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5.6 Photosynthesis

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5.6.1 PHOTOSYNTHESIS & RESPIRATION

Photosynthesis & Respiration

- Photosynthesis occurs in autotrophic organisms such as plants, algae and cyanobacteria
- In the process of photosynthesis, light energy is trapped and used to convert simple inorganic compounds into complex organic compounds. **Energy is stored** within these organic compounds
- Respiration occurs in all living organisms
- Respiration is the process by which **energy is released** from organic molecules in living cells. The process can be aerobic (using oxygen) or anaerobic (without using oxygen)
- There are several similarities and differences between photosynthesis and the two types of respiration
- For example, the coenzyme NADP is used in photosynthesis whereas the coenzyme NAD is used in both aerobic and anaerobic respiration

5.6 Photosynthesis

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Comparing Photosynthesis & Respiration Table

Photosynthesis	Aerobic Respiration	Anaerobic Respiration
Occurs in photosynthetic organisms (plants, algae and cyanobacteria)	Occurs in all living organisms	Lactate fermentation occurs in the muscle cells of animals and ethanol fermentation occurs in plant and fungi
Stores energy	Releases energy	Releases energy
Converts light energy into chemical energy	Converts chemical energy into a more accessible form of chemical energy	Converts chemical energy into a more accessible form of chemical energy
Anabolic reaction (builds molecules)	Catabolic reaction (breaks down molecules)	Catabolic reaction (breaks down molecules)
Carbon dioxide + water → glucose + oxygen	Glucose + oxygen → carbon dioxide + water	Glucose –> lactate or ethanol
ATP produced by photophosphorylation	ATP produced by substrate–level phosphorylation and by oxidative phosphorylation	ATP produced by substrate–level phosphorylation
Occurs in chloroplasts	Occurs in mitochondria	Occurs in cytosol/ cytoplasm
Requires light	Does not require light	Does not require light
Requires a supply of carbon dioxide	Requires a supply of oxygen	Occurs in the absence of oxygen
The coenzyme NADP is used	The coenzyme NAD is used	The coenzyme NAD is used
Electron transport chain involved	Electron transport chain involved	No electron transport chain involved
End products: Oxygen and glucose	End products: Carbon dioxide and water	End products: Lactate or ethanol

Exam Tip

Remember, energy is never created or destroyed; it is converted from one form to another!

5.6 Photosynthesis

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5.6.2 CHLOROPLAST STRUCTURE & FUNCTION

Chloroplast Structure & Function

- Chloroplasts are the **organelles** in plant cells where **photosynthesis** occurs
- These organelles are roughly 2 – 10 μm in diameter (they are larger than mitochondria)
- Each chloroplast is surrounded by a **double-membrane envelope**
  - Each of the envelope membranes is a phospholipid bilayer
  - The outer membrane is permeable to a range of ions and small molecules
  - The inner membrane contains transport proteins that only allow certain molecules or ions to enter or leave the chloroplast
- Chloroplasts are filled with a cytosol-like fluid known as the **stroma**
  - CO_2, sugars, enzymes and other molecules are dissolved in the stroma
- A separate **system of membranes** is found in the stroma
  - This membrane system consists of a series of flattened fluid-filled sacs known as **thylakoids**
  - The thylakoid membranes contain **pigments**, **enzymes** and **electron carriers**
  - These thylakoids stack up to form structures known as **grana** (singular – granum)
  - Grana are connected by membranous channels called stroma **lamellae**, which ensure the stacks of sacs are connected but distanced from each other
  - The membranes of the grana create a **large surface area**
  - This membrane system provides a large number of pigment molecules that ensure **as much light as necessary is absorbed**
  - The pigment molecules are arranged in light-harvesting clusters known as **photosystems**
  - In a photosystem, the different pigment molecules are arranged in **funnel-like structures** in the thylakoid membrane (each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre)
- The stroma also contains small (70S) **ribosomes**, a loop of **DNA** and **starch grains**:
  - The loop of DNA codes for some of the **chloroplast proteins** (other chloroplast proteins are coded for by the DNA in the plant cell nucleus)
  - The proteins coded for by this loop of chloroplast DNA are **produced at the 70S ribosomes**
  - Sugars formed during photosynthesis are **stored** as **starch** inside starch grains

5.6 Photosynthesis

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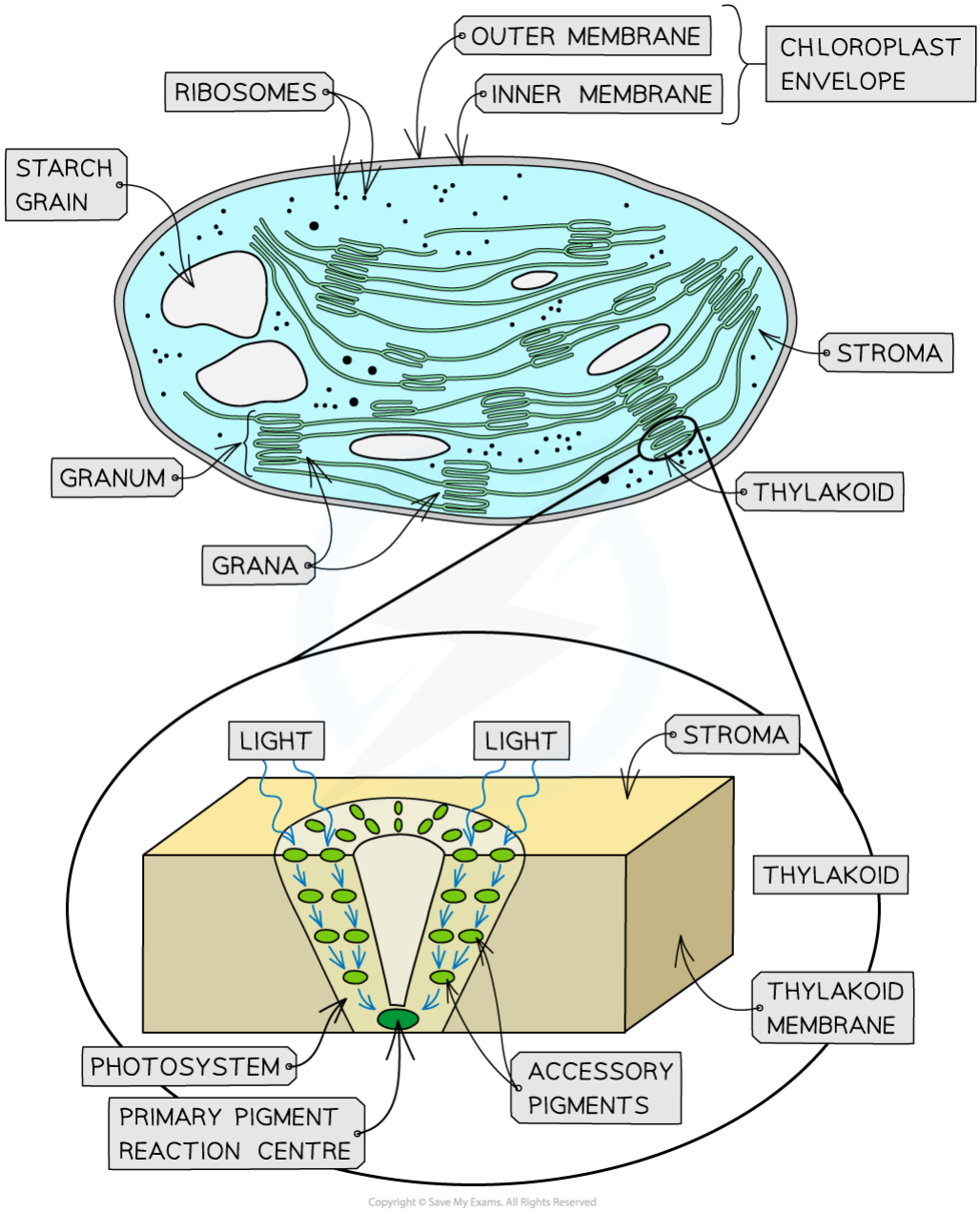

An example of a photosystem in a chloroplast thylakoid membrane: a light-harvesting cluster of photosynthetic pigments involved in the light-dependent stage of photosynthesis

5.6 Photosynthesis

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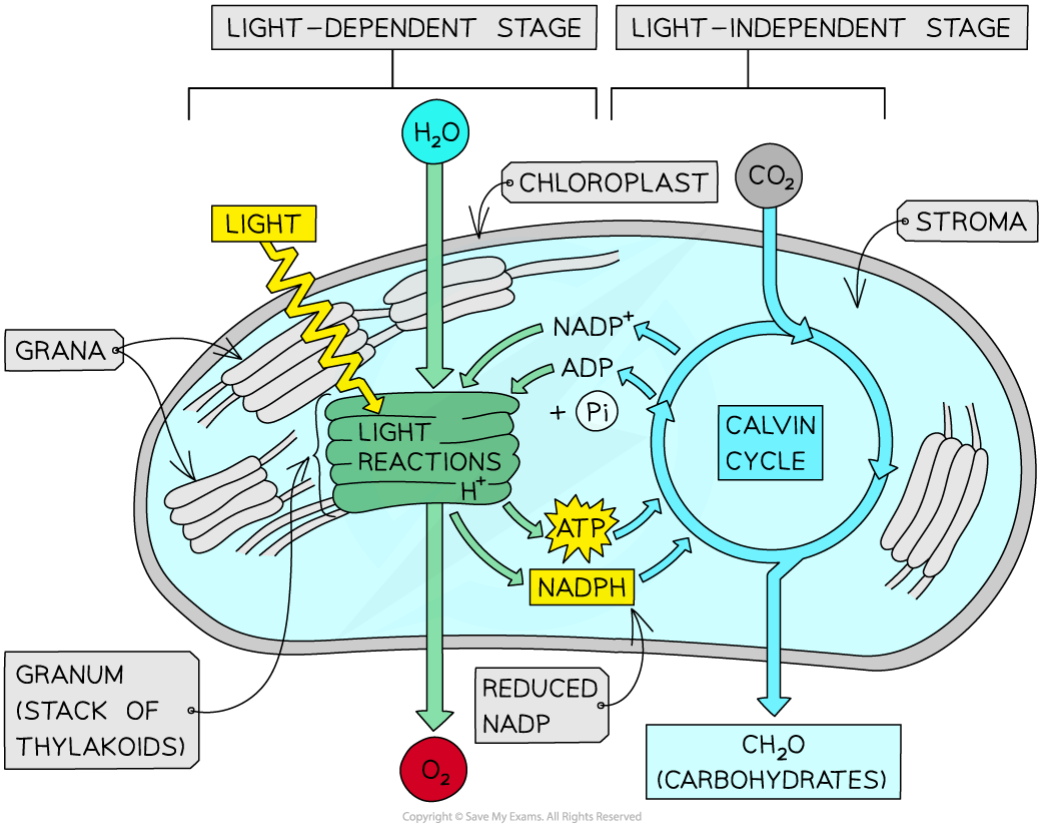
Location of the stages of photosynthesis

- The process of photosynthesis is made up of two stages, the light-dependent stage and the light-independent stage
- These stages take place in specific locations within the chloroplast
- The first stage, the **light-dependent stage** takes place on the **thylakoid membranes** of the grana
    - Light becomes trapped within the reaction centres of the grana
- The **light-independent stage** takes place in the **stroma**



The location of the different stages of photosynthesis. The products of the light-dependent reaction are used in the light-independent reaction.

5.6 Photosynthesis

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Adaptations of chloroplasts to photosynthesis

- Chloroplasts are **specialised** organelles that are adapted to carry out photosynthesis
- **Stroma:** The gel-like fluid contains **enzymes** that catalyse the reactions of the light-independent stage. The stroma **surrounds the grana and membranes**, making the transport of products from the light-dependent stage into the stroma **rapid**
- **Grana:** The granal stacks create a **large surface area** for the presence of many photosystems which allows for the maximum absorption of light. It also provides more membrane space for electron carriers and ATP synthase enzymes
- **DNA:** The chloroplast DNA (cpDNA) contains genes that code for some of the **proteins and enzymes** used in photosynthesis
- **Ribosomes:** The presence of ribosomes allows for the **translation** of proteins coded by cpDNA
- **Inner membrane of chloroplast envelope:** The selective **transport proteins** present in the inner membrane control the flow of molecules between the stroma and cytosol (the cytoplasm of the plant cell)

Exam Tip

Make sure you can identify the structures of a chloroplast on a diagram AND that you can explain the function of each of these structures.

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5.6.3 PHOTOSYNTHETIC PIGMENTS

Photosynthetic Pigments

- Chloroplasts contain several different **photosynthetic pigments** within the **thylakoids**, which **absorb different wavelengths of light**
- There are **two types** of pigments: **chlorophylls** and **carotenoids**
    - Accessory pigments that surround the primary pigment absorb both similar and different wavelengths of light to chlorophyll, this **expands the wavelength range that can be absorbed from light** for use in photosynthesis

Chloroplast Pigments Table

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Yellow–green Blue–green
Carotenoids	β carotene Xanthophyll	Orange Yellow

- **Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
    - They reflect green light, causing plants to appear green
- **Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum

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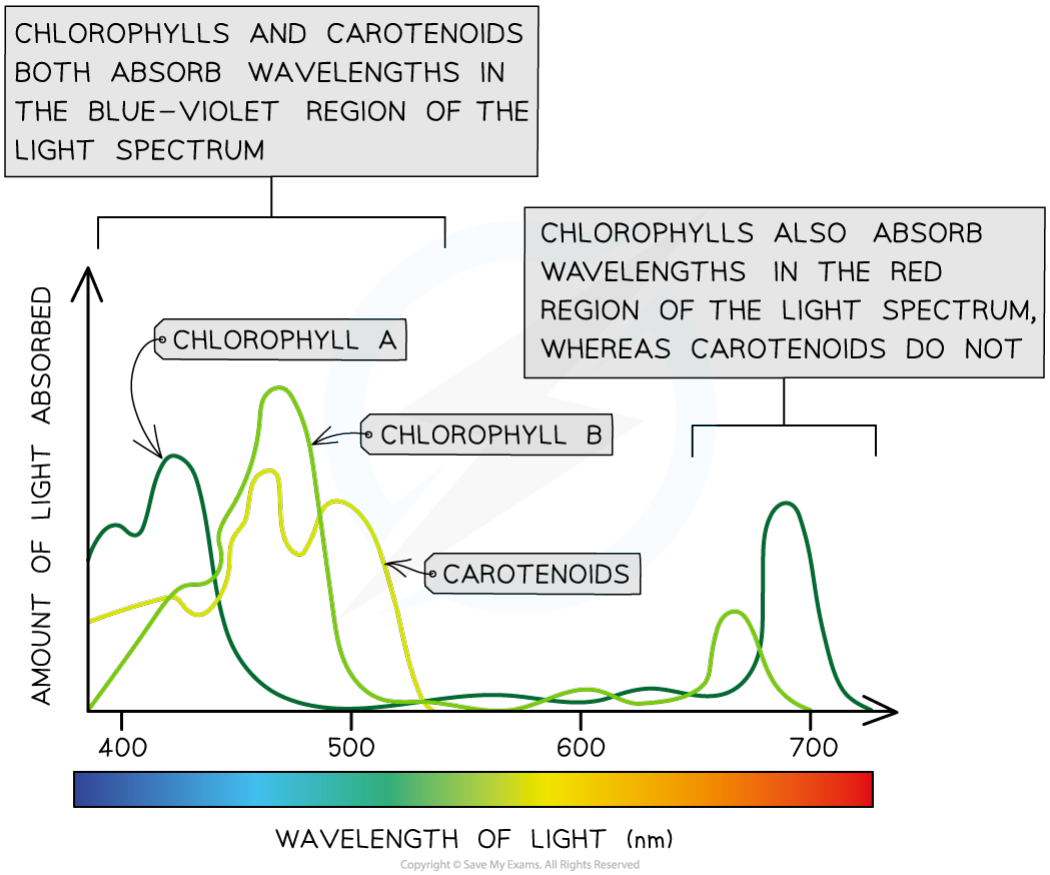

Chlorophyll and carotenoids absorb light across the visible light spectrum to use in the light-dependent reaction of photosynthesis

Pigments and photosystems

- Within chloroplasts thylakoids stack up to form structures known as **grana** (singular – granum)
- The thylakoid membrane system provides a large number of pigment molecules in an arrangement that ensures **as much light as necessary is absorbed**
- The pigment molecules are arranged in light-harvesting clusters known as **photosystems**
- In a photosystem, the different pigment molecules are arranged in **funnel-like structures** in the thylakoid membrane (each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre)
- There are two different photosystems, each with a specific form of chlorophyll *a*
- **Photosystem 1** (PSI), often referred to as **P700**
    - The chlorophyll *a* in this system has a maximum absorption of light at 700nm
- **Photosystem 2** (PSII), often referred to as **P680**
    - The chlorophyll *a* in this system has a maximum absorption of light at 680nm

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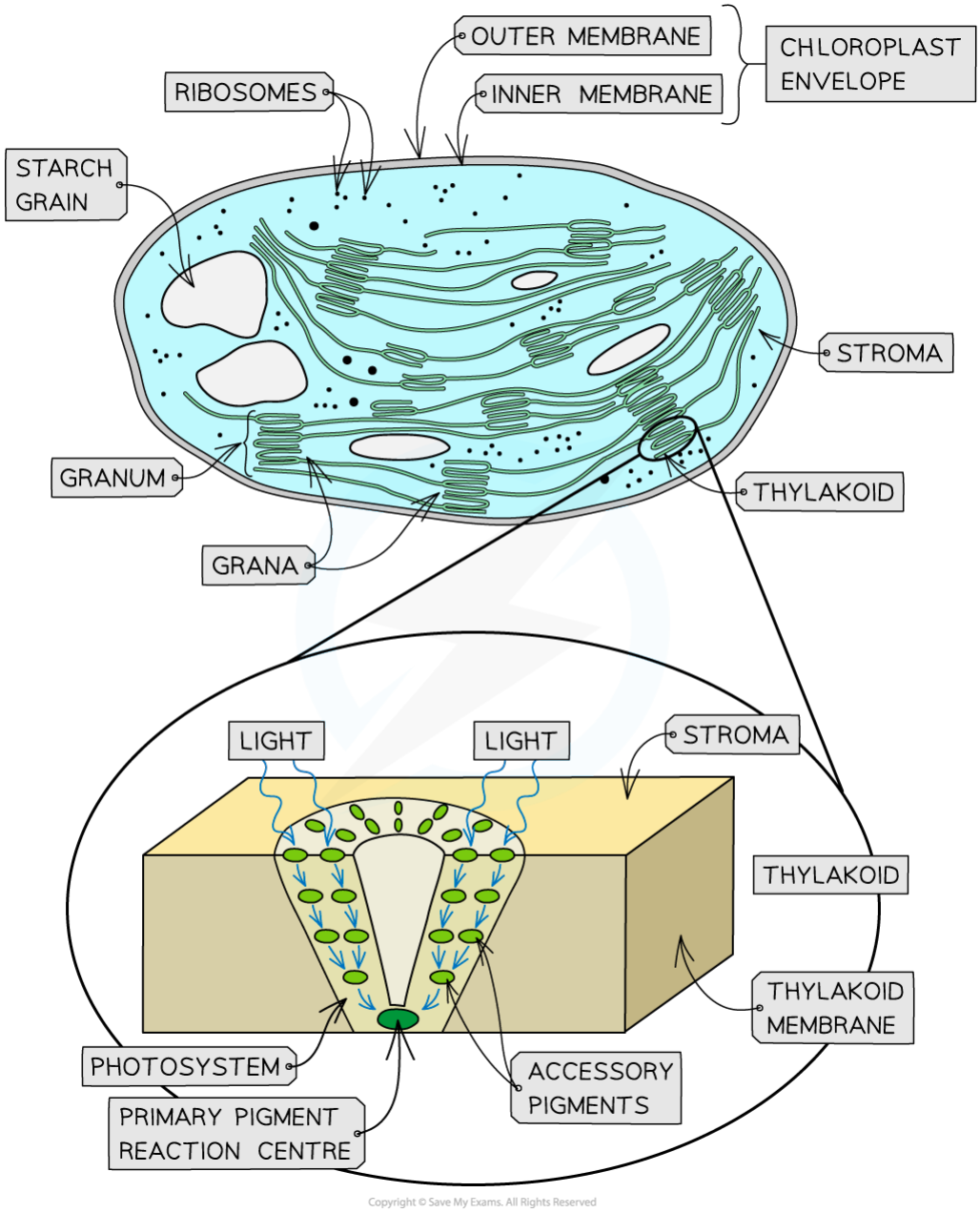

An example of a photosystem in a chloroplast thylakoid membrane: a light-harvesting cluster of photosynthetic pigments involved in the light-dependent stage of photosynthesis

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Exam Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*.

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5.6.4 PRACTICAL: INVESTIGATING PHOTOSYNTHETIC PIGMENTS WITH CHROMATOGRAPHY

Practical: Investigating Photosynthetic Pigments with Chromatography

- Chloroplasts contain several different **photosynthetic pigments** within the **thylakoids**, which **absorb different wavelengths of light**
- Chromatography can be used to **separate and identify chloroplast pigments** that have been extracted from a leaf

Chloroplast pigments table

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Yellow–green Blue–green
Carotenoids	β carotene Xanthophyll	Orange Yellow

Chromatography

- **Chromatography** is an experimental technique that is used to **separate mixtures**:
  - **Different components** within the mixture travel through the material at **different speeds due to their size and charge**
  - This causes the different components to **separate**
  - A retardation factor ($\mathbf{R_f}$) can be calculated for each component of the mixture
- Two of the most common techniques for separating these photosynthetic pigments are:
  - **Paper chromatography** – the mixture of pigments is passed through paper (cellulose)
  - **Thin-layer chromatography** – the mixture of pigments is passed through a thin layer of adsorbent (eg. silica gel), through which the mixture travels faster and separates more distinctly

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Apparatus

- Leaf sample
- Distilled water
- Pestle and mortar
- Filter paper
- Capillary tube
- Chromatography solvent
- Acetone
- Pencil
- Ruler

Method

- Draw a straight line in pencil approximately 1cm above the bottom of the filter paper being used
    - Do not use a pen as the ink will separate into pigments within the experiment and obscure the results
- Cut a section of leaf and place it in a mortar
    - It is important to choose a healthy leaf that has been in direct sunlight so you can be sure it contains many active photosystems
- Add 20 drops of acetone and use the pestle to grind up the leaf sample and release the pigments
    - Acetone is an organic solvent and therefore fats, such as the lipid membrane, dissolve in it
    - Acetone and mechanical pressure are used to break down the cell, chloroplast and thylakoid membranes to release the pigments
- Extract some of the pigment using a capillary tube and spot it onto the centre of the pencil line you have drawn
- Suspend the paper in the chromatography solvent so that the level of the solvent is below the pencil line and leave the paper until the solvent has reached the top of the paper
    - The mixture is **dissolved** in the **solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
- Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to
    - The pigment should have separated out and there should be different spots on the paper at different heights above the pencil line, these are the separate pigments
- Calculate the Rf value for each spot

R_f value = distance travelled by component (pigment) ÷ distance travelled by the solvent

- Always measure to the centre of each spot

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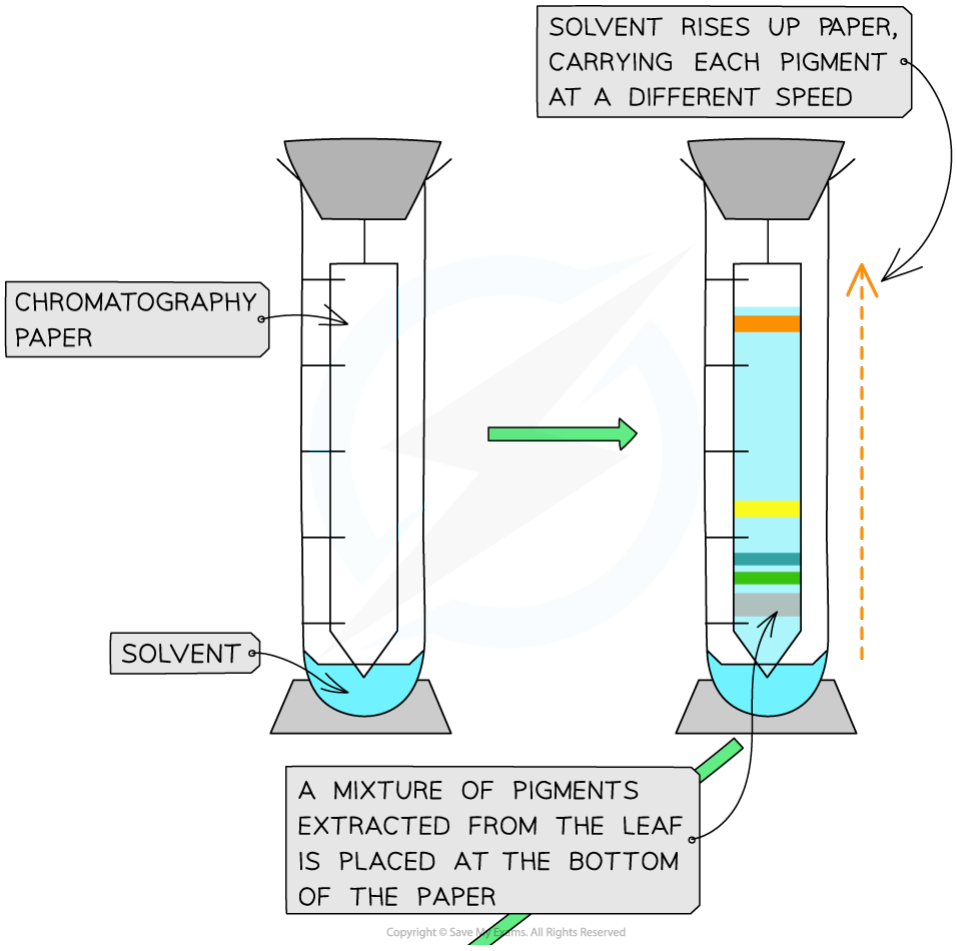
Results

- Chromatography can be used to **separate and identify chloroplast pigments** that have been extracted from a leaf as each pigment will have a unique R_f value
- The R_f value demonstrates how far a dissolved pigment travels through the stationary phase
  - Molecules with a higher affinity to the stationary phase, such as large molecules, will travel slower and therefore have a **smaller R_f value**
  - Molecules that are more soluble in the mobile phase will travel faster and therefore have a **larger R_f value**
- Although specific R_f values depend on the solvent that is being used, in general:
  - **Carotenoids** have the **highest R_f values** (usually close to 1)
  - **Chlorophyll *b*** has a **much lower R_f value**
  - **Chlorophyll *a*** has an R_f value somewhere **between** those of carotenoids and chlorophyll *b*
  - **Small R_f values** indicate the pigment is **less soluble** and/or **larger** in size



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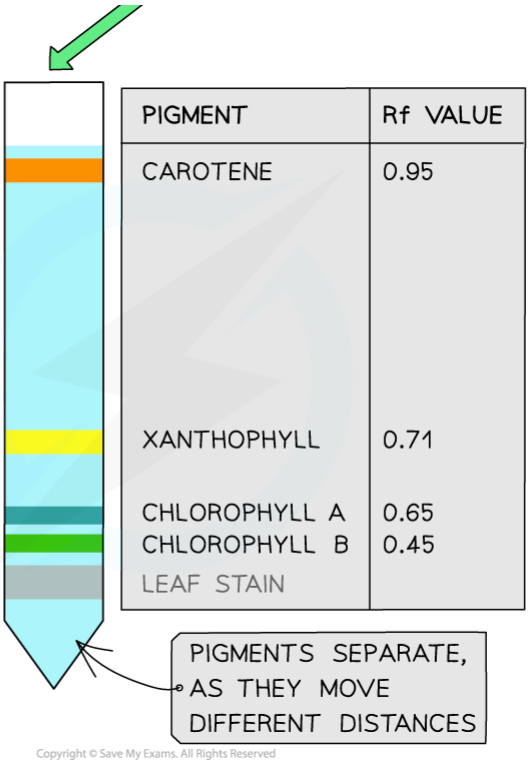

Paper chromatography is used to separate photosynthetic pigments. These pigments can be identified by their R_f values. In this example, a line of mixture (rather than a spot) is added to the paper.

Limitations

- Paper chromatography is not as specific as other chromatography techniques
  - It is sufficient to separate and distinguish different pigments and to calculate their R_f value
- Chromatography does not give data on the amount of each pigment present or the wavelengths that they absorb
  - Colorimetry can be used to calculate these values

Exam Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*.

Make sure you learn the approximate R_f values for the different pigments within chloroplasts (or at least their values relative to each other). This means you should be able to identify different chloroplast pigments based on their R_f values alone.

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5.6.5 THE LIGHT-DEPENDENT STAGE

The Light-Dependent Stage

- Photosynthesis occurs in two stages: the **light-dependent stage**, which takes place in the **thylakoids**, and the **light-independent stage**, which takes place in the **stroma**
- During the **light-dependent** stage of photosynthesis:
  - Light energy is used to breakdown water (photolysis) to produce hydrogen ions, electrons and oxygen in the **thylakoid lumen**
  - A **proton gradient** is formed due to the photolysis of water resulting in a high concentration of hydrogen ions in the thylakoid lumen
  - Electrons travel through an **electron transport chain** of proteins within the membrane
  - **Reduced NADP** (NADPH) is produced when hydrogen ions in the stroma and electrons from the electron transport chain combine with the **carrier molecule NADP**
  - **ATP** is produced during a process known as **photophosphorylation**

Photophosphorylation & chemiosmosis

- Photophosphorylation is the name for the overall process of using light energy and the electron transport chain to phosphorylate ADP to ATP
  - The light-dependent reaction is sometimes called 'photophosphorylation'
- During **photophosphorylation**, energetic (excited) **electrons** are passed along a chain of electron carriers (known as the **electron transport chain**)
- The electron carriers are alternately **reduced** (as they **gain** an electron) and then **oxidised** (as they **lose** the electron by passing it to the next carrier)
- The excited electrons **gradually release their energy** as they pass through the electron transport chain
- The released energy is used to **actively transport protons (H^+ ions)** across the thylakoid membrane, from the **stroma** (the fluid within chloroplasts) to the **thylakoid lumen** (the space within thylakoids)
  - A **'proton pump'** transports the protons across the thylakoid membrane, from the stroma to the thylakoid lumen
  - The energy for this active transport comes from the excited electrons moving through the electron transport chain
- This creates a **proton gradient**, with a **high concentration** of protons in the **thylakoid lumen** and a **low concentration** in the **stroma**
- Protons then return to the stroma (moving down the proton concentration gradient) by **facilitated diffusion** through **transmembrane ATP synthase** enzymes in a process known as **chemiosmosis**
- This process provides the energy needed to **synthesise ATP** by adding an inorganic

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phosphate group (P_i) to ADP (**ADP + P_i → ATP**)

- The whole process is known as **photophosphorylation** as **light** provides the initial **energy source** for ATP synthesis
- The photophosphorylation of ADP to ATP can be **cyclic** or **non-cyclic**, depending on the pattern of electron flow in photosystem I or photosystem II or both
    - In **cyclic** photophosphorylation, **only photosystem I** is involved
    - In **non-cyclic** photophosphorylation, **both photosystem I and photosystem II** are involved
- **Photosystems** are collections of photosynthetic pigments that absorb light energy and transfer the energy onto electrons, each photosystem contains a **primary pigment**
    - **Photosystem II** has a primary pigment that absorbs light at a wavelength of 680nm and is therefore called **P680**
    - Photosystem II is at the beginning of the electron transport chain and is where the photolysis of water takes place
    - **Photosystem I** has a primary pigment that absorbs light at a wavelength of 700nm and is therefore called **P700**
    - Photosystem I is in the middle of the electron transport chain
    - The energy carried by the ATP is then used during the **light-independent** reactions of photosynthesis

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Cyclic photophosphorylation

- Cyclic photophosphorylation involves **photosystem 1 (PSI) only**
- **Light is absorbed** by photosystem 1 (located in the thylakoid membrane) and passed to the photosystem I **primary pigment** (P700)
- An **electron** in the primary pigment molecule (ie. the chlorophyll molecule) is **excited** to a **higher energy level** and is emitted from the chlorophyll molecule in a process known as **photoactivation**
- This excited electron is captured by an electron acceptor, transported via a **chain of electron carriers** known as an **electron transport chain** before being passed back to the chlorophyll molecule in photosystem 1 (hence: cyclic)
- As electrons pass through the electron transport chain they provide energy to transport protons (H^+) from the stroma to the thylakoid lumen via a **proton pump**
- A build-up of protons in the thylakoid lumen can then be used to drive the **synthesis of ATP** from ADP and an inorganic phosphate group (P_i) by the process of **chemiosmosis**

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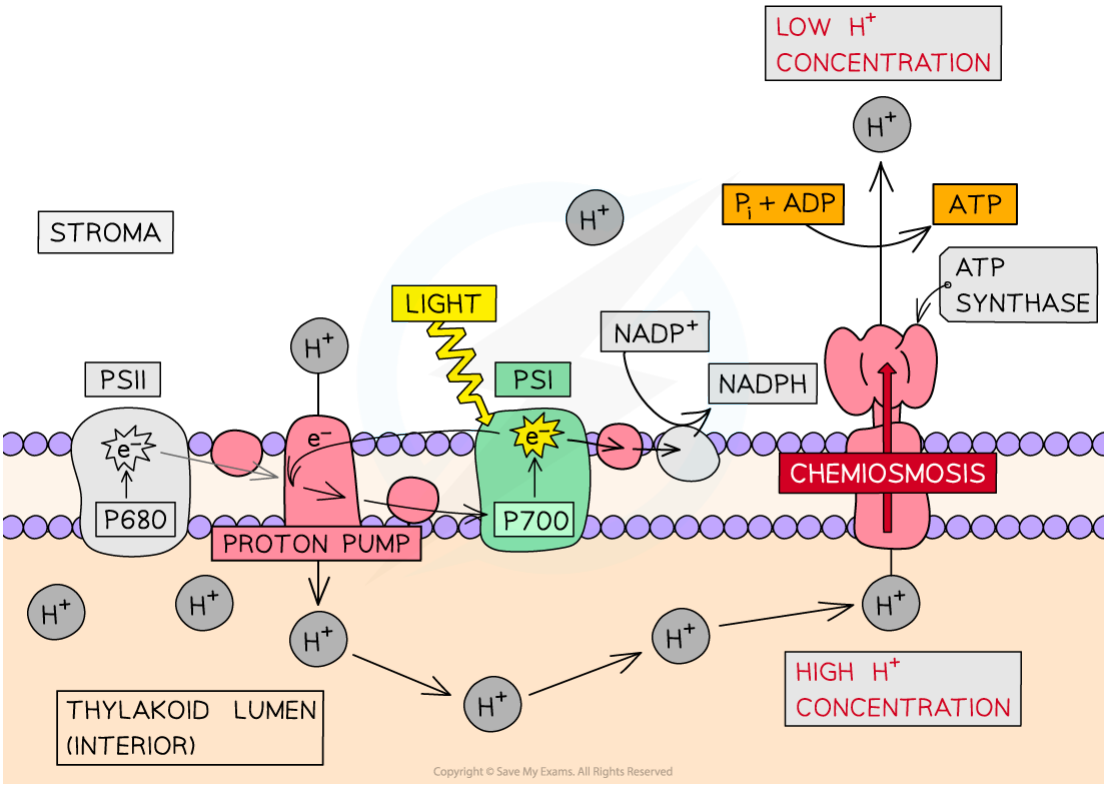

Cyclic photophosphorylation

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Non-cyclic photophosphorylation

- **Light is absorbed** by **photosystem 2** (located in the thylakoid membrane) and passed to the photosystem 2 **primary pigment (P680)**
- Two **electrons** in the primary pigment molecule (ie. the chlorophyll molecule) are **excited** to a **higher energy level** and are emitted from the chlorophyll molecule in a process known as **photoionisation**
- Each excited electron is passed down a **chain of electron carriers** known as an **electron transport chain**, before being passed on to photosystem 1
- During this process **chemiosmosis** occurs:
  - The energy given by the electrons moving through the electron transport chain enables H^+ ions (protons) to pass from a low concentration in the stroma to a high concentration in the thylakoid lumen
  - The creation of this proton gradient across the membrane later drives the synthesis of ATP in **photophosphorylation**
- Photosystem 2 contains a **water-splitting enzyme** called the **oxygen-evolving complex** which catalyses the breakdown (**photolysis**) of water by light:

$$\mathbf{H_2O \rightarrow 2H^+ + 2e^- + \frac{1}{2}O_2}$$

- As the excited electrons **leave** the primary pigment of photosystem 2 and are passed on to photosystem 1, they are **replaced** by **electrons** from the **photolysis** of water
- At the **same time** as photoactivation of electrons in photosystem 2, **electrons in photosystem 1 (PSI) also undergo photoionisation**
- The excited electrons from photosystem 1 also pass along an **electron transport chain**, alternatively **reducing and oxidising proteins** as they are accepted then passed on
- These electrons **combine** with **hydrogen ions** (produced by the photolysis of water and transported out of the thylakoid lumen by ATP synthase) and the **carrier molecule NADP** to give **reduced NADP**:

$$\mathbf{2H^+ + 2e^- + NADP \rightarrow \text{reduced NADP}}$$

- The reduced NADP (NADPH) then passes to the **light-independent reactions** to be used in the **synthesis of carbohydrates**
- The electrons lost by photosystem 1 are replaced by the de-energised electrons from photosystem 2

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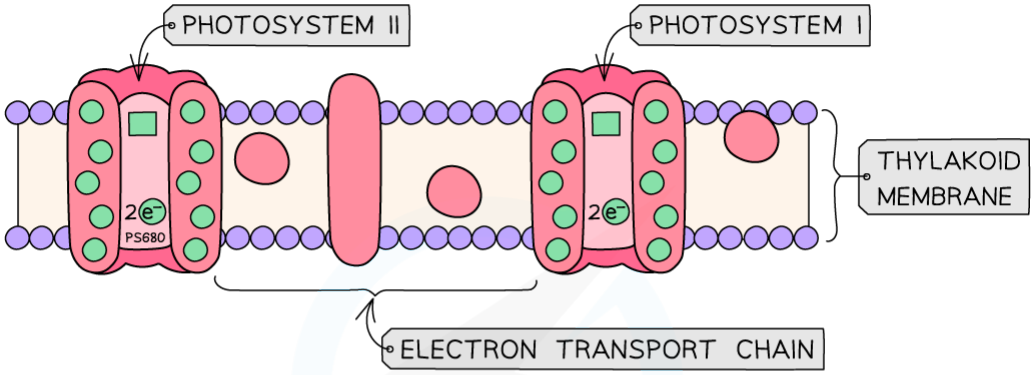
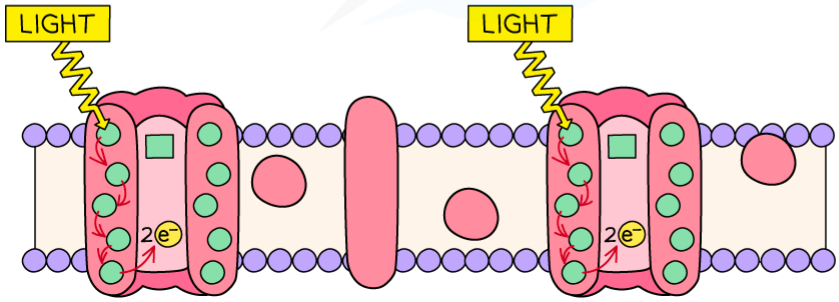
1 NON-CYCLIC PHOTOPHOSPHORYLATION INVOLVES BOTH PHOTOSYSTEM I AND PHOTOSYSTEM II



2 LIGHT IS ABSORBED BY BOTH PHOTOSYSTEMS. THE LIGHT IS PASSED TO THE PRIMARY PIGMENT OF EACH PHOTOSYSTEM



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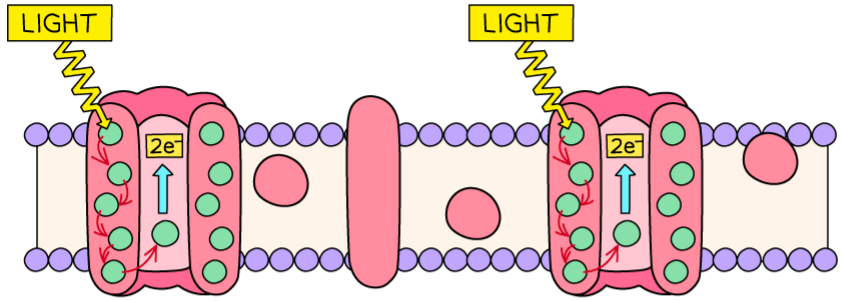
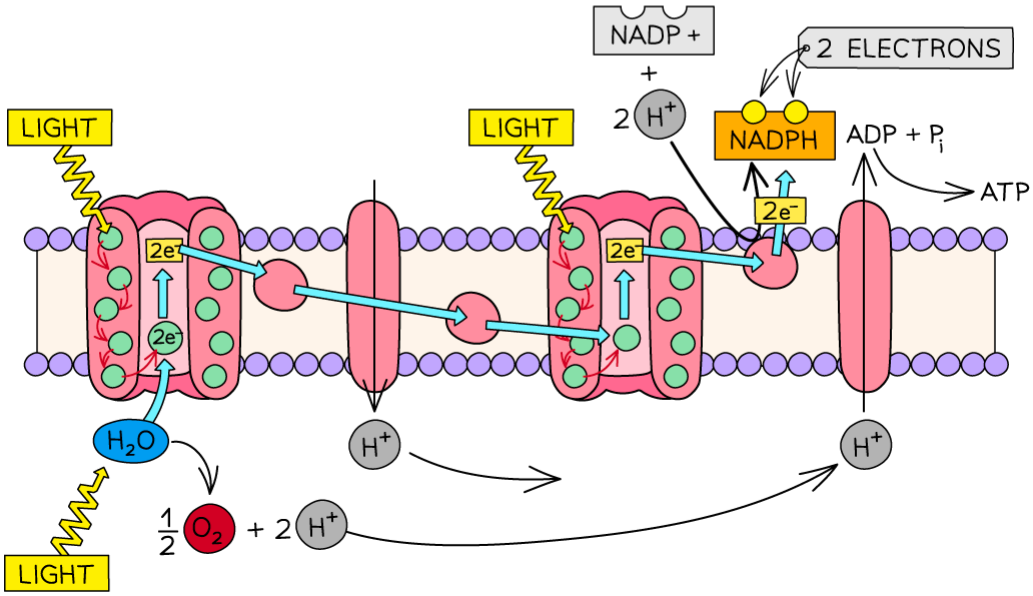
3 PHOTOACTIVATION – ELECTRONS IN THE PRIMARY PIGMENT MOLECULE OF EACH PHOTOSYSTEM ARE EXCITED TO A HIGHER ENERGY LEVEL



4 EXCITED ELECTRONS FROM PHOTOSYSTEM II ARE PASSED TO PHOTOSYSTEM I VIA AN ELECTRON TRANSPORT CHAIN, RELEASING SUFFICIENT ENERGY TO SYNTHESISE ATP



5 ELECTRONS FROM THE PHOTOLYSIS OF WATER REPLACE THOSE LOST FROM PHOTOSYSTEM II

6 EXCITED ELECTRONS FROM PHOTOSYSTEM I AND HYDROGEN IONS FROM THE PHOTOLYSIS OF WATER BOTH COMBINE WITH NADP TO FORM REDUCED NADP

Light-dependent photophosphorylation leads to the production of ATP and NADP

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Comparison of Cyclic Photophosphorylation & Non-cyclic Photophosphorylation Table

	Cyclic photophosphorylation	Non-cyclic photophosphorylation
Photosystems involved	Photosystem 1 (PSI)	Photosystem 2 (PSII)
Photolysis of water	No	Yes
Electron donor	P700 in PSI	Water
Final electron acceptor	P700 in PSI	NADP
Products	ATP	ATP, oxygen and reduced NADP

Exam Tip

Make sure you know the difference between the two forms of photophosphorylation!

Cyclic photophosphorylation differs from non-cyclic photophosphorylation in two key ways:

- Cyclic photophosphorylation **only** involves photosystem I (whereas non-cyclic photophosphorylation involves photosystems I and II)
- Cyclic photophosphorylation does **not** produce reduced NADP (whereas non-cyclic photophosphorylation does)

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5.6.6 USING THE PRODUCTS OF THE LIGHT-DEPENDENT REACTION

Using the Products of the Light-Dependent Reaction

- Photosynthesis occurs in two stages: the **light-dependent stage**, which takes place in the **thylakoids**, and the **light-independent stage**, which takes place in the **stroma**
- During the **light-dependent** stage of photosynthesis:
  - **Reduced NADP** is produced when hydrogen ions combine with the **carrier molecule NADP** using electrons from the **photolysis** of water
  - **ATP** is produced from ADP and P_i by **ATP synthase** in a process called **photophosphorylation** (ADP + P_i → ATP)
  - Photophosphorylation uses the **proton (H^+) gradient** generated by the photolysis of water
  - **Energy from ATP** and **hydrogen from reduced NADP** are passed from the light-dependent stage to the light-independent stage of photosynthesis
- The energy and hydrogen are used during the **light-independent** reactions (known collectively as the **Calvin cycle**) to produce complex organic molecules, including (but not limited to) **carbohydrates**, such as:
  - **Starch** (for storage)
  - **Sucrose** (for translocation around the plant)
  - **Cellulose** (for making cell walls)

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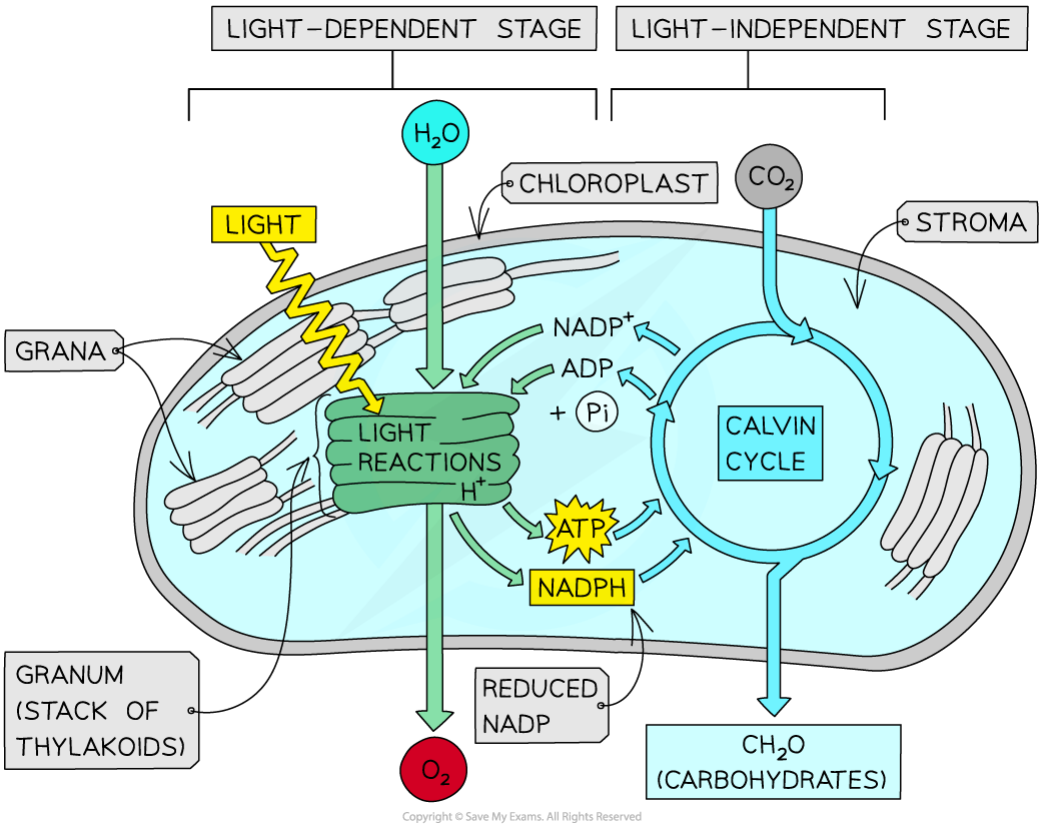

Photosynthesis occurs in two, closely-linked stages: the light-dependent stage, which takes place in the thylakoids, and the light-independent stage, which takes place in the stroma

Exam Tip

Remember, the whole purpose of the light-dependent stage is to produce ATP and reduced NADP, which are then used to complete the process of photosynthesis through the light-independent stage.

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5.6.7 THE LIGHT-INDEPENDENT STAGE

The Light-Independent Stage

- The **light-independent** stage of photosynthesis is sometimes referred to as the **Calvin cycle**
- This stage produces complex organic molecules, including (but not limited to) **carbohydrates**, such as:
  - **Starch** (for storage)
  - **Sucrose** (for translocation around the plant)
  - **Cellulose** (for making cell walls)
- The light-**independent** stage does not require energy from light and can therefore take place in light or darkness. However, as it requires inputs of ATP and reduced NADP from the light-dependent stage, it cannot continue indefinitely in darkness, as these inputs will run out
- There are **three main steps** within the **Calvin cycle**:
  - **Rubisco** catalyses the **fixation of carbon dioxide** by combination with a molecule of **ribulose bisphosphate** (RuBP), a 5C compound, to yield two molecules of **glycerate 3-phosphate** (GP), a 3C compound
  - **GP is reduced** to **triose phosphate** (TP) in a reaction involving reduced NADP and ATP
  - **RuBP is regenerated** from TP in reactions that use ATP

Carbon fixation

- Carbon dioxide combines with a five-carbon (5C) sugar known as **ribulose bisphosphate** (RuBP)
- An **enzyme** called **rubisco** (ribulose bisphosphate carboxylase) catalyses this reaction
- The resulting six-carbon (6C) compound is **unstable** and splits in two
- This gives two molecules of a three-carbon (3C) compound known as **glycerate 3-phosphate** (GP)
- The carbon dioxide has been **'fixed'** (it has been removed from the external environment and has become part of the plant cell)
- Glycerate 3-phosphate (GP) is not a carbohydrate but the next step in the Calvin cycle converts it into one

Reduction of glycerate 3-phosphate

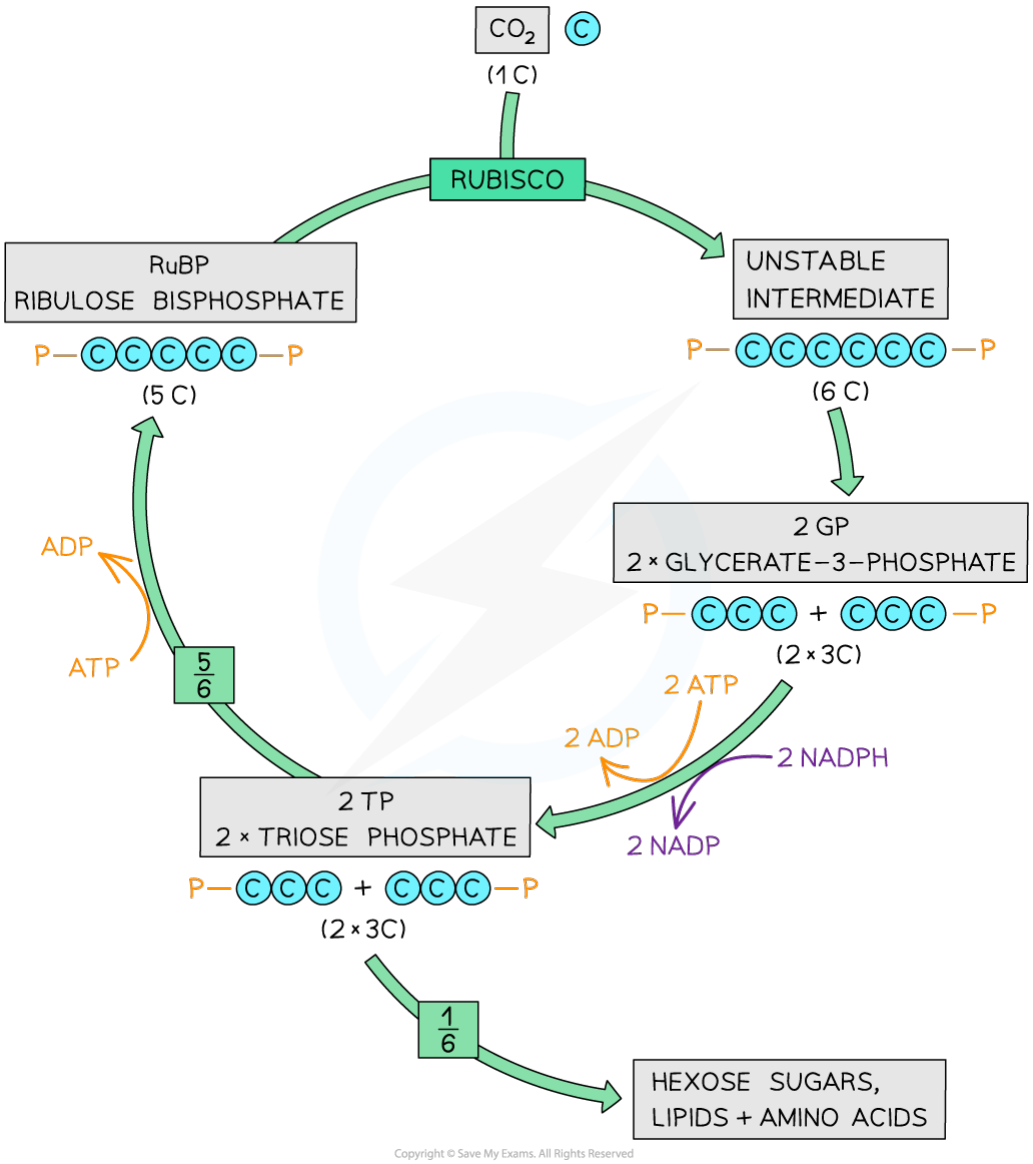
- **Energy from ATP** and **hydrogen from reduced NADP** – both produced during the light-dependent stage of photosynthesis – are used to **reduce glycerate 3-phosphate** (GP) to a phosphorylated three-carbon (3C) sugar known as **triose phosphate** (TP)
- **One-sixth** of the triose phosphate (TP) molecules are used to produce useful organic molecules needed by the plant

5.6 Photosynthesis

YOUR NOTES

Regeneration of ribulose bisphosphate

- **Five-sixths** of the triose phosphate (TP) molecules are used to **regenerate ribulose bisphosphate** (RuBP)
- This process **requires ATP**



The Calvin cycle

5.6 Photosynthesis

YOUR NOTES ⬇

Exam Tip

Make sure to learn when in the Calvin cycle ADP and NADP are produced as you will be expected to know it!

5.6 Photosynthesis

YOUR NOTES ⬇

5.6.8 USES OF TRIOSE PHOSPHATE

Uses of Triose Phosphate

- During the light-independent stage **energy from ATP** and **hydrogen from reduced NADP** (both products of the light-dependent stage) are used to **reduce glycerate 3-phosphate** (GP) to a phosphorylated three-carbon (3C) sugar known as **triose phosphate** (TP)
- **One-sixth** of the triose phosphate (TP) molecules are used to produce useful organic molecules needed by the plant:
  - Triose phosphates can condense to become **hexose phosphates** (6C), which can be used to produce **starch, sucrose or cellulose**
  - Triose phosphates can be converted to **glycerol** while glycerate 3-phosphates can be converted to **fatty acids.** These molecules join together to form **lipids for cell membranes**
  - Triose phosphates can be used in the **production of amino acids for protein synthesis**
- **Five-sixths** of the triose phosphate (TP) molecules are used to **regenerate ribulose bisphosphate** (RuBP). This process **requires ATP**

YOUR NOTES ⬇

5.6 Photosynthesis

5.6.9 FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

Factors That Limit the Rate of Photosynthesis

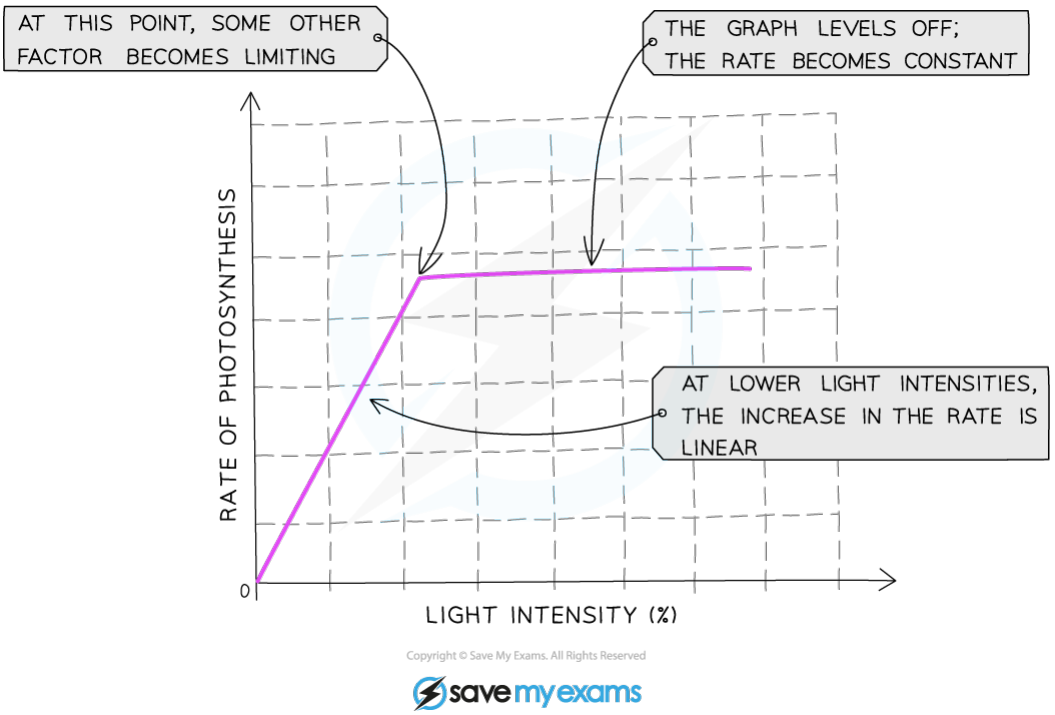
- Plants need several factors for **photosynthesis** to occur:
  - The presence of **photosynthetic pigments**
  - A supply of **carbon dioxide**
  - A supply of **water**
  - **Light** energy
  - A **suitable temperature**
- If there is a **shortage** of any of these factors, photosynthesis cannot occur at its **maximum possible rate**
- The main external factors that affect the rate of photosynthesis are:
  - **Light intensity**
  - **Carbon dioxide concentration**
  - **Temperature**
- These are known as **limiting factors** of photosynthesis
- If any one of these factors is **below the optimum level** for the plant, its rate of photosynthesis will be **reduced**, even if the other two factors are at the optimum level
- Although a lack of water can reduce the rate of photosynthesis, water shortages usually affect other processes in the plant before affecting photosynthesis and is therefore not one of the main limiting factors

Light intensity

- When temperature and carbon dioxide concentration remain constant, changes in light intensity affect the rate of photosynthesis
- **The rate of photosynthesis increases as light intensity increases**:
  - The greater the light intensity, the more energy supplied to the plant and therefore **the faster the light-dependent stage** of photosynthesis can occur
  - This **produces more ATP and reduced NADP for the Calvin cycle** (light-independent stage), which can then also occur at a greater rate
  - During this stage of the graph below, light intensity is said to be a limiting factor of photosynthesis
- At some point, if light intensity continues to increase, the relationship above will no longer apply and the rate of photosynthesis will reach a **plateau**
- At this point, **light intensity is no longer a limiting factor** of photosynthesis – another factor is limiting the rate of photosynthesis
- The factors which could be limiting the rate when the line on the graph is horizontal include **temperature** being too low or too high, or not enough **carbon dioxide**

5.6 Photosynthesis

YOUR NOTES



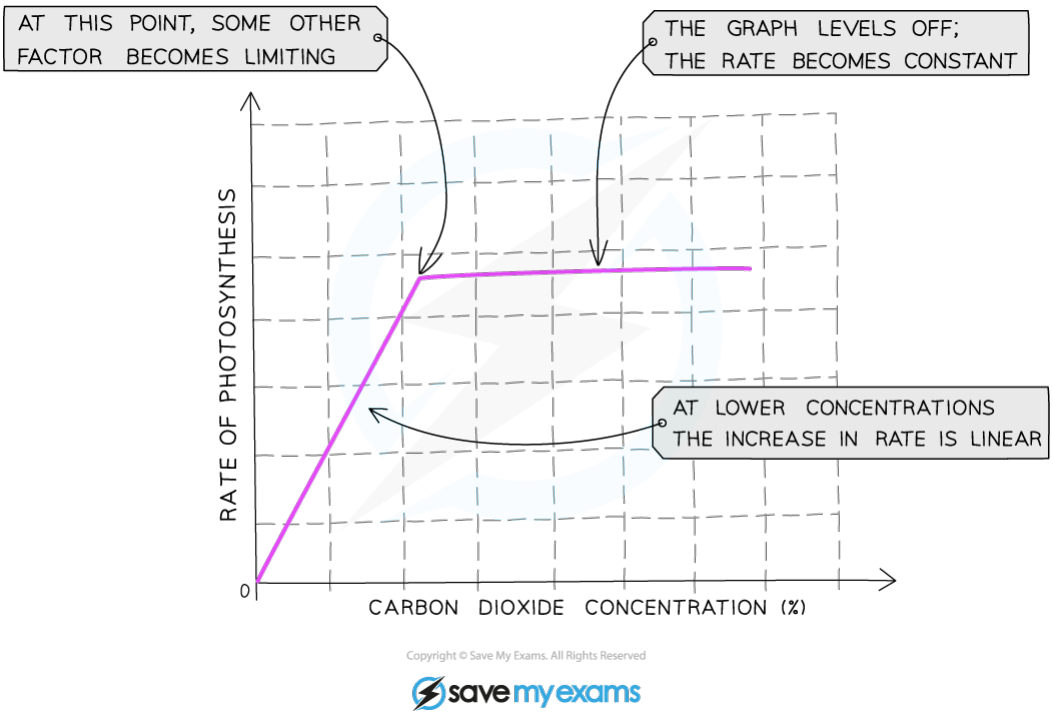
The effect of light intensity on the rate of photosynthesis

Carbon dioxide concentration

- **The rate of photosynthesis increases as carbon dioxide concentration increases**:
  - Carbon dioxide is one of the raw materials required for photosynthesis
  - It is required for the **light-independent stage of photosynthesis,** when CO_2 is combined with the five-carbon compound ribulose bisphosphate (RuBP) during **carbon fixation**
  - This means the more carbon dioxide that is present, the faster this step of the **Calvin cycle** can occur and **the faster the overall rate of photosynthesis**
- This trend will continue until some other factor required for photosynthesis prevents the rate from increasing further because it is in short supply
- The natural level of CO_2 in the atmosphere is **0.04%**, it is therefore not advisable to increase the CO_2 concentration much higher than this as it can become **toxic**
- The factors which could be limiting the rate when the line on the graph is horizontal include **temperature** being too low or too high, or not enough **light**

5.6 Photosynthesis

YOUR NOTES



The effect of carbon dioxide concentration on the rate of photosynthesis

5.6 Photosynthesis

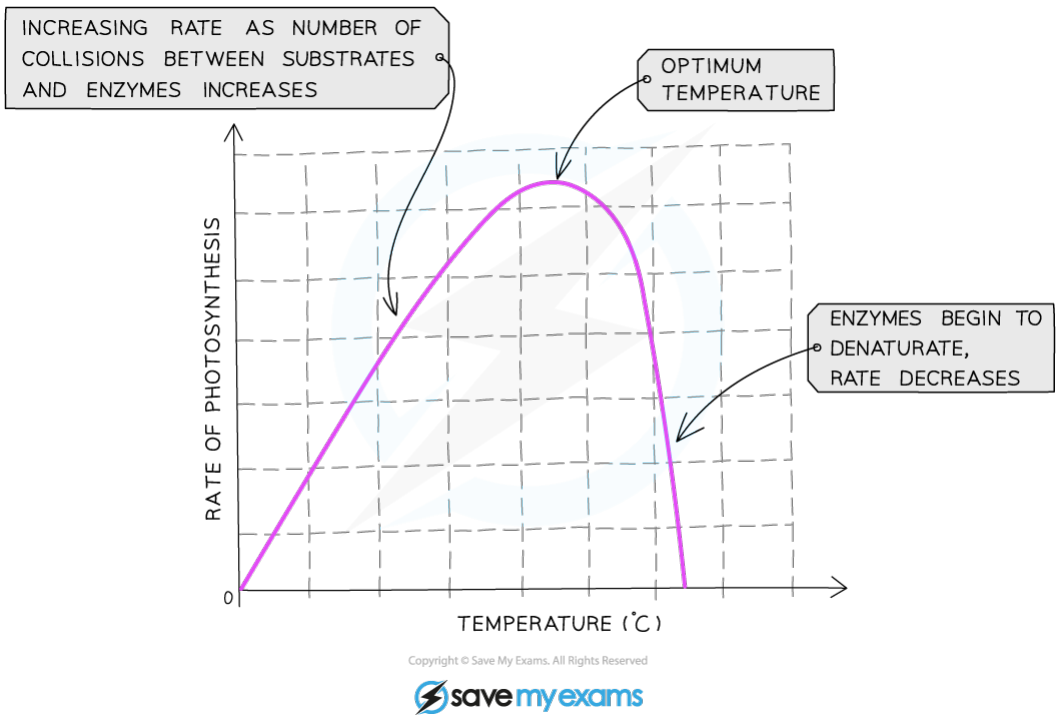
YOUR NOTES ⬇

Temperature

- **As temperature increases the rate of photosynthesis increases as the reaction is controlled by enzymes**
- However, as the reaction is controlled by enzymes, **this trend only continues up to a certain temperature** beyond which the enzymes begin to **denature** and the rate of reaction **decreases**
- For most metabolic reactions, temperature has a large effect on reaction rate
- For photosynthesis, **temperature has no significant effect on the light-dependent reactions**, as these are driven by energy from light rather than the kinetic energy of the reacting molecules
- However, **the Calvin cycle is affected by temperature, as the light-independent reactions are enzyme-controlled reactions** (eg. rubisco catalyses the reaction between CO_2 and the five-carbon compound ribulose bisphosphate)
- As long as there is enough light to produce ATP and NADPH in the light-dependent reaction, increasing temperature up to an **optimum temperature** (this will vary by species and what its natural habitat is) will increase the rate of the light-independent reactions and therefore the rate of photosynthesis
- Although the rate of enzymatic reactions is the main component affected by temperature, other components of the process can also be affected:
    - Increasing temperature causes **stomata** on the leaf to close in order to **reduce water loss**, when the stomata are closed CO_2 cannot enter the leaves – therefore, a balance must be met here
    - The light-dependent reaction relies on a proton gradient forming across the **thylakoid membrane** – it is important that a too high or too low temperature does not affect the **permeability** of the membrane which may lead to a dissipation of the proton gradient

5.6 Photosynthesis

YOUR NOTES



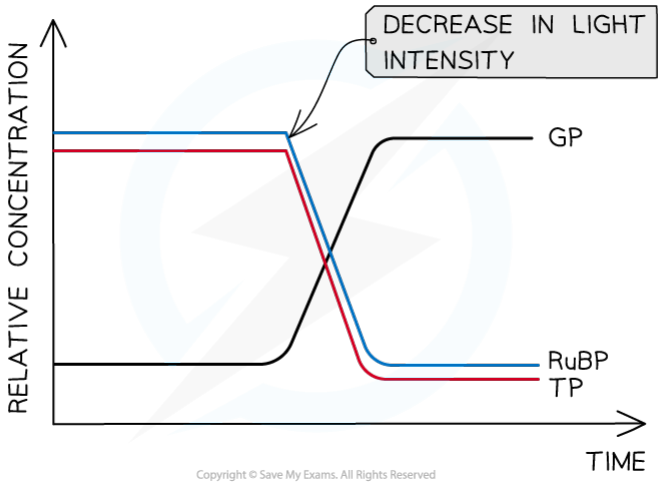
The effect of temperature on the rate of photosynthesis

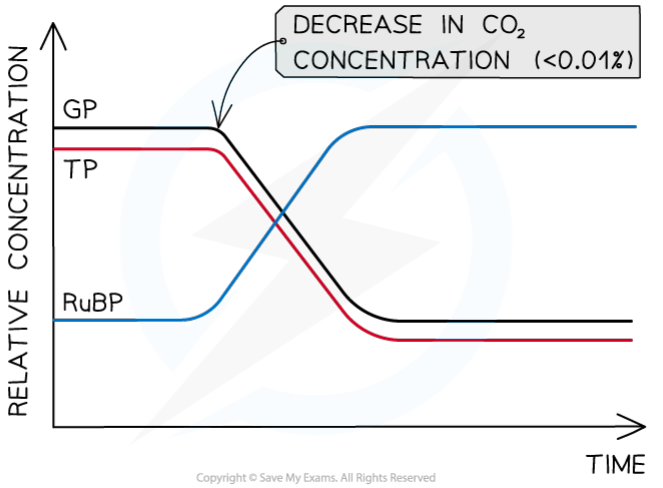
The effect of the light intensity and carbon dioxide concentration on levels of GP, TP and RuBP

- The concentrations of glycerate 3-phosphate (GP), triose phosphate (TP) and ribulose bisphosphate (RuBP) within chloroplasts can be affected by changes to light intensity and carbon dioxide concentration
- A decrease in light intensity causes a **decrease in TP and RuBP** concentrations but a **slight increase in GP** concentration
    - When there is less light available the **light-dependent stage stops** and does not form any more products needed for the light-independent stage (ATP and NADPH)
    - As a consequence, GP builds up as it is **not converted to TP**
    - A lack of TP means that RuBP will not form
    - Over time the fixation of carbon dioxide will stop and the concentration of GP will plateau
- Very low concentrations of carbon dioxide (less than 0.01%) causes a **decrease in the concentration of GP and TP** but an **increase in RuBP** concentration
    - RuBP **accepts carbon dioxide** so when there is a lack of carbon dioxide molecules it remains **unfixed and builds up**
    - The lack of carbon dioxide fixation prevents GP and TP molecules from forming

5.6 Photosynthesis

YOUR NOTES





A decrease in light intensity and carbon dioxide concentration has different effects on the concentrations of GP, TP and RuBP.

5.6 Photosynthesis

YOUR NOTES ⬇

Agricultural practices balance limiting factors

- An understanding of the effect of limiting factors on the rate of photosynthesis can be used to **increase crop yields** in **protected environments**, such as **glasshouses**
- In the most sophisticated glasshouses, for example, **sensors** can be used to monitor the **light intensity**, the **humidity** of the atmosphere and the **carbon dioxide concentration** around the crops
    - This means that plants could continue to grow through the night if they are kept lit with artificial lighting
    - Plants can be grown out of their natural season and habitat because the temperature can be kept constant all year round
- All these factors can be **managed** by a computer and their levels **adjusted** to ensure the crop can **photosynthesis at the highest rate possible**
- Water can be supplied by irrigation systems throughout the glasshouse or fields which sometimes contain added fertilisers or growth nutrients such as nitrates to aid plant growth
- Natural pests that may spread disease or eat the crops can be controlled within agricultural settings by pesticides or by separating the plants from the unfiltered outside air
- This **maximises the yield** of the crop
- Farmers have to find a **balance** between crop **yield** and the **cost** of maintaining 24-hour lighting and year-round heating as well as the **environmental implications** this has

Exam Tip

Interpreting graphs of limiting factors can be confusing for many students, but it's quite simple.

In the section of the graph where the rate is increasing (the line is going up), the limiting factor is whatever the label on the x-axis (the bottom axis) of the graph is.

In the section of the graph where the rate is not increasing (the line is horizontal), the limiting factor will be something other than what is on the x-axis – choose from temperature, light intensity or carbon dioxide concentration.

5.6 Photosynthesis

YOUR NOTES ⬇

5.6.10 PRACTICAL: INVESTIGATING FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

Practical: Investigating Factors Affecting the Rate of Photosynthesis

- Investigations to determine the effects of light intensity, carbon dioxide concentration and temperature on the **rate of photosynthesis** can be carried out using **aquatic plants**, such as *Elodea* or *Cabomba* (types of **pondweed**)
- The effect of these limiting factors on the rate of photosynthesis can be investigated in the following ways:
  - **Light intensity** – change the distance (d) of a light source from the plant (light intensity is proportional to $1/d^2$)
  - **Carbon dioxide concentration** – add different quantities of sodium hydrogencarbonate ($NaHCO_3$) to the water surrounding the plant, this dissolves to produce CO_2
  - **Temperature (of the solution surrounding the plant)** – place the boiling tube containing the submerged plant in water baths of different temperatures
- Whilst changing one of these factors during the investigation (as described below), **ensure the other two remain constant**
  - For example, when investigating the effect of light intensity on the rate of photosynthesis, a glass tank should be placed in between the lamp and the boiling tube containing the pondweed to absorb heat from the lamp – this prevents the solution surrounding the plant from changing temperature

Apparatus

- Distilled water
- Test tube
- Beaker
- Lamp
- Aquatic plant, algae or algal beads
- Ruler
- Sodium hydrogen carbonate solution
- Thermometer
- Test tube plug
- Syringe

5.6 Photosynthesis

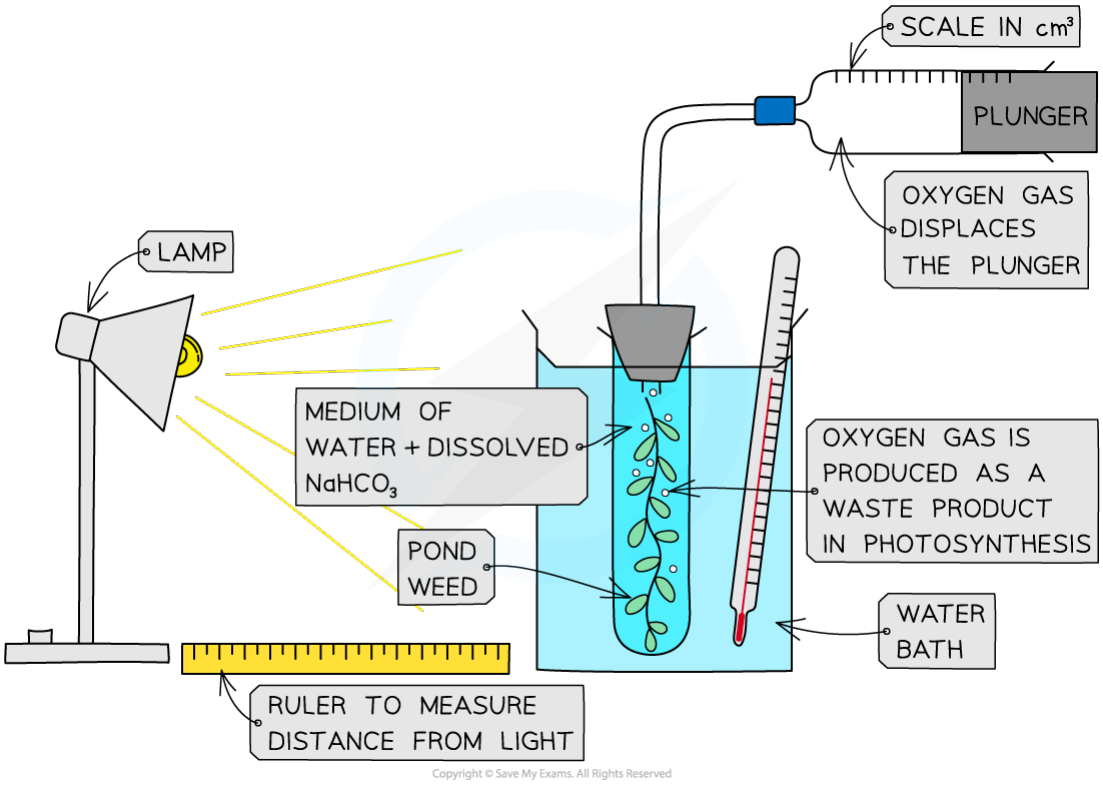
YOUR NOTES ⬇

Method

- Ensure the water is **well aerated** before use by **bubbling air through it**
  - This will ensure oxygen gas given off by the plant during the investigation form **bubbles** and **do not dissolve in the water**
- Ensure the plant has been **well illuminated** before use
  - This will ensure that the plant contains all the enzymes required for photosynthesis and that any changes of rate are due to the independent variable
- Set up the apparatus in a **darkened room**
  - Ensure the pondweed is submerged in **sodium hydrogen carbonate solution (1%)** – this ensures the pondweed has a controlled **supply of carbon dioxide** (a reactant in photosynthesis)
- Cut the stem of the pondweed **cleanly** just before placing into the boiling tube
- Measure the volume of gas collected in the gas-syringe in a **set period of time** (eg. 5 minutes)
- **Change the independent variable** (ie. change the light intensity, carbon dioxide concentration or temperature depending on which limiting factor you are investigating) and repeat step 5
- Record the results in a table and plot a graph of volume of oxygen produced per minute against the distance from the lamp (if investigating light intensity), carbon dioxide concentration, or temperature

5.6 Photosynthesis

YOUR NOTES



The effect of light intensity on an aquatic plant is measured by the volume of oxygen produced

Results – Light Intensity

- The closer the lamp, the higher the light intensity (intensity ∝ $1/d^2$)
- Therefore, the volume of oxygen produced should increase as the light intensity is increased
- At a point, the volume of oxygen produced will stop changing even if the light is moved closer
    - This is when the light stops being the limiting factor and the temperature or concentration of carbon dioxide is limiting the rate of photosynthesis
    - The effect of these variables could then be measured by increasing the temperature of water (by using a water bath) or increasing the concentration of sodium hydrogen carbonate respectively
- The results should be displayed on a graph of light intensity vs. rate of photosynthesis
    - **Rate of photosynthesis = volume of oxygen produced ÷ time elapsed**

5.6 Photosynthesis

YOUR NOTES ⬇

Limitations

- Algae is often used in experiments on photosynthesis and respiration rates but it can be very hard to maintain consistency in the number of algae and it can be hard to handle directly in the water
    - Immobilised algae beads are beads of jelly with a known surface area and volume that contain algae, therefore it is easier to ensure a standard quantity
    - Immobilised algae beads are easy and cheap to grow, they are also easy to keep alive for several weeks and can be reused in different experiments
    - The method is the same for algae beads though it is important to ensure sufficient light coverage for all beads

5.6 Photosynthesis

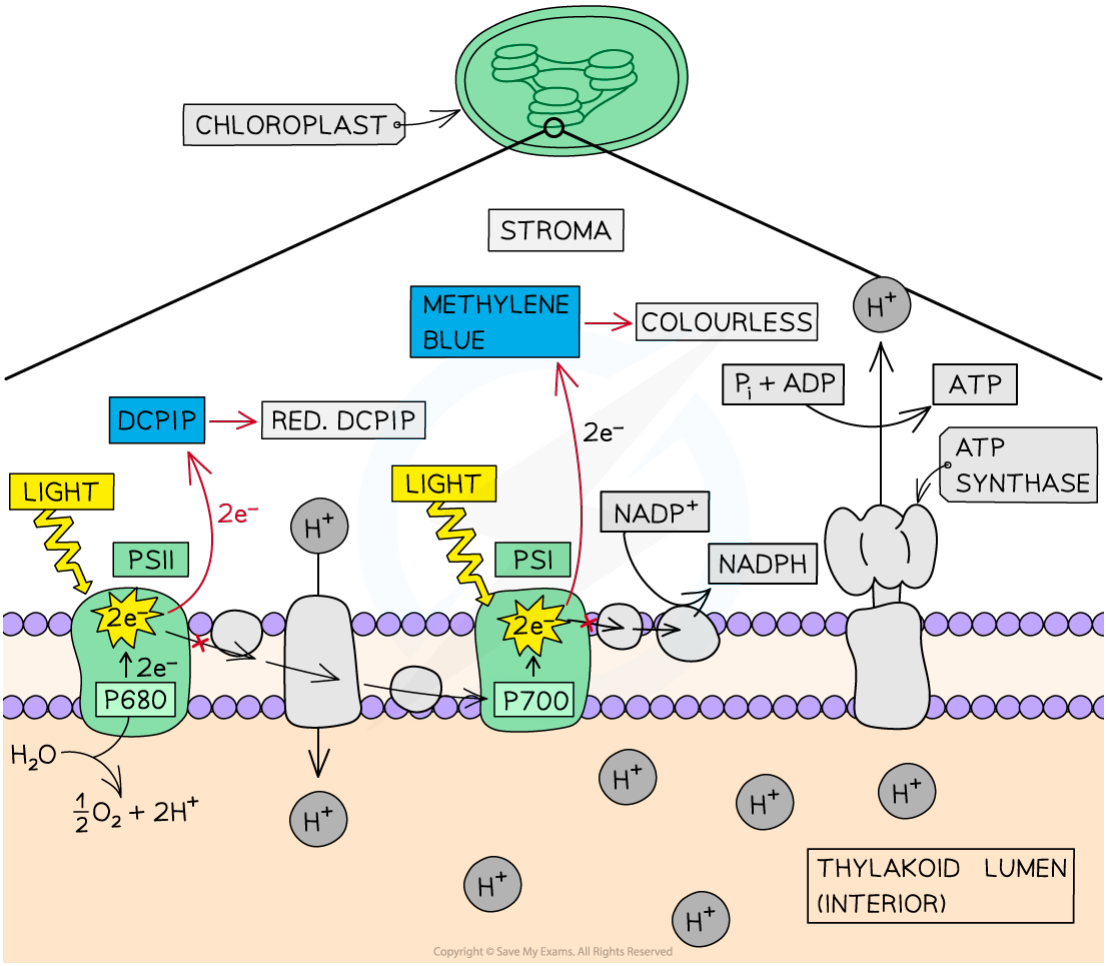
YOUR NOTES ⬇

Practical: Measuring the rate of the light-dependent stage of photosynthesis

- The **light-dependent reactions** of photosynthesis take place in the thylakoid membrane and involve the **release of high-energy electrons** from chlorophyll *a* molecules
- These electrons are **picked up by the electron acceptor NADP** in a reaction catalysed by the enzyme **dehydrogenase**
- However, if a **redox indicator** (such as **DCPIP** or **methylene blue**) is present, the indicator **takes up the electrons instead of NADP**
- This causes the indicator to **change colour**
    - DCPIP: oxidised (**blue**) → accepts electrons → reduced (**colourless**)
    - Methylene blue: oxidised (**blue**) → accepts electrons → reduced (**colourless**)
    - *The colour of the reduced solution may appear green because chlorophyll produces a* ***green colour***
- The **rate** at which the redox indicator changes colour from its **oxidised** (blue) state to its **reduced** (colourless) state can be used as a measure of the **rate of dehydrogenase activity** and therefore, the **rate of the light-dependent stage of photosynthesis**
    - When light is at a higher intensity, or at more preferable light wavelengths, the rate of photoactivation of electrons is faster, therefore the rate of reduction of the indicator is faster

5.6 Photosynthesis

YOUR NOTES ⬇



The light activates electrons from chlorophyll molecules during the light-dependent reaction. Redox indicators accept the excited electrons from the photosystem, becoming reduced and therefore changing colour.

Apparatus

- Leaves
- Isolation medium
- Pestel and mortar
- Lamp
- Test tubes
- Stopwatch
- Aluminium Foil
- DCPIP or methylene blue indicator
- Buffer solution

5.6 Photosynthesis

YOUR NOTES ⬇

Method – Measuring light as a limiting factor

- Leaves are crushed in a liquid known as an **isolation medium**
  - This produces a concentrated leaf extract that contains a **suspension of intact and functional chloroplasts**
  - The medium must have the **same water potential** as the leaf cells so the chloroplasts don't shrivel or burst and contain a **buffer** to keep the pH constant
  - The medium should also be **ice-cold** (to avoid damaging the chloroplasts and to maintain membrane structure)
- The experiment should be set up in a dark room so that the light source and intensity can be controlled
  - The room should be at an adequate temperate for photosynthesis and maintained throughout, as should carbon dioxide concentration
- Small tubes are set up with **different intensities**, or **different colours (wavelengths)** of light shining on them
  - If different intensities of light are used, they must all be of the same wavelength (same colour of light) – light intensity is altered by changing the distance between the lamp and the test tube
  - If different wavelengths of light are used, they must all be of the same light intensity – the lamp should be the same distance in all experiments
- **DCPIP** or **methylene blue** indicator is added to each tube, as well as a small volume of the leaf extract
- A control that is not exposed to light (wrapped in aluminium foil) should also be set up to ensure the affect on colour is due to the light
- The **time taken** for the redox indicator to go **colourless** (or green, as the chlorophyll may also colour the solution) is recorded
  - This is a measure of the **rate of photosynthesis**

Results

- A graph should be plotted of absorbance against time for each distance from the light
- As the light intensity decreases, the rate of photosynthesis also decreases
  - This is because the lowered light intensity will slow the rate of photoionisation of the chlorophyll pigment, so the overall rate of the light dependent reaction will be slower
  - This means that less electrons are released by the chlorophyll, hence the DCPIP accepts less electrons. This means that it will take longer to turn from blue to colourless
- When the DCPIP is blue, the absorbance is higher. The rate at which the absorbance decreases can therefore be used to determine the activity of the dehydrogenase enzyme
  - A higher rate of decrease, shown by a steep gradient on the graph, indicates that the dehydrogenase is highly active.

5.6 Photosynthesis

YOUR NOTES ⬇

Limitations

- This experiment is not measuring the rate of dehydrogenase activity directly (through measuring the rate of substrate use or product made) but is instead predicting what the rate would be by measuring the rate of electron transfer from the photosystems
- The concentration of DCPIP will depend on the number of chloroplasts in a sample and therefore the number of light-dependent electron transport chains
    - It is therefore important to control the amount of leaf used to produce the chloroplast sample and also how much time is spent crushing the leaf to release the chloroplast
    - It is also a good idea to measure a specific wavelength absorption by each sample on the colorimeter before and after the experiment so you can get a more accurate change in oxidised DCPIP concentration
    - Results should also be repeated and the mean value calculated
- The time taken to go colourless is subjective to each person observing and therefore one person should be assigned the task of deciding when this is

Exam Tip

Learn the 3 limiting factors and how each one can be altered in a laboratory environment:

• Light intensity – the distance of the light source from the plant (intensity $\propto 1/d^2$)

• Temperature – changing the temperature of the water bath the test tube sits in

• Carbon dioxide – the amount of $NaHCO_3$ dissolved in the water the pondweed is in

Also, remember that the variables not being tested (the control variables) must be kept constant.