

CORE IDEA 3: ENERGY AND EQUILIBRIUM
TOPIC 3.1 PHOTOSYNTHESIS

Learning Outcomes:

- (a) Identify components of chloroplasts in drawings, photomicrograph and electron micrographs.
 - (b) Explain the absorption and action spectra of photosynthetic pigments.
 - (c) With reference to the chloroplast structure, describe and explain how light energy is harnessed and converted into chemical energy during the light-dependent reactions of photosynthesis.
 - (d) Outline the three phases of Calvin cycle in C₃ plants: (i) CO₂ fixation, (ii) PGA reduction and (iii) ribulose biphosphate (RuBP) regeneration, indicating the roles of rubisco, ATP and reduced NADP in these processes that ultimately allow synthesis of sugars.
 - (e) Discuss limiting factors in photosynthesis and carry out investigations on the effect of limiting factors such as temperature, light intensity and carbon dioxide concentration on the rate of photosynthesis.
 - (f) Outline chemiosmosis in photosynthesis. (names of complexes in the ETC are not required.)
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References:

Campbell and Reece. *Biology*. (11th edition). Chapter 11: Photosynthetic Processes, pg.259 – 283 (or corresponding chapter in other editions)

Note: This textbook is available in our library. You may wish to borrow it to supplement your reading when necessary.

1) Introduction

- The Chloroplast
- Photosynthetic Pigments
- Absorption and Action Spectra
- Stages of Photosynthesis

2) Light-Dependent Stage

- Non-cyclic Photophosphorylation
- Cyclic Photophosphorylation
- Chemiosmosis

3) Light-Independent Stage**4) Factors Affecting Rate of Photosynthesis**

- Light Intensity
- Carbon Dioxide Concentration
- Temperature

1 INTRODUCTION

- Most plants synthesise their own organic molecules from carbon dioxide and other inorganic raw materials (such as water and minerals) obtained from the environment.
- They use light as a source of energy to synthesise these organic compounds. This light is absorbed by photosynthetic pigments present in the chloroplasts of leaf cells or green organs of the plant.
- Photosynthesis is the conversion of **light energy** to **chemical energy** that is stored in sugars or other organic compounds
- The process of photosynthesis can be summarised by the following equation:

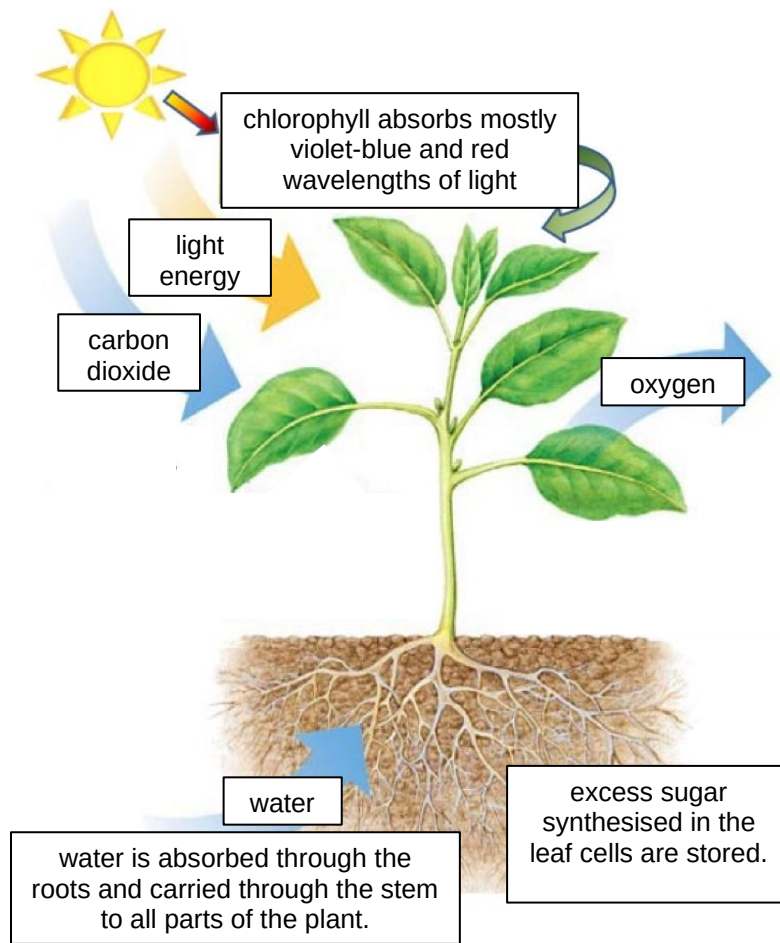
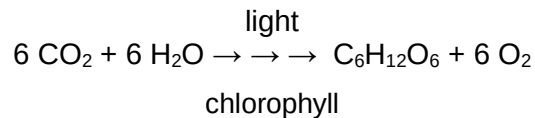


Fig. 1.1: Plants as autotrophs

1.1 THE CHLOROPLAST

Learning Outcome (a):

Identify components of chloroplasts in drawings, photomicrograph and electron micrographs.

- Chloroplasts, found in plants and algae, are the **sites of photosynthesis**. They convert light energy to chemical energy by absorbing light and using it to drive the synthesis of organic compounds (such as sugars) from carbon dioxide and water.
- Chloroplast are 3-10 μm in diameter, and are **visible under the light microscope**.
- The chloroplast is a **double membrane-bound** organelle, with an **outer and inner chloroplast membrane**.

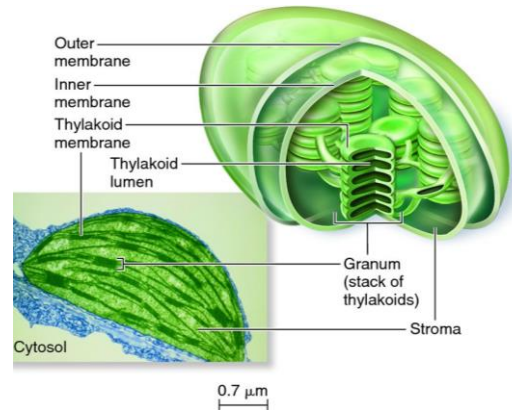


Fig. 1.1.1: Structure of chloroplast

Part of chloroplast	Structure	Function
Thylakoid	Chloroplast contains an internal membrane system known as the <u>thylakoid system</u> Thylakoids are flat, interconnected sacs bound by a membrane known as <u>thylakoid membrane</u>. Thylakoid membrane is embedded with photosynthetic pigments (e.g. chlorophyll), proteins such as ATP synthase and electron transport chain. Within the thylakoid is the thylakoid space / lumen. Thylakoids will stack up to form a <u>granum</u> (plural: grana), which are connected to each other by <u>intergranal lamellae</u>.	Site of Light-dependent Stage Thylakoid membrane: 1. Affords a <u>large surface area</u> to accommodate the <u>electron transport chain</u> and to absorb light energy. 2. <u>Impermeable to charged particles</u> such as of H^+ ions, enabling the generation of a <u>proton gradient</u>. 3. Contains <u>ATP synthase</u>, which phosphorylates ADP to ATP by chemiosmosis.
Stroma	The fluid <u>outside</u> the thylakoid system is called <u>stroma</u> and it contains starch grains, enzymes, circular double-stranded DNA and ribosomes.	Site of Light-independent Stage/ Calvin Cycle

1.2 PHOTOSYNTHETIC PIGMENTS

- Photosynthetic pigments absorb light for the plant's use. Different pigments absorb different wavelengths of light. Non-absorbed wavelengths are reflected.
 - E.g. Blue and red light are absorbed by chlorophyll, causing green light to be reflected. This gives leaves their signature green colour.
 - E.g. Blue and green light are absorbed by carotenoids, causing yellow, orange, red light to be reflected. Hence leaves appear to be such colours during autumn as chlorophyll levels decrease.

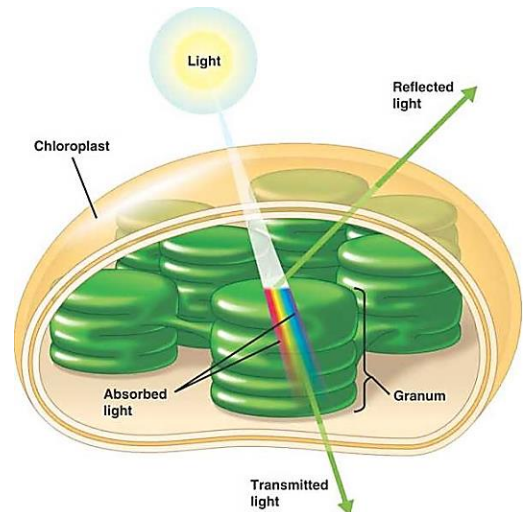


Fig. 1.2.1: Absorption of light by pigments in the chloroplast

- Chloroplasts contain the following photosynthetic pigments:
 - Chlorophyll**
 - Chlorophyll (e.g. chlorophyll *a* and *b*) absorbs light maximally at the blue-violet (400 – 500nm) and red regions (600 – 700nm) of the visible light spectrum. Green light is reflected to give chlorophyll its characteristic colour. (refer to 1.4 Absorbance and Action Spectra)
 - Chlorophyll functions to absorb light energy for photoexcitation of electrons during photoactivation.
 - Carotenoids**
 - Carotenoids [e.g. carotene (yellow / orange) and xanthophylls (yellow)] act as accessory pigments to broaden the spectrum of light absorbed for photosynthesis.
 - It also serves in photoprotection, preventing damage by absorbing excess light energy.

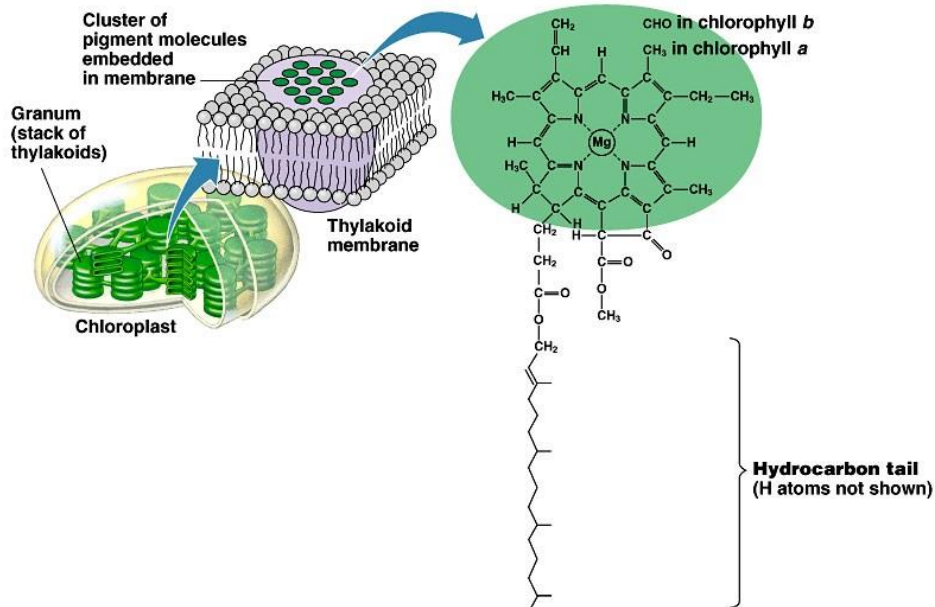


Fig. 1.2.2: Structure of chlorophyll

- These photosynthetic pigments are found in **photosystems** which are **embedded on the thylakoid membrane**. Photosystems are protein complexes containing:
 - **Reaction Centre:**
 - contains special chlorophyll a molecules (e.g. P680 or P700) with a primary electron acceptor.
 - **Light Harvesting Complex:**
 - made up of hundreds of accessory pigment molecules (e.g. chl a, chl b and carotenoids).
 - Light Harvesting Complex surrounds the reaction centre and channels energy absorbed from light to it.

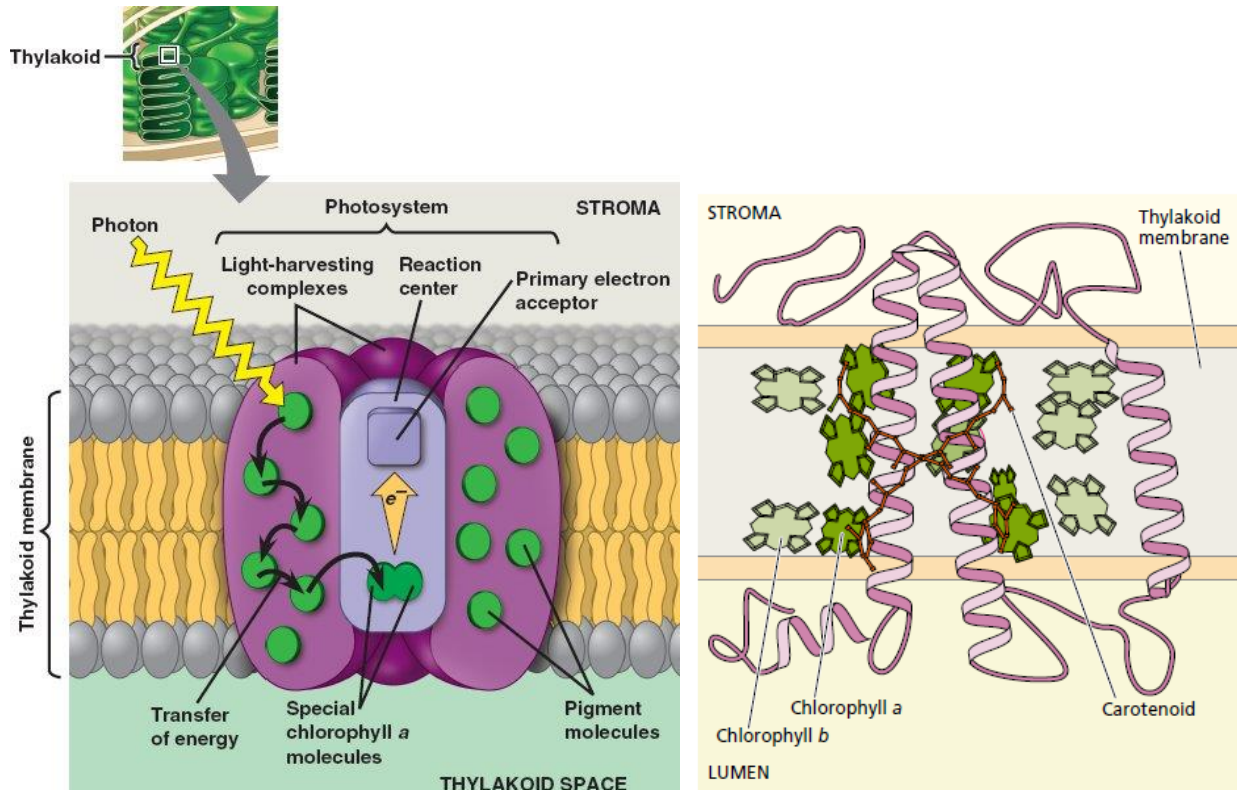
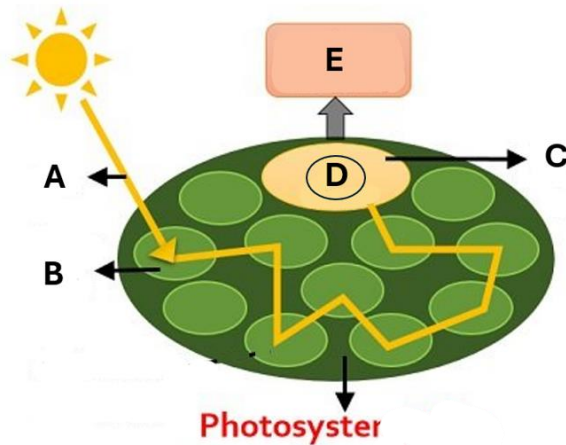


Fig. 1.2.3: A photosystem embedded in the thylakoid membrane

- There are two photosystems present in chloroplasts – **photosystem I (PS I)** absorbs light optimally at **700 nm**, while **photosystem II (PS II)** absorbs light optimally at **680 nm**.

CHECKPOINT 1.2

1 Name the components A, B, C, D and E in the diagram below.



- A _____
- B _____
- C _____
- D _____
- E _____

2 Where is B found in the photosystem?

- A Reaction Centre
- B Primary Electron Donor
- C Primary Electron Acceptor
- D Light Harvesting Complex

3 Which options below is the primary electron donor?

- A Carotenoids
- B Accessory pigments
- C Chlorophyll
- D Special chlorophyll a

4 What is the purpose of having a primary electron donor?

- A Photoprotection
- B Photoexcitation during photoactivation
- C Donate an electron to primary electron acceptor
- D Convert light energy to chemical energy

*there can be more than 1 right answer for this question

1.3 ABSORPTION & ACTION SPECTRA

Learning Outcome (b):

Explain the absorption and action spectra of photosynthetic pigments.

- An **absorption spectrum** plots the **amount of light absorbed** at each wavelength by a particular pigment.
- An **action spectrum** plots the **rate of photosynthesis** at each wavelength due to all pigments. It is a record of the amount of photosynthesis occurring at each wavelength of light.

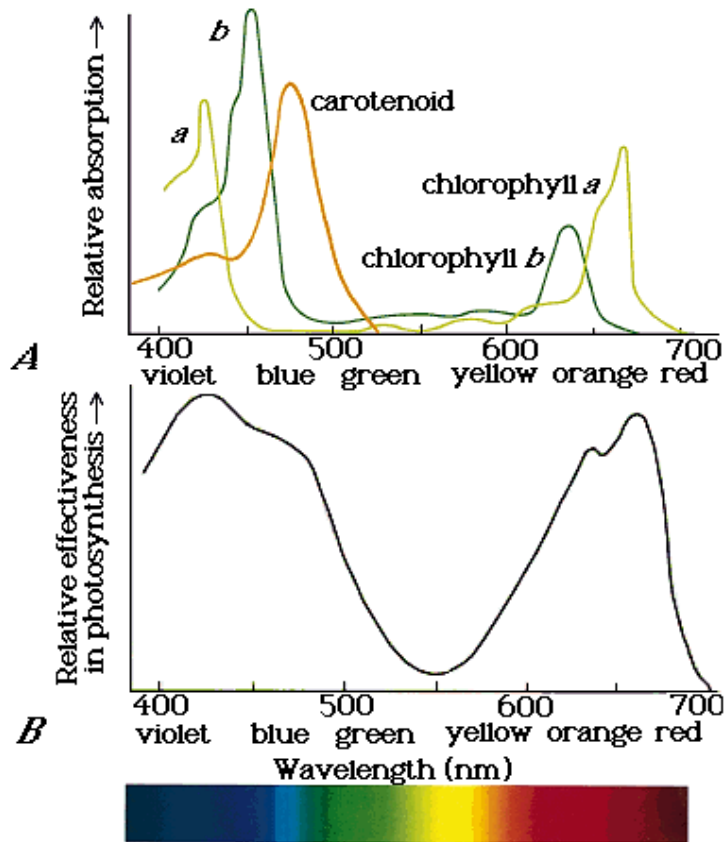


Fig. 1.3.1: Absorption spectrum (A) and action spectrum (B)

- From the absorption spectra, chlorophyll absorbs violet-blue and red light best. From the action spectra, photosynthesis is also the most effective at violet-blue and red light.

- However, comparing the absorption spectra of chlorophyll and the action spectrum does not result in an exact match. Hence, it can be concluded that other accessory pigments (e.g. carotenoids) with different absorption spectra are also involved in photosynthesis.

1.4 STAGES OF PHOTOSYNTHESIS

- Photosynthesis occurs in two main stages:

1) Light-dependent Stage

- The light-dependent stage involves the **conversion of light energy to chemical energy**, where light energy absorbed by chlorophyll results in flow of electrons that produces **ATP** and **reduced nicotinamide adenine dinucleotide phosphate (reduced NADP / NADPH)**.

2) Light-independent Stage

- The light-independent stage utilises chemical energy (from light-dependent stage) in the **Calvin cycle** to **reduce carbon dioxide** in order to **synthesise sugars**.

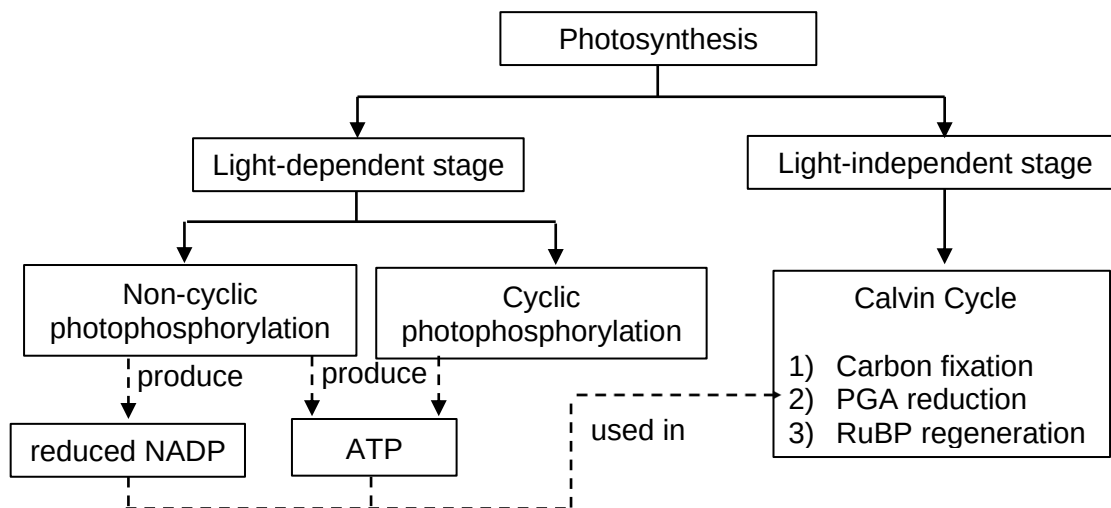


Fig. 1.4.1: Overview of photosynthesis

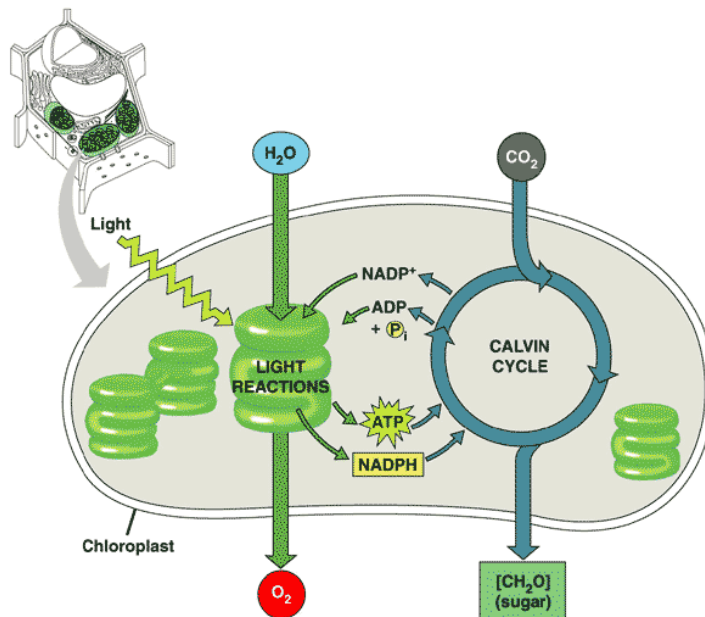


Fig. 1.4.2: Overview of photosynthesis within chloroplast

2 LIGHT-DEPENDENT STAGE

Learning Outcome (c):

With reference to the chloroplast structure, describe and explain how light energy is harnessed and converted into chemical energy during the light-dependent reactions of photosynthesis.

- The light-dependent stage occurs in the **presence of light** and is located on the **thylakoid membrane**. It can occur via **non-cyclic** or **cyclic photophosphorylation**.

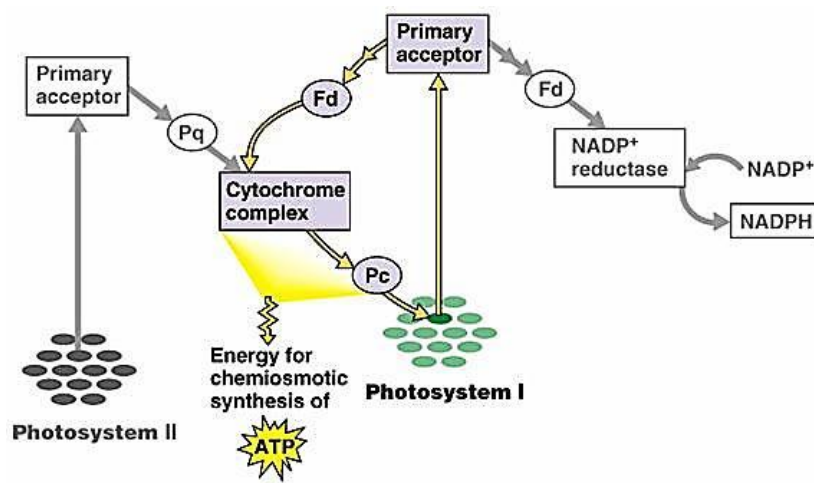


Fig. 2: Overview of cyclic and non-cyclic photophosphorylation

2.1 NON-CYCLIC PHOTOPHOSPHORYLATION

- Non-cyclic photophosphorylation involves absorption of light by both **PSI** and **PSII**, and the subsequent production of **ATP** and **reduced NADP / NADPH**.

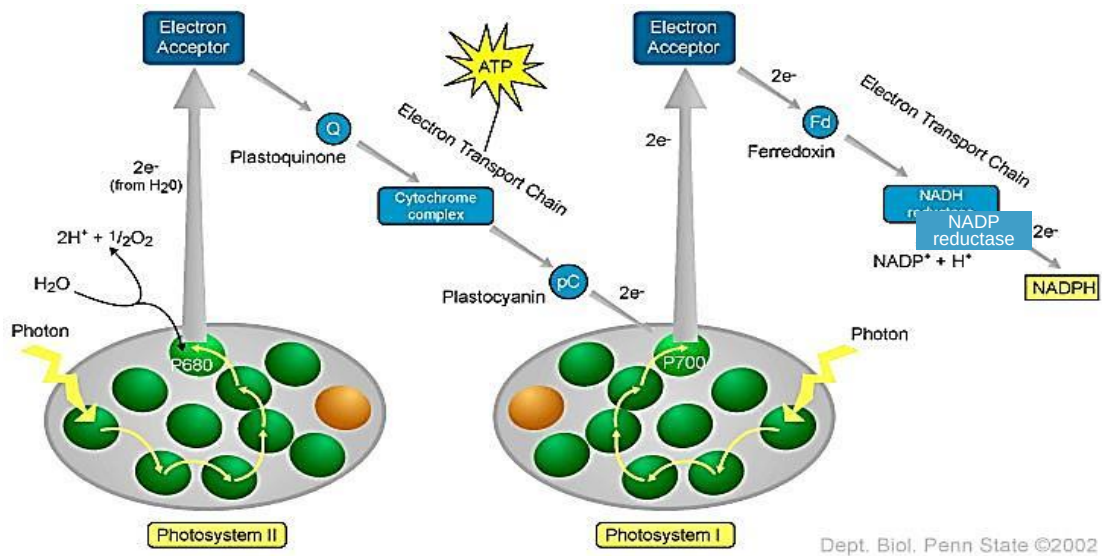


Fig. 2.1.1: Non-cyclic photophosphorylation

- A **photon** of light is collected by the accessory pigments in the **light-harvesting complex (LHC)**. The energy is then passed on to neighbouring pigments and finally to a pair of special chlorophyll a (P680) in the **reaction centre** of PSII.

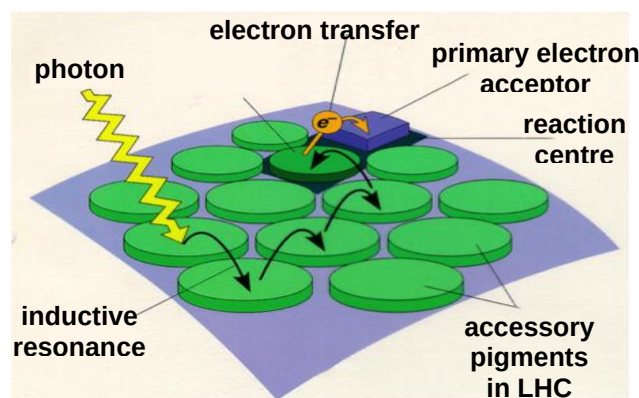


Fig. 2.1.2: Energy transfer

- The special chlorophyll a (P680) is **photoactivated**, where a pair of electrons is **photoexcited** (raised by light energy) to a higher energy level.

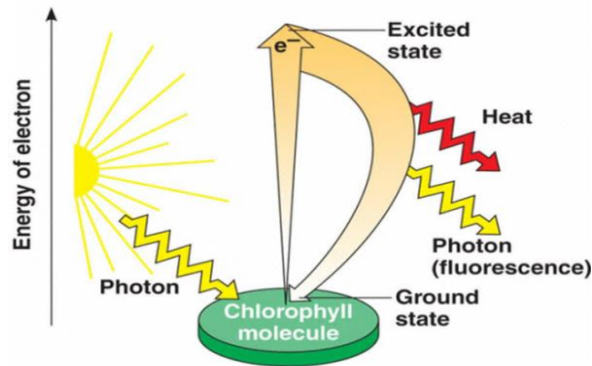
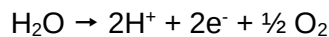


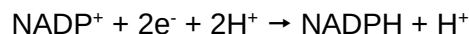
Fig. 2.1.3: Photoactivation of special chlorophyll a

3. The pair of excited electrons is then passed onto the **primary electron acceptor** in the reaction centre of PSII.
4. The pair of electrons from the primary electron acceptor will be passed through a series of electron carriers in the **electron transport chain (ETC)** present in the thylakoid membrane. Each electron carrier in the chain has an energy level lower than the one preceding it. The electrons are **passed down the series of electron carriers** (in the ETC) through repeated oxidation and reduction (redox) reactions. **Energy is lost** as electrons are passed along the ETC.
**Energy lost is coupled to proton pumping in chemiosmosis (ref to 2.3 Chemiosmosis)*

5. The **electron deficit in P680** will then be replaced by electrons from the **photolysis** of a molecule of water mediated by **Manganese (Mn)-containing enzyme** (aka oxygen-evolving complex). H⁺ ions are released and **oxygen** is evolved (as a by-product). Water is therefore the **primary electron donor**.



6. As photoactivation of a pair of special chlorophyll a (P700) in Photosystem I (PSI) occurs simultaneously as P680 in PSII, PSI will also have an electron deficit. Hence, the pair of electrons lost from PSII (which has travelled through the electron transport chain) will be accepted by PSI.
7. The photoexcited pair of electrons in P700 will be accepted by another **primary electron acceptor** in reaction centre of PSI.
8. **NADP** will act as the **final electron acceptor** and accepts the pair of electrons (from PSI) and 2H⁺ ions to become **reduced NADP / NADPH**. This process is catalysed by **NADP reductase**.



Recall your knowledge on REDOX reactions!

Oxidation: loses e^- , loses H^+ , gains O_2 , increase in oxidation state

Reduction: gains e^- , gains H^+ , loses O_2 , decrease in oxidation state

!! Since $NADP^+$ gains e^- and H^+ , $NADP$ is reduced to form $NADPH$!!

Experimental Evidence (Further Reading):**Does the oxygen produced during photosynthesis come from carbon dioxide or water?**

- An experiment was conducted using radioactive isotope labelling, where the oxygen atom in water was labelled with heavy oxygen, ^{18}O . The experimental results produced the following equation:



- Therefore, three conclusions were made from this experiment:
 - (i) Photosynthesis is a two-stage process.
 - Stage 1:** Water molecules are hydrolysed using light energy to form hydrogen and oxygen (photolysis of water). Oxygen is released as a by-product.
 - Stage 2:** Hydrogen combines with carbon dioxide to produce carbohydrate.
 - (ii) Oxygen produced comes from the two water molecules.
 - (iii) A new water molecule is produced during photosynthesis.

CHECKPOINT 2.1

1 Arrange steps involved in the light-dependent reactions of photosynthesis in the correct sequence.

1. Electrons lost by P680 in PSII are passed through the electron transport chain.
2. NADP⁺ is reduced to NADPH at the end of the light reactions.
3. Light energy excites electrons in P700 of PSI, leading to photoactivation.
4. Electrons lost from P700 in PSI are replaced by electrons originally excited in PSII.
5. Light energy excites electrons in P680 of PSII, leading to photoactivation.

- A** 3 → 4 → 5 → 1 → 2
B 3 → 5 → 1 → 4 → 2
C 5 → 1 → 3 → 4 → 2
D 5 → 3 → 1 → 4 → 2

2 Which of the follow molecule is the final electron acceptor?

- A** ATP
B NADP
C NADPH
D Water

3 After photoactivation, both PSI and PSII experience an electron deficit.

Which of the following options show the correct sources of electrons that replenish the electron deficits in each photo system?

	PSI	PSII
A	Photolysis of water	NADPH
B	Electrons from PSII	Photolysis of water
C	NADPH	Photolysis of oxygen
D	Electrons from PSII	NADPH

2.2 CYCLIC PHOTOPHOSPHORYLATION

- In the absence of water and a higher demand for ATP, cyclic photophosphorylation occurs to produce only **ATP**. Only **PSI** is involved in this process; hence transfer of electrons takes a cyclic path where **P700** is both the **primary electron donor** and the **final electron acceptor**.
- and a higher demand for ATP, cyclic photophosphorylation occurs to produce only **ATP**. Only **PSI** is involved in this process; hence transfer of electrons takes a cyclic path where **P700** is both the **primary electron donor** and the **final electron acceptor**.

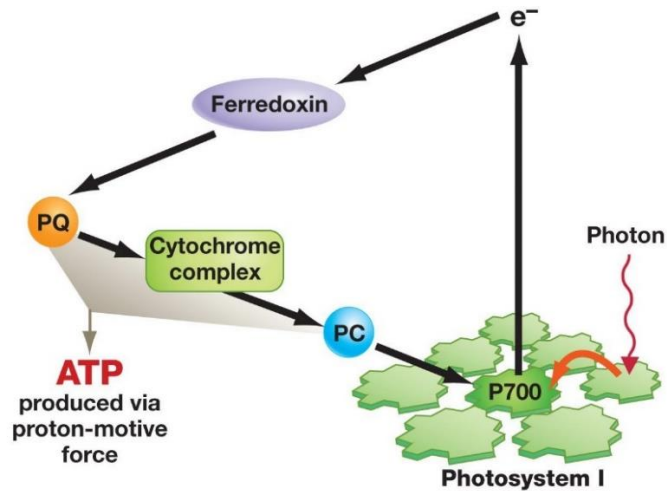
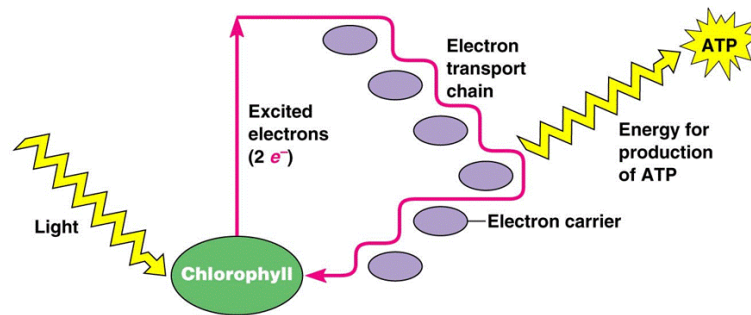
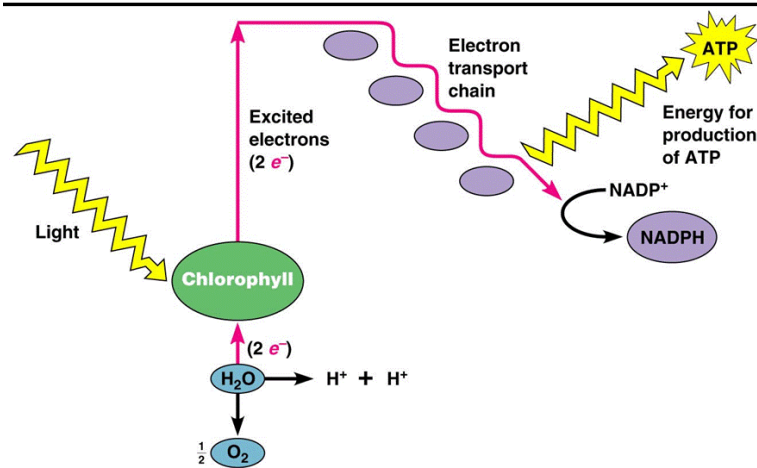


Fig. 2.2.1: Cyclic photophosphorylation

1. A photon of light is collected by the accessory pigments in the **light-harvesting complex (LHC)**. The energy is then passed on to neighbouring pigments and finally to a pair of **special chlorophyll a (P700)** in the **reaction centre** of PSI.
2. The special chlorophyll a (P700) is therefore **photoactivated**, where a pair of electrons is **photoexcited** (raised by light energy) to a higher energy level. The pair of excited electrons is then passed onto the primary electron acceptor in the reaction centre of PSI.
3. The excited electrons (high energy) are **passed down the electron transport chain (ETC)** before returning back to **PSI** to replace the electron deficit.
4. During the transfer of electrons in the ETC, the electron carriers undergo repeated oxidation-reduction (redox) reactions and energy is lost from the electrons.



(a) Cyclic photophosphorylation



(b) Noncyclic photophosphorylation

Fig. 2.2.2: Overview of (a) cyclic and (b) non-cyclic photophosphorylation.

CHECKPOINT 2.2

1 Which of the follow molecule is the final electron acceptor?

- A** Both processes involve PSI and PSII, and produce ATP and NADPH
- B** Non-cyclic photophosphorylation produces oxygen, while cyclic photophosphorylation does not.
- C** In cyclic photophosphorylation, electrons from PSII are transferred back to PSI
- D** Both processes involve photolysis of water to replenish electrons in PSI.

2 Compare non-cyclic photophosphorylation and cyclic photophosphorylation

	Non-Cyclic Photophosphorylation	Cyclic Photophosphorylation
Photosystems involved		
Products		
Electron path		
Electron donor for PSI		
Photolysis of water		
Final Electron Acceptor		

2.3 CHEMIOSMOSIS

Learning Outcome (I) modified:

Outline chemiosmosis in photosynthesis.

- Chemiosmosis refers to the process where **ATP is synthesized** through the **movement of protons down an electrochemical gradient**.
- Energy is released as the excited electrons (high energy) are passed along the ETC. This energy is used to **pump H^+** ions from the **stroma** to the **thylakoid lumen**, resulting in **accumulation of H^+** ions in the thylakoid lumen.

As the thylakoid membrane is impermeable to ions, a **proton gradient** across the thylakoid membrane is established. H^+ ions produced by **photolysis of water** also contributes to the proton gradient.

- H^+ ions to diffuse from the **thylakoid lumen** back to the **stroma** through **ATP synthase**, which **phosphorylates ADP** with inorganic phosphate, P_i , to **synthesise ATP**.
- Since the absorption of light energy leads to flow of electrons in the ETC which is coupled with ADP phosphorylation, ATP is thus said to be produced via **photophosphorylation**.

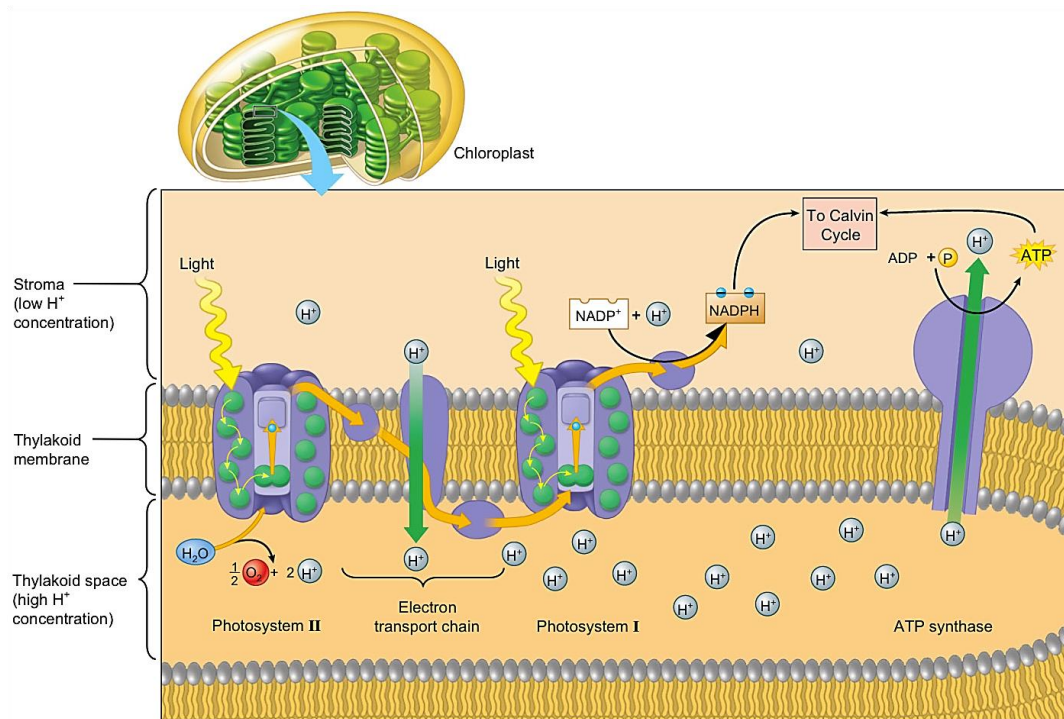


Fig. 2.3.1: Overview of chemiosmosis in light-dependent stage

CHECKPOINT 2.3

- 1** The process of ATP synthesis in photosynthesis involves chemiosmosis. Arrange the following steps of this process in the correct sequence.

1. A proton gradient is established across the thylakoid membrane due to H⁺ ions accumulating in the lumen.
2. Energy released from electron transfer in the ETC is used to pump H⁺ ions into the thylakoid lumen.
3. H⁺ ions diffuse from the thylakoid lumen back to the stroma through ATP synthase, driving ATP synthesis.
4. Light absorption excites electrons, which flow through the electron transport chain.
5. Photolysis of water releases H⁺ ions, which contributes to the proton gradient.

- A** 4 → 1 → 2 → 5 → 3
B 5 → 4 → 2 → 1 → 3
C 4 → 2 → 1 → 5 → 3
D 5 → 2 → 4 → 1 → 3

- 2** Chemiosmosis plays a crucial role in ATP synthesis during the light-dependent reactions of photosynthesis.

Which of the following correctly describes the role of chemiosmosis in the process?

- A** Chemiosmosis pumps H⁺ ions into the thylakoid lumen, creating an electrochemical gradient that directly powers ATP synthase.
- B** Chemiosmosis involves the diffusion of H⁺ ions from the thylakoid lumen to the stroma through ATP synthase, releasing energy to phosphorylate ADP.
- C** Chemiosmosis moves electrons across the thylakoid membrane, creating the proton gradient required for ATP synthesis.
- D** Chemiosmosis uses energy from the photolysis of water to drive the active transport of ATP through the thylakoid membrane.

3 LIGHT-INDEPENDENT STAGE

Learning Outcome (d):

Outline the three phases of Calvin cycle in C₃ plants: (i) CO₂ fixation, (ii) PGA reduction and (iii) ribulose biphosphate (RuBP) regeneration, indicating the roles of rubisco, ATP and reduced NADP in these processes that ultimately allow synthesis of sugars.

- The light-independent stage (Calvin cycle / C₃ cycle) can occur in the absence of light and is located in the **stroma**. It involves a series of chemical reactions that convert carbon dioxide to sugar, in the form of **triose phosphate** (aka glyceraldehyde phosphate), using **ATP** and **reduced NADP / NADPH** from the light-dependent stage.
- The light-independent stage involves 3 main phases: (i) CO₂ fixation, (ii) phosphoglycerate (PGA) reduction and (iii) ribulose biphosphate (RuBP) regeneration.

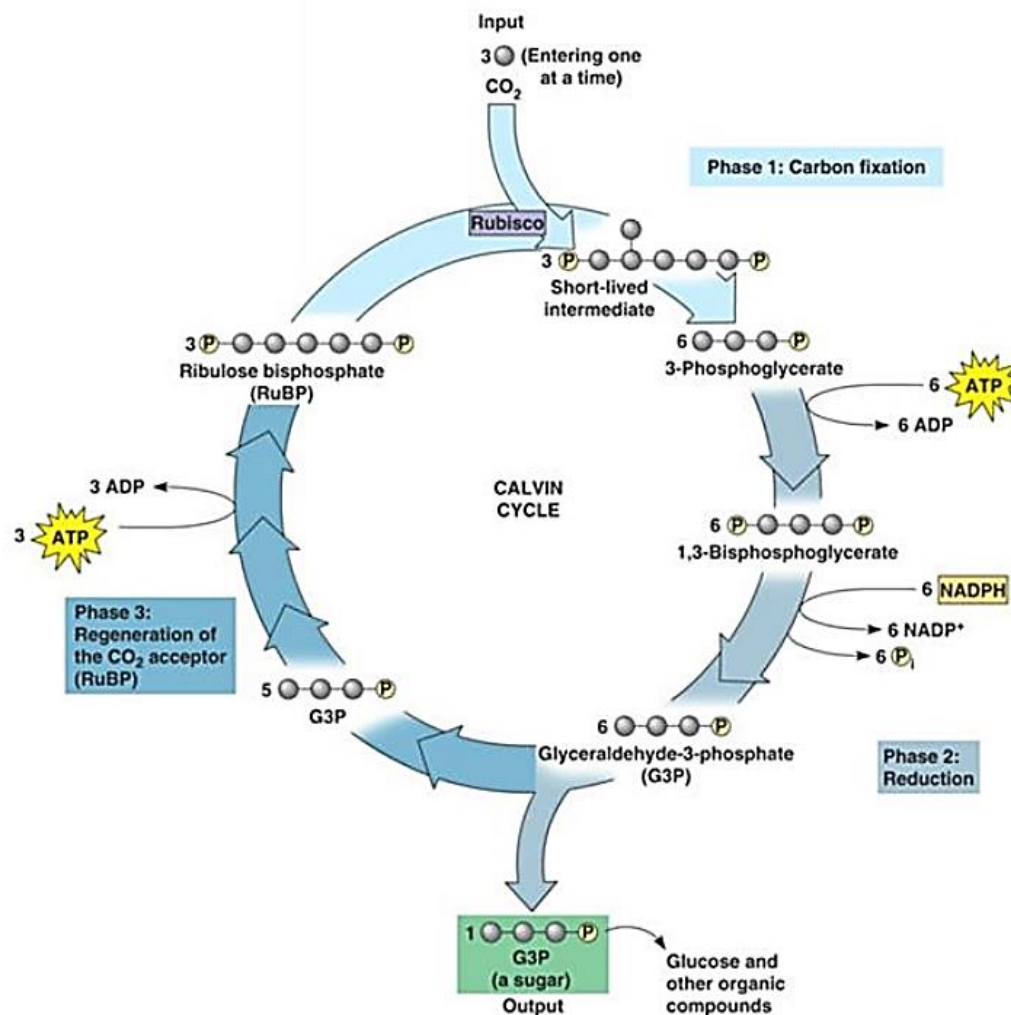


Fig. 3.1: Overview of light-independent stage

Phase 1: CO₂ Fixation

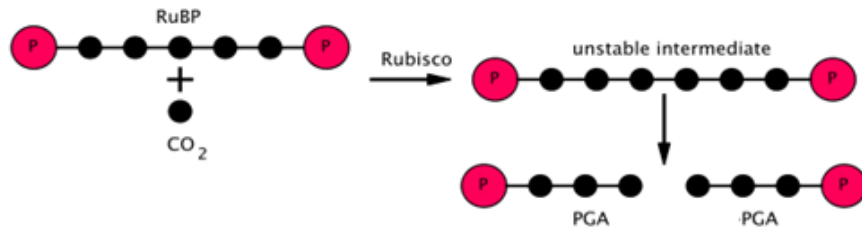


Fig. 3.2: Carbon fixation

- One **carbon dioxide (1C)** molecule is added to one molecule of **ribulose biphosphate (RuBP)(5C)** to form a 6C unstable intermediate, catalysed by the enzyme **RuBP carboxylase (Rubisco)**. The 6C intermediate immediately splits to **two molecules** of **3-phosphoglycerate (PGA)(3C)**, also known as glycerate-3-phosphate.

Phase 2: PGA Reduction

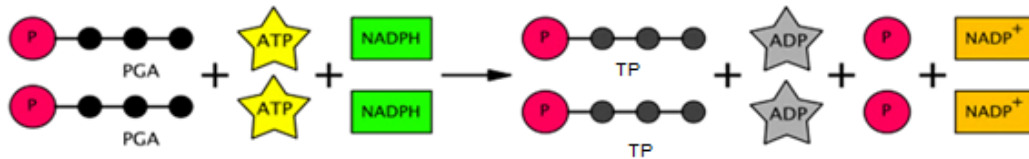


Fig. 3.3: PGA reduction

- PGA (3C)** is **reduced to triose phosphate (TP)(3C)**, also known as glyceraldehyde-3-phosphate. Energy for the reaction is provided by ATP (from light-dependent stage) which forms ADP + Pi. Reducing power for the reduction reaction is provided by **reduced NADP** (from light-dependent stage) which is regenerated into NADP for use in the light-dependent stage.
- Out of the 6 molecules of TP produced, only **1 TP** (final product) exits the cycle and is converted to carbohydrates / lipids / protein for storage or usage in the cell.

Phase 3: RuBP Regeneration

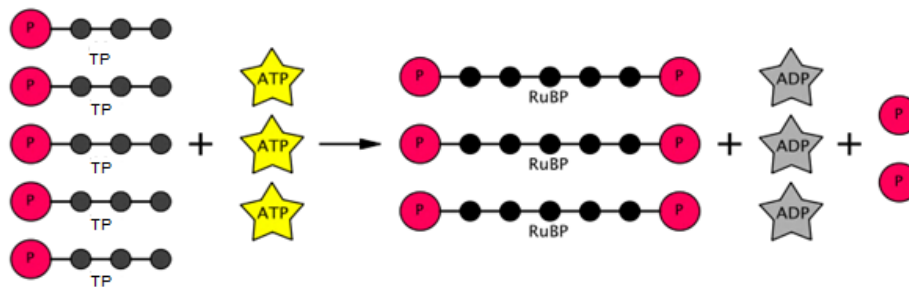
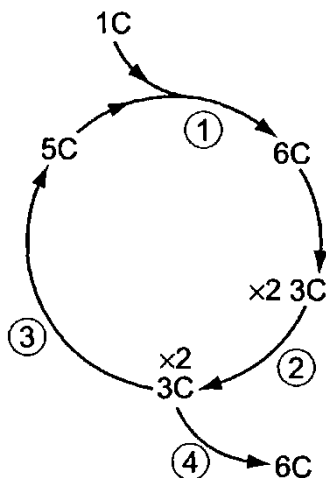


Fig. 3.4: RuBP Regeneration

- The remaining 5 molecules of TP are rearranged to form 3 molecules of RuBP (5C). This occurs via a series of steps that require ATP (from light-dependent stage). RuBP is regenerated and re-enters the CO₂ fixation stage.

CHECKPOINT 3

- 1 The diagram shows some phases of the Calvin cycle and the numbers of carbon atoms in the compounds involved.



Which reactions occur at phases 1 to 4?

A

phase 1	$\text{RuBP} + \text{CO}_2$
phase 2	$\text{ATP} \rightarrow \text{ADP} + \text{P}_i$ $\text{NADP} \rightarrow \text{reduced NADP}$
phase 3	$\text{ATP} \rightarrow \text{ADP} + \text{RuBP}$
phase 4	triose $\rightarrow$ hexose

B

phase 1	triose $\rightarrow$ hexose
phase 2	$\text{ADP} + \text{P}_i \rightarrow \text{ATP}$ $\text{NADP} \rightarrow \text{reduced NADP}$
phase 3	$\text{ADP} + \text{RuBP} \rightarrow \text{ATP}$
phase 4	glycerate 3-phosphate $\rightarrow$ triose

C

phase 1	triose $\rightarrow$ hexose
phase 2	$\text{ADP} + \text{P}_i \rightarrow \text{ATP}$ $\text{reduced NADP} \rightarrow \text{NADP}$
phase 3	$\text{ADP} + \text{RuBP} \rightarrow \text{ATP}$
phase 4	glycerate 3-phosphate $\rightarrow$ triose

D

phase 1	$\text{RuBP} + \text{CO}_2$
phase 2	$\text{ATP} \rightarrow \text{ADP} + \text{P}_i$ $\text{reduced NADP} \rightarrow \text{NADP}$
phase 3	$\text{ATP} \rightarrow \text{ADP} + \text{RuBP}$
phase 4	triose $\rightarrow$ hexose

- 2** The Calvin cycle (light-independent stage) involves three key phases: CO₂ fixation, PGA reduction, and RuBP regeneration. Arrange the following steps of the cycle in the correct sequence.
1. PGA is reduced to triose phosphate (TP) using ATP and reduced NADP from the light-dependent stage.
 2. RuBP is regenerated from the remaining 5 TP molecules in a series of steps requiring ATP, allowing it to re-enter the CO₂ fixation stage.
 3. A molecule of CO₂ combines with RuBP to form an unstable 6C intermediate, which splits into two molecules of PGA.
 4. Out of six molecules of TP produced, one exits the cycle and is converted into carbohydrates, lipids, or proteins.

Which of the following correctly describes the role of chemiosmosis in the process?

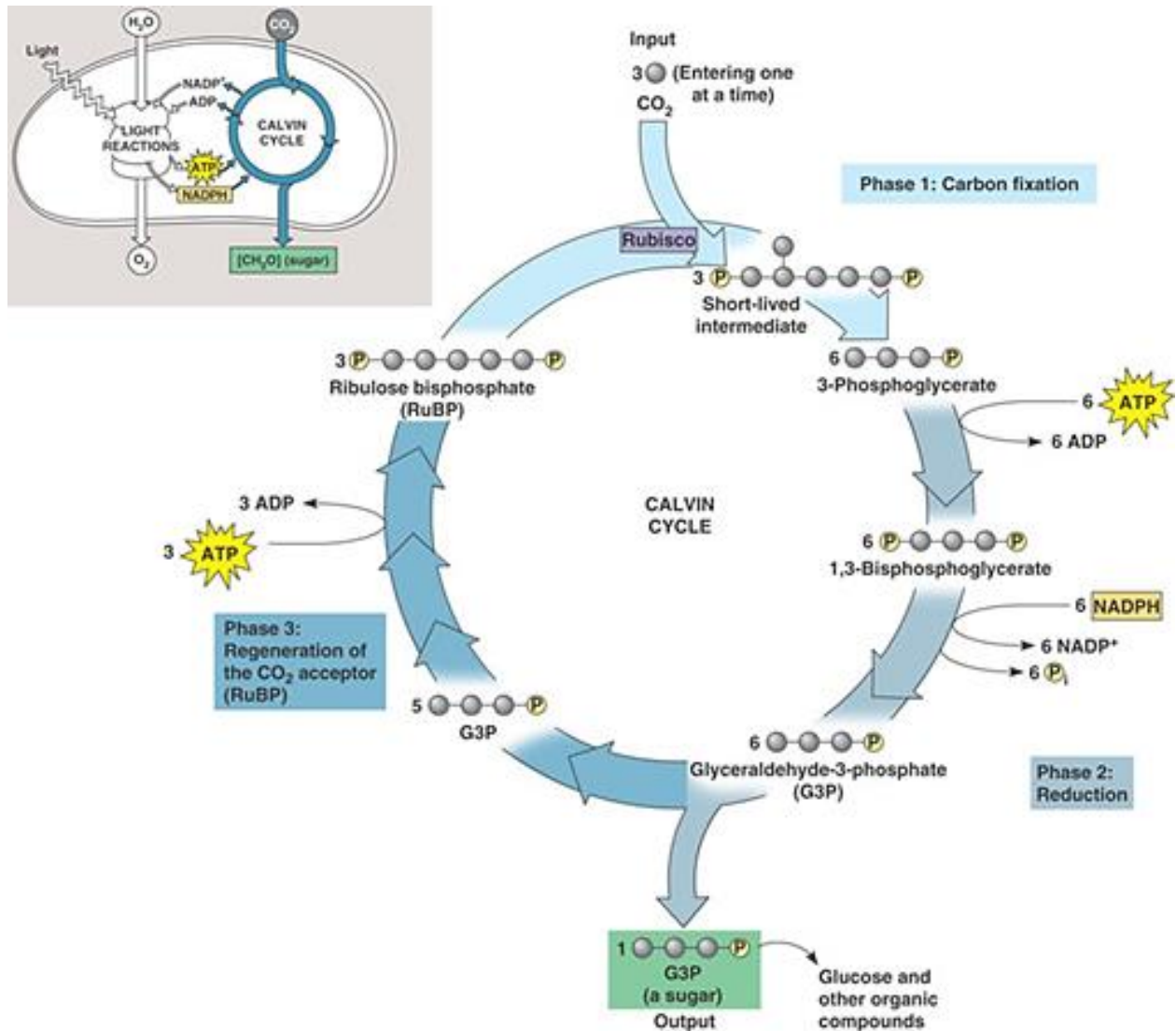
- A** 3 → 1 → 4 → 2
B 1 → 3 → 2 → 4
C 3 → 1 → 4 → 2
D 3 → 1 → 4 → 2
- 3** Which of the following statements correctly describes the Calvin cycle?
**There can be more than one correct answer for this question*
- A** The Calvin cycle occurs in the thylakoid membrane and produces oxygen as a by-product.
- B** ATP and reduced NADP are required in the Calvin cycle for the reduction of RuBP into triose phosphate (TP)
- C** The Calvin cycle occurs in the stroma and begins with the fixation of carbon dioxide into RuBP, forming an unstable 6C intermediate.
- D** The conversion of PGA to triose phosphate used to regenerate NADP from NADPH, and ADP from ATP for the light-dependent reactions.

DID YOU KNOW?

The PGA reduction in fact consists of 2 steps.

First, each molecule of PGA receives a phosphate group from ATP, a molecule called 1,3-bisphosphoglycerate. ADP is produced as a by-product.

Second, the 1,3-bisphosphoglycerate molecules are reduced by reduced NADP and loses one of its phosphate groups, turning into a triose phosphate (3C sugar), also called glyceraldehyde 3-phosphate (G3P). This step produces NADP and P_i .



4 FACTORS AFFECTING RATE OF PHOTOSYNTHESIS

Learning Outcome (e):

Discuss limiting factors in photosynthesis and carry out investigations on the effect of limiting factors such as temperature, light intensity and carbon dioxide concentration on the rate of photosynthesis.

- The overall rate of a series of reactions is limited by the slowest reaction. Environmental factors that are in short supply (limited) will affect the rate of reaction and prevent the rate from increasing. These environmental factors are known as the limiting factors.
- Limiting factor is defined as any factor that when increased in quantity, increases the rate of reaction.** Limiting factors in photosynthesis include light intensity, CO₂ concentration, temperature and chlorophyll concentration.
- In the investigation of rate of photosynthesis, photosynthetic rate is calculated from either as volume of CO₂ assimilated over time or O₂ produced over time.
 - In an investigation of a limiting factor in photosynthesis, the rate of photosynthesis is plotted against the chosen factor.
 - If the rate of photosynthesis increases as the amount of the factor increases, the factor is said to be limiting.
 - If the rate of photosynthesis remains the same (plateaus) as the amount of the factor increases, the factor is not limiting anymore. Another factor has become limiting.

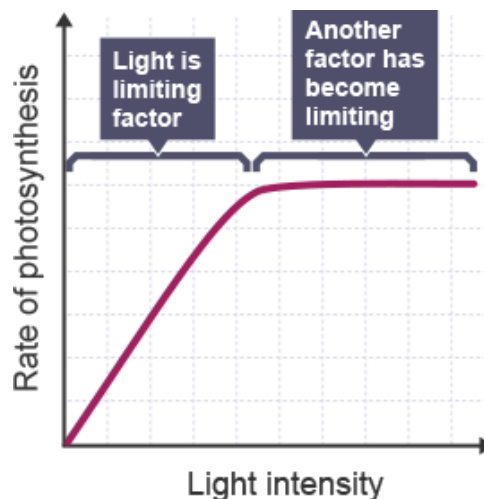


Fig. 4.1: Effect of light intensity on rate of photosynthesis

4.1 LIGHT INTENSITY

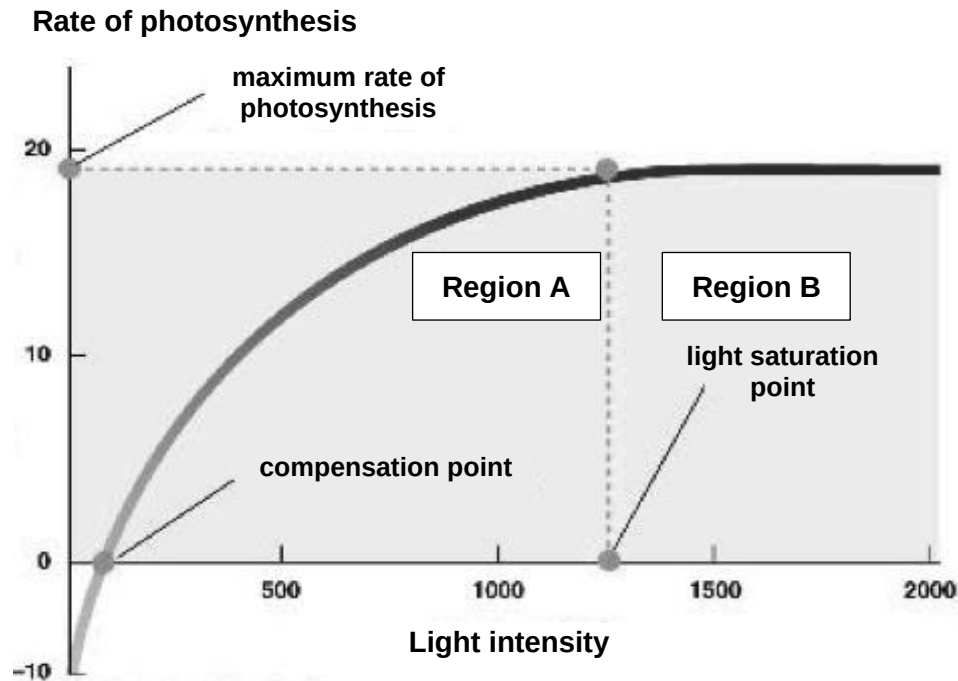


Fig. 4.1.1: Effect of light intensity on rate of photosynthesis

- **Region A:** Increasing light intensity increases rate of light-dependent reactions to produce increased amounts of ATP and reduced NADP. This results in increased rate of light-independent reactions, resulting in increase in rate of photosynthesis. Light intensity is the limiting factor.
- **Region B:** Further increase in light intensity causes no further increase in rate of photosynthesis, indicating that other factors are limiting the rate of reaction.
- **Light saturation point:** The light intensity at which the rate of photosynthesis is the maximum, as ATP and reduced NADP are produced at maximum rate. The reaction is saturated with respect to light intensity.
- **Compensation point:** Defined as the light intensity at which the **rate of photosynthesis is equal to the rate of respiration**. At this point, there is **no net gaseous exchange** between the plant and its environment. Compensation point is usually reached at low light intensities i.e. sunrise and sunset.

4.2 CARBON DIOXIDE CONCENTRATION

- At low CO_2 concentration, an increase in concentration of CO_2 results in an increase in rate of CO_2 fixation during Calvin cycle, where CO_2 combines with RuBP, catalysed by RuBP carboxylase (Rubisco). This results in increase in rate of light-independent reactions and an increase in rate of photosynthesis. CO_2 concentration is the limiting factor.
- At high CO_2 concentrations, further increase in CO_2 concentration does not result in increase in rate of photosynthesis. Other factors (e.g. light intensity) are limiting.
- NB: Carbon dioxide is the major limiting factor of photosynthesis under normal atmospheric conditions as it is at a low CO_2 concentration of 0.04%.*

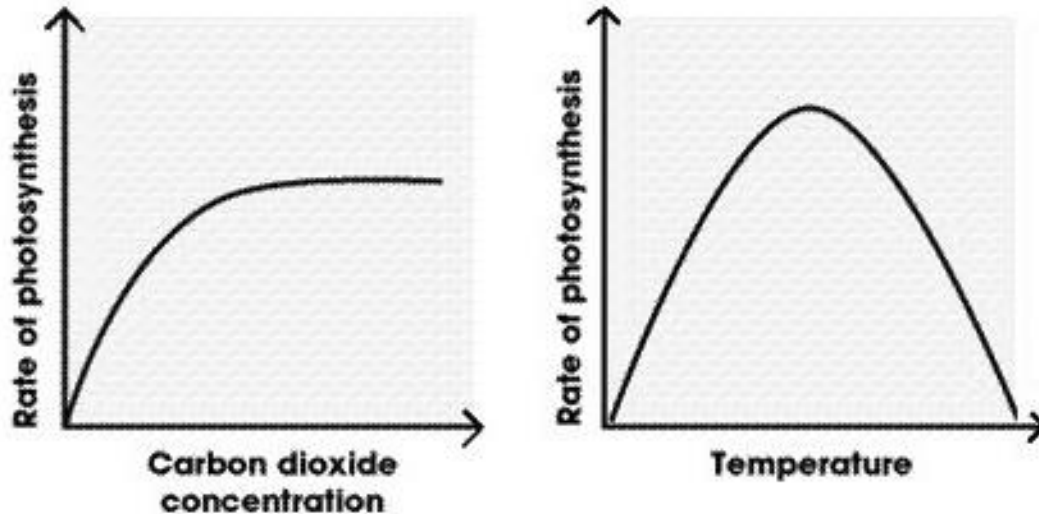


Fig. 4.2.1: Effect of carbon dioxide concentration (left) and temperature (right) on rate of photosynthesis

4.3 TEMPERATURE

- An increase in temperature results in an increase in rate of enzyme-catalysed reactions in photosynthesis, due to an increase in frequency of effective collisions between enzyme and substrate molecules. This results in an increase in rate of ES complex formation, and subsequent product formation. (*Ref to Topic 1.3 Enzymes*)
- NB: There exist an optimum temperature range for which the enzymes involved are most effective. However, due to the vast number of enzymes involved, the optimum temperature occurs over a broad range.*

CHECKPOINT 4

Most of the questions in TYS challenge you to **apply your understanding of the factors influencing photosynthesis, in conjunction with the light-dependent and light-independent stages.**

Therefore, relying solely on memorizing the factors is not the most effective strategy. You should be doing more practice question and **map the trends of the graph to a molecular level.**

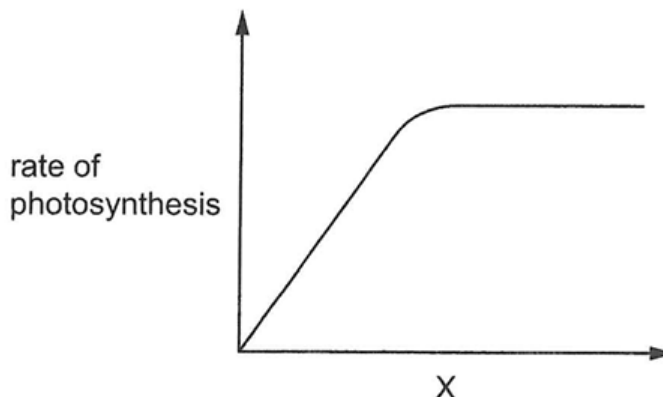
- e.g. Why does the graph plateau off at high carbon dioxide concentration?
 - Rubisco is the enzyme used during carbon fixation in Calvin's cycle
 - At high carbon dioxide concentrations, their active sites are fully filled, and hence rate of reaction plateaus.
- You should do the same for both light intensity (in relation to photosystems) and temperature (in relation to enzymes involved)

- 1 The rate of photosynthesis in a plant was measured while one variable was altered, and all other conditions were kept constant.

The graph below shows the rate of photosynthesis at different values of a variable, X:

Which variables could be represented by X?

1. Light intensity
2. Carbon dioxide concentration
3. Oxygen concentration
4. Temperature



- A 1, 2, and 4
B 2 and 4 only
C 1, and 2 only
D 3 and 4 only

*Modified TYS question

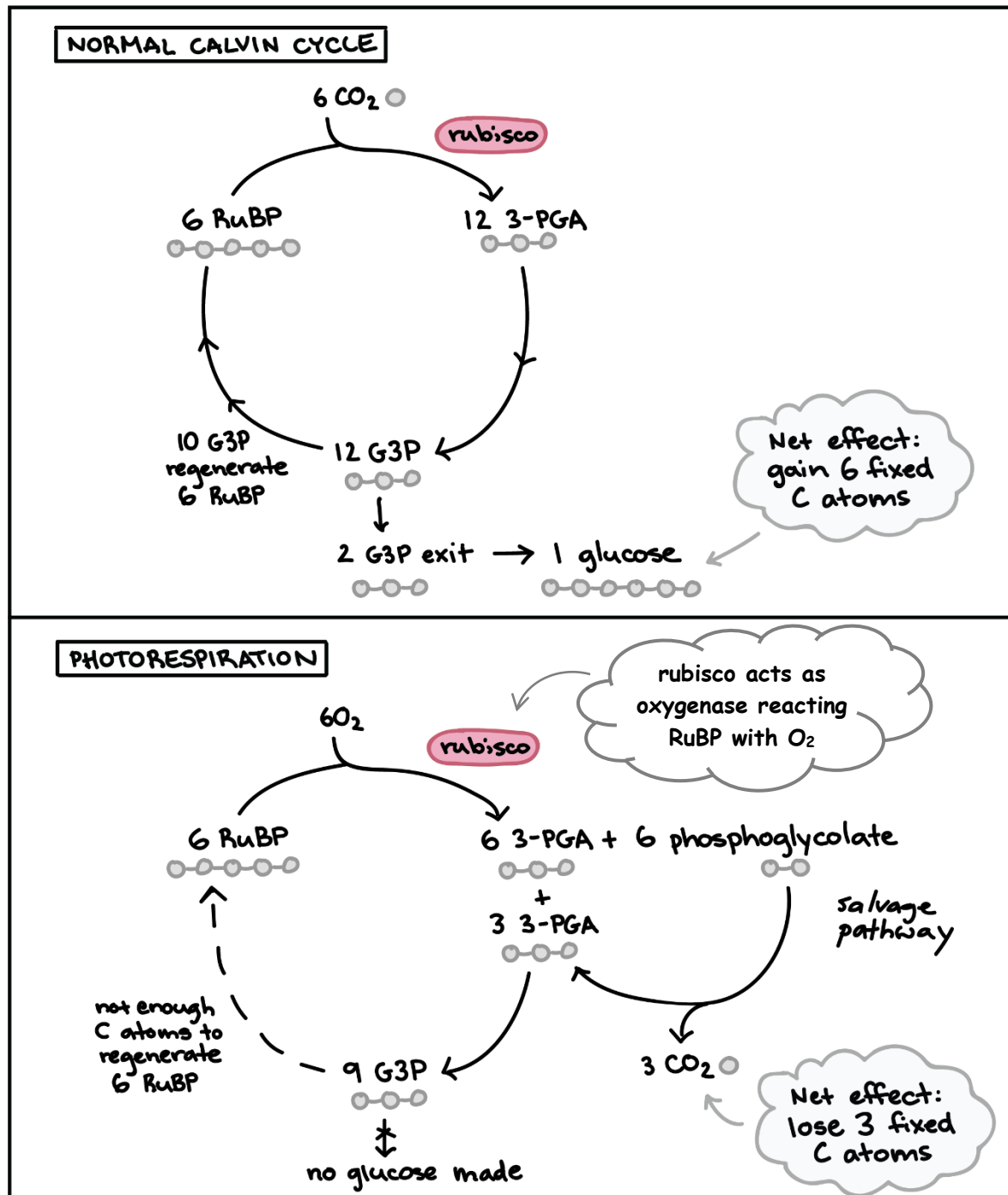
- 2 Which row correctly describes both the light-dependent reactions and the Calvin cycle during a condition of low light availability?

	Light-dependent reactions	Calvin cycle
A	Few electrons are available for electron transport	The synthesis of reduced NADP is slow
B	photosystem I is inactive	the concentration of triose phosphate is high
C	the synthesis of ATP is slow	the concentration of RuBP is low
D	the thylakoid space contains a high concentration of H ⁺ ions	the concentration of PGA is low

* TYS 2019 P1 Q21

DID YOU KNOW?

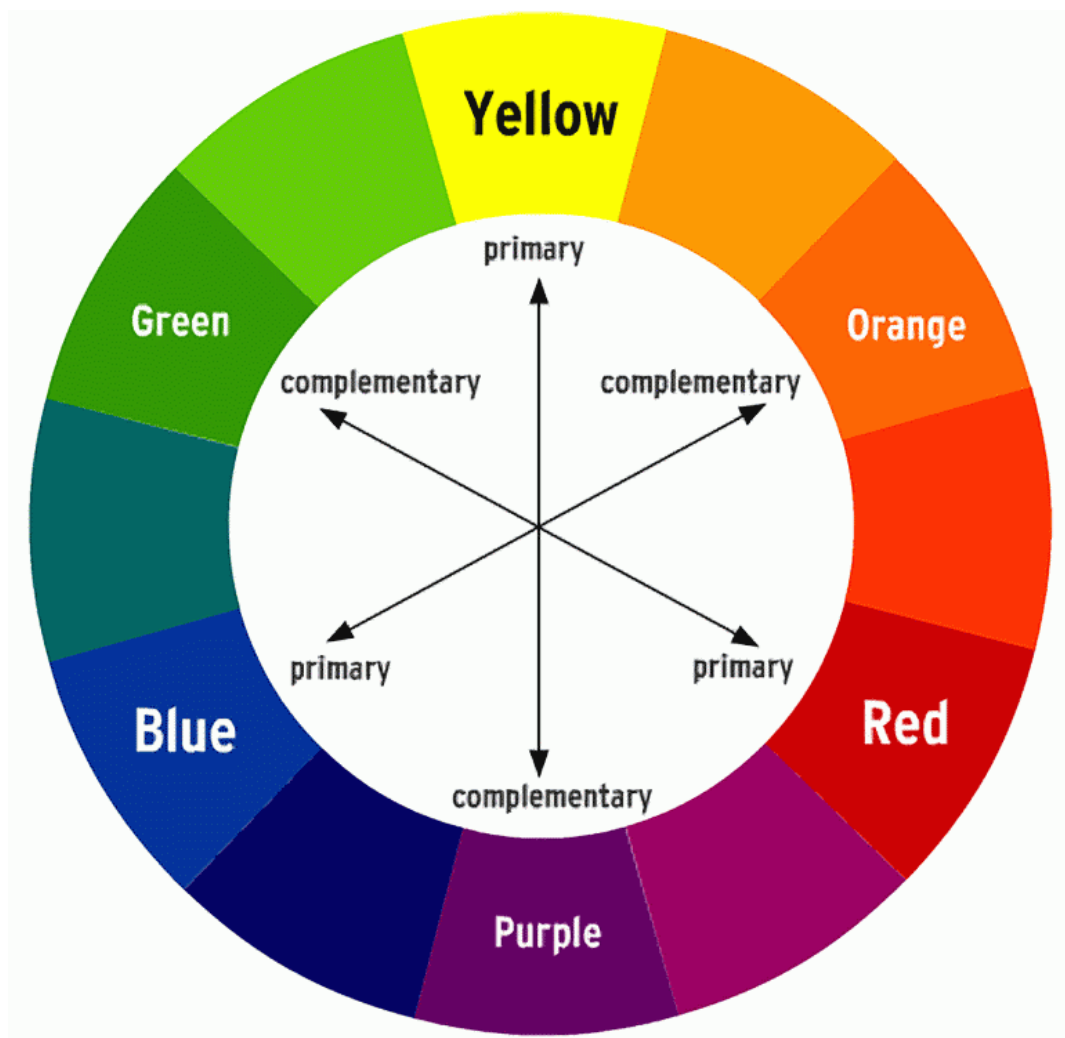
High O_2 concentrations inhibit photosynthesis as O_2 competes with CO_2 for RuBP carboxylase binding site. This is because RuBP carboxylase can also act as RuBP oxygenase. This process is known as photorespiration, which results in decrease in production of glyceraldehyde-3-phosphate and wastage of ATP and reduced NADP.



ANNEX 1: Why are leaves red in autumn?

For further reading, not included in your lecture notes

- A colour wheel is where complementary colours are arranged opposite one another.
- If RED is absorbed, the complementary GREEN light will be reflected. Hence many leaves appear to be green during summer as chlorophyll is the photosynthetic pigment that absorbs red light.
- If GREEN is absorbed, the complementary RED light will be reflected. Hence many leaves appear to be red/orange in autumn as chlorophyll is broken down, and carotenoids mainly absorb green light.



ANNEX 2: Experiments to extract photosynthetic pigments

Understanding such experiments may help you tackle novel questions, especially questions that require you to identify the types of photosynthetic pigments present in the plant.

1. Extraction:

- The extraction is made from fresh green leaves ground up with **aqueous propanone (acetone)** and sand.

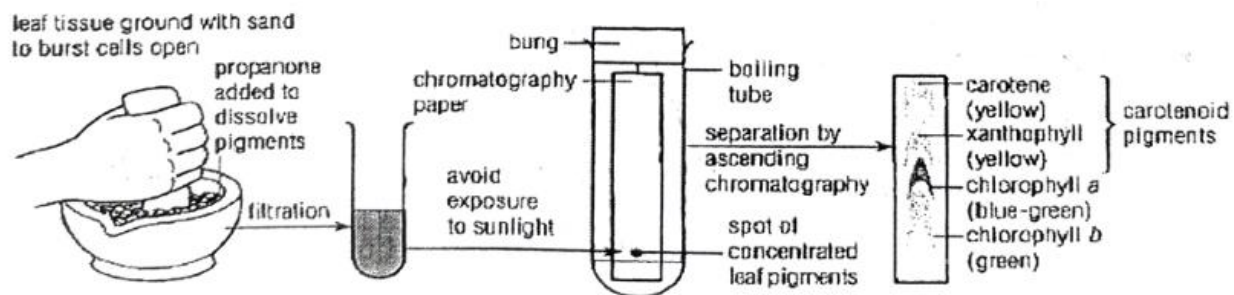
2. Chromatographic Separation:

- Separation is carried out on a narrow strip of filter paper, suspended in a boiling tube.
- Separation is complete in approximately **30–60 minutes**.

3. Identification of pigment

- At the end of the chromatographic run, the **solvent front** is marked, and the **Rf values** of the pigments are calculated using the formula:

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



Loading the chromatogram: the chromatogram is loaded at a point just above the line of contact with the solvent; a tiny spot of pigment extract is spotted and allowed to dry, and that spot is neatly respotted. Loading is repeated until a dark green spot is produced.

Running the chromatogram: a solvent mixture that causes the individual pigments to be separated during the run is selected; the loaded chromatogram is lowered into the solvent and the solvent travels up the paper. The run is stopped when the solvent front nears the top of the paper.