	Calvin cycle intermediates	Nitrogen and phosphate

Interdependence of the light-dependent and light-independent reactions

The light-dependent reactions supply ATP and reduced NADP to the Calvin cycle; the Calvin cycle returns ADP, phosphate and NADP. Neither can continue for long without the other.

- **Lack of light** stops the light-dependent reactions, so no ATP or reduced NADP is made. GP cannot be converted to TP, so GP accumulates and RuBP is not regenerated. The Calvin cycle stops.
- **Lack of CO₂** stops carbon fixation, so ATP and reduced NADP are not used. NADP is not regenerated, so photosystem I has no electron acceptor and the electron carriers stay reduced. Electrons from photosystem II cannot be passed on, so **photosystem II stops functioning**, and photolysis and oxygen production stop.



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The two sets of reactions **depend on each other**: ATP and reduced NADP go one way, ADP, Pi and NADP come back.

Exam tip: For a change in conditions, predict what builds up and what runs out. Darkness: GP rises and RuBP falls. Low CO₂: RuBP rises and GP falls.

Quick check

1. Write the word equation for photosynthesis.
2. Where does the oxygen released by photosynthesis come from?
3. How is an R_f value calculated?
4. What is the difference between an absorption spectrum and an action spectrum?
5. **HL only** What are the substrates and product of the reaction catalysed by Rubisco?

Quick check answers

Enzymes and metabolism

1. The complex network of interdependent and interacting chemical reactions occurring in living organisms.
2. Anabolic: protein synthesis, glycogen formation or photosynthesis. Catabolic: hydrolysis in digestion or oxidation of glucose in respiration.
3. Both the substrate and the enzyme's active site.
4. The activation energy is lowered; the overall energy change is unchanged.
5. Threonine deaminase, the first enzyme of the pathway, at its allosteric site.

Cell respiration

1. Adenosine triphosphate; a nucleotide.
2. glucose $\rightarrow$ lactate
3. It absorbs carbon dioxide, so the movement of the drop measures oxygen uptake.
4. Citrate (6C) and oxaloacetate (4C).
5. It is the terminal electron acceptor: it accepts electrons and protons, forming water, so electrons keep flowing along the chain.

Photosynthesis

1. carbon dioxide + water $\rightarrow$ glucose + oxygen
2. The splitting of water.
3. Distance moved by the pigment $\div$ distance moved by the solvent front.
4. An absorption spectrum shows light absorbed by a pigment at each wavelength; an action spectrum shows the rate of photosynthesis at each wavelength.
5. Substrates: RuBP and CO₂. Product: glycerate 3-phosphate (GP).