Energy & Equilibrium – Photosynthesis

1. Overview of Topic

All living things require energy to grow and survive. Most living organisms get their energy either directly or indirectly from sunlight. Producers derive their energy from the sun via photosynthesis while consumers such as herbivores, carnivores and omnivores get their energy from eating other organisms.

Photosynthesis is the process by which plants, some bacteria and some protists use light energy to produce glucose from CO_2 and water. The oxidation of glucose during respiration can yield ATP to fulfil an organism's energy needs.

Photosynthesis is also a source of carbon, which is needed for the synthesis of other organic compounds, e.g. proteins, lipids and nucleic acids. In this chapter, we will look at the location, importance and mechanisms of photosynthesis.

2. Learning Outcomes

a. Identify components of chloroplasts and mitochondria in drawings, photomicrographs 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 harnesses and converted into chemical energy during the light-dependent reactions of photosynthesis.
d. Outline the three phases of the Calvin cycle in C3 plants:
    i. CO_2 fixation,
    ii. PGA reduction and
    iii. Ribulose bisphosphate (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.

3. References

Campbell, N.A. and Reece, J.B. (2008). Biology, 9th edition. Pearson.

Contents

4. Introduction

An autotroph is an organism that can produce its own food and are therefore also called producers. Most autotrophs use a process called photosynthesis to make their own food – energy from the sun is used to convert CO_2 and water into glucose. Plants are one of the most well-known examples of autotrophs, but there are many other autotrophic organisms such as phytoplankton and some bacteria.

Photosynthesis takes place in the chloroplast in two stages:

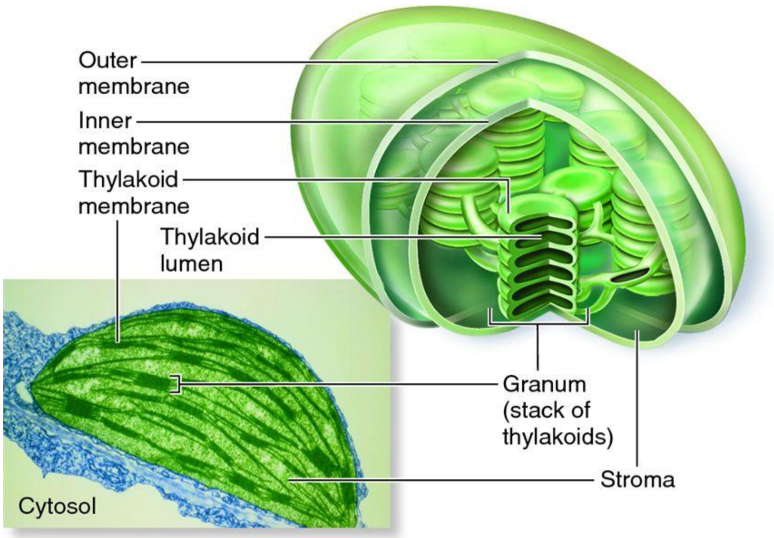
PHOTO SYNTHESIS

- **Light-dependent stage**
- Light energy is converted into **chemical energy in the form of NADPH and ATP**. These products are then used to drive the reactions in the next stage of photosynthesis.

- **light-independent stage** (a.k.a **Calvin cycle**)
- **Fixing** atmospheric **carbon** in the form of CO_2 into **sugar** molecules using NADPH and ATP from light-dependent reaction.

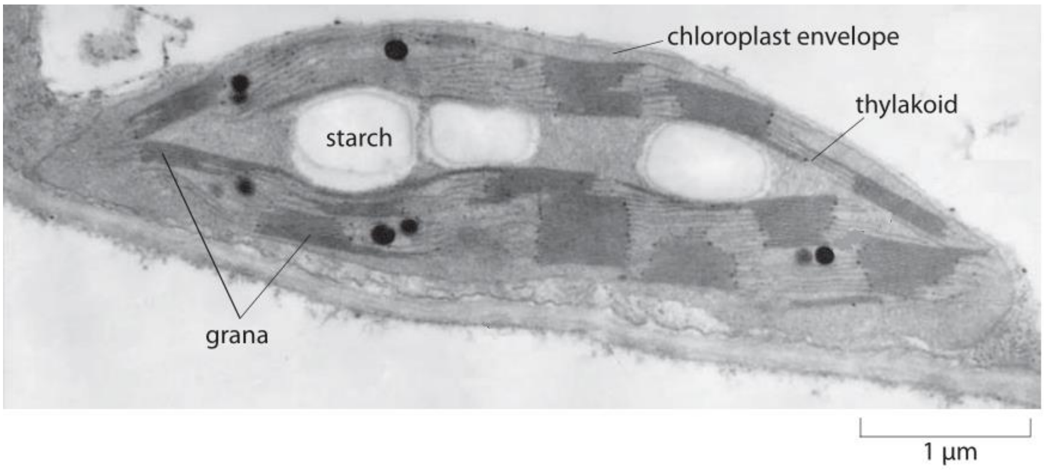
Light-independent stage will continue to take place so long as the products of the light-dependent stage have not run out.

5. Chloroplast – The Site of Photosynthesis



Structure of chloroplast.

Notes to self



Electron micrograph of chloroplast.

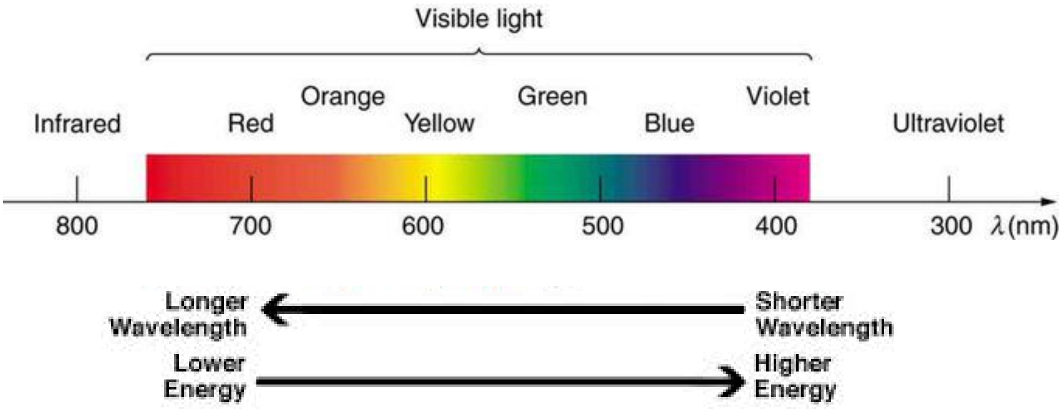
Chloroplast envelope (double membrane) separates the inside of the chloroplast and the cytoplasm of the cell.

The dense fluid within the chloroplast containing soluble enzymes and other organic substances is called the **stroma**.

Stacks of **thylakoids** form a **granum** (singular) and are found within the chloroplast. Intergranal lamella link the grana (plural).

Photosynthetic pigments (e.g. **chlorophyll** and **carotenoids**) are located on the thylakoid membrane and allow plant cells to capture light energy for photosynthesis.

6. Light & Photosynthetic Pigments



The electromagnetic spectrum.

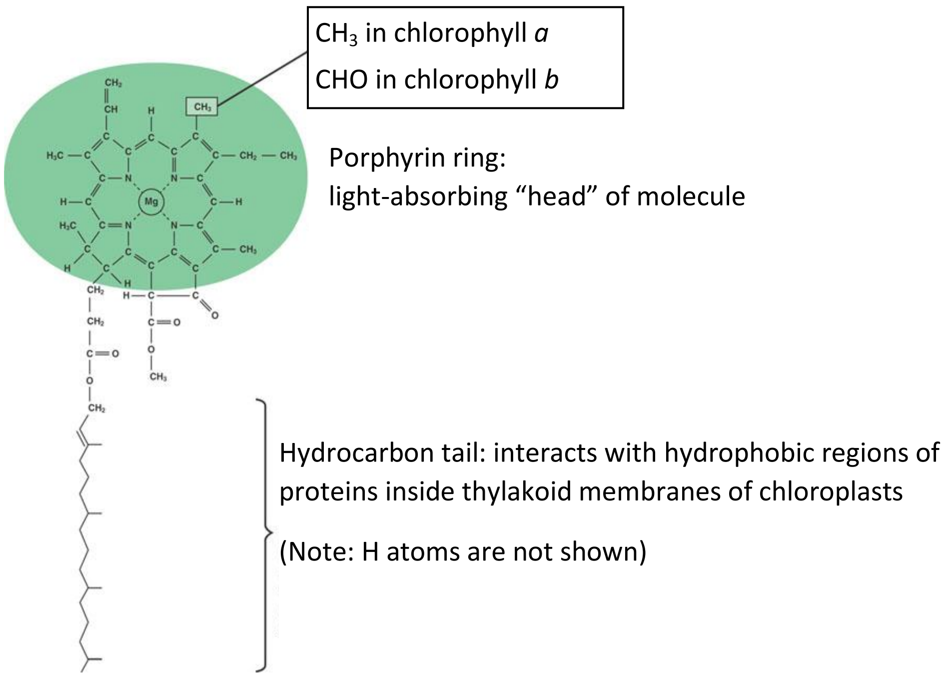
The sun gives off a full range of light of varying wavelengths called the electromagnetic spectrum. However, Earth's atmosphere only allows certain wavelengths of light through, one of which is visible light. Out of all the light wavelengths that gets pass the Earth's atmosphere, only **visible light** is **absorbed by the photosynthetic pigments** in plants for photosynthesis.

Notes to self

There are several types of photosynthetic pigments but we will focus only on the following:

- **Chlorophyll a** is the most abundant photosynthetic pigment. They exist as several forms (e.g. **P700** and **P680**) with different absorption peaks. They are the only pigments that **participate directly in photosynthesis**.
- **Chlorophyll b** and **carotenoids** are **accessory pigments**. They play an **indirect role** in photosynthesis and **channel light energy absorbed to chlorophyll a**.

  Other than their indirect role in photosynthesis, carotenoids have a **photoprotective role**. They **absorb and dissipate excess light energy** that could damage chlorophyll or quench reactive oxidative molecules that are dangerous to the cell. Additionally, they add colour to fruits and flowers which help in dispersal and pollination respectively.



Structure of a chlorophyll molecule.

7. Absorption & Action Spectrum

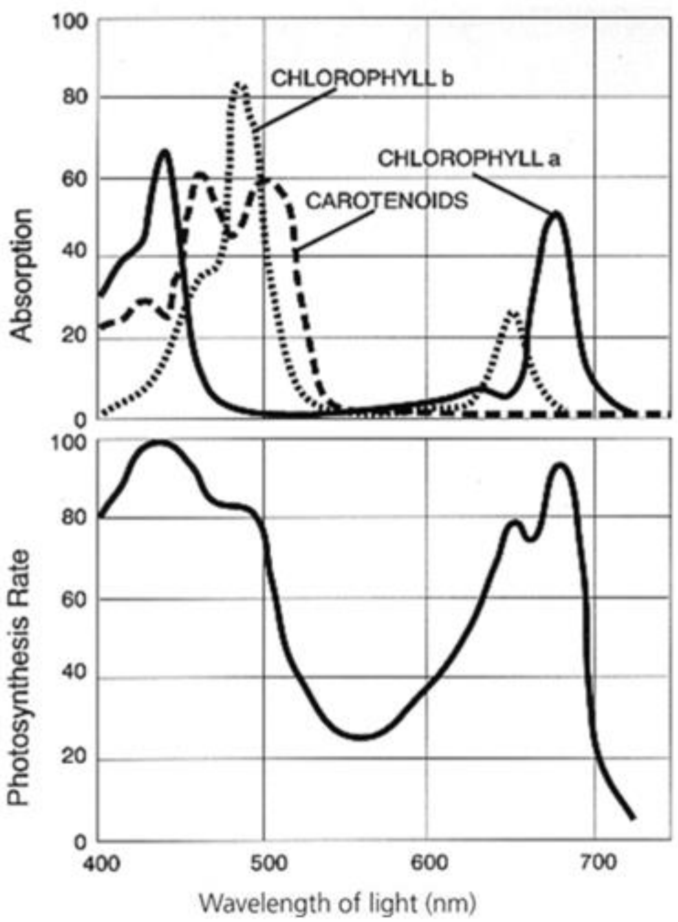
Notes to self

The ability of a pigment to absorb various wavelengths of light can be measured by a spectrophotometer. A spectrophotometer is a device that directs beams of light of different wavelengths through a solution of pigment and measures the fraction of the light transmitted at each wavelength.

A pigment's **absorption spectrum** is a graph showing the **set of light wavelengths absorbed by the pigment**.

An **action spectrum** is a graph showing the **effectiveness of different light wavelengths in stimulating photosynthesis**.

This is obtained by measuring the **rate of photosynthesis** at various light wavelengths.



Absorption spectrum and action spectrum of photosynthetic pigments.

Q: Note down any significant observations about the absorption spectrum.

Q: The absorption spectrum of chlorophyll a does not exactly match the absorption spectrum. Why?

8. Photoactivation of Photosynthetic Pigments

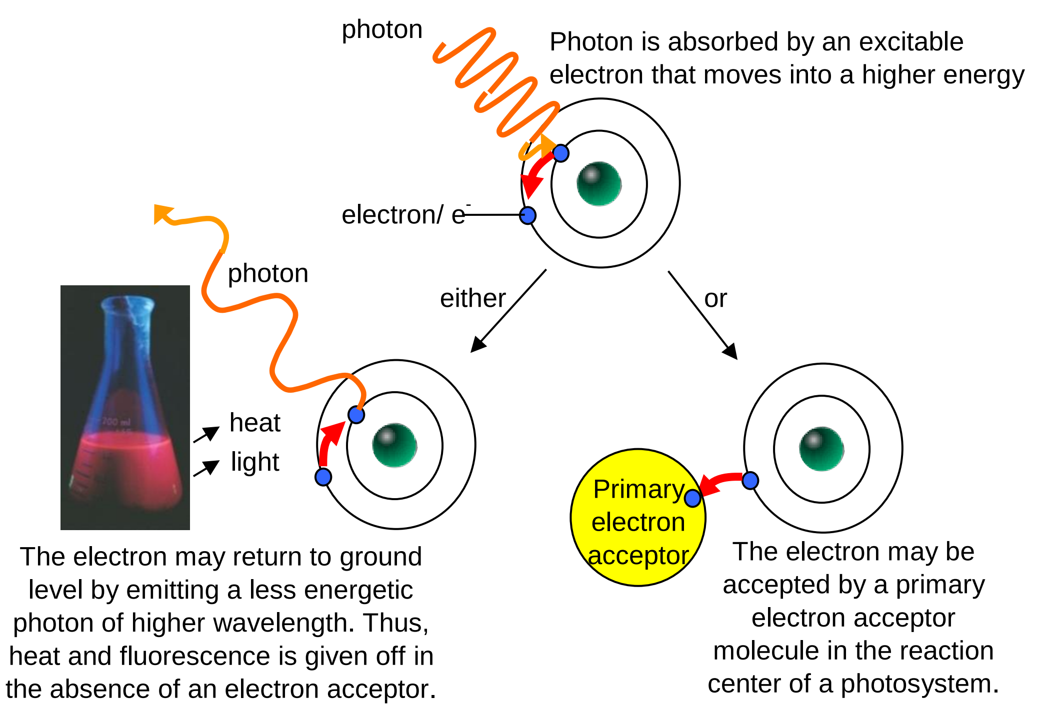
Notes to self

When a chlorophyll molecule absorbs a **photon** of light (a photon is a discrete packet of energy that is transmitted in light as a subatomic particle), one of the pigment molecule's **electrons is elevated from its ground state to its excited state** (i.e. the photon boosts an electron to an orbital of higher potential energy).

Different pigments absorb only photons corresponding to specific wavelengths, which is why **each pigment has a unique absorption spectrum**.

The electron cannot remain excited for long since the **excited state is unstable**. The electrons quickly fall back to ground state orbital resulting in excess energy released as heat and fluorescence. **Energy released** in this process can be **passed to another chlorophyll molecule** by **resonance**.

Alternatively, instead of falling to the unexcited state, **electron is captured by the primary electron acceptor** molecule in the reaction center of a **photosystem**.

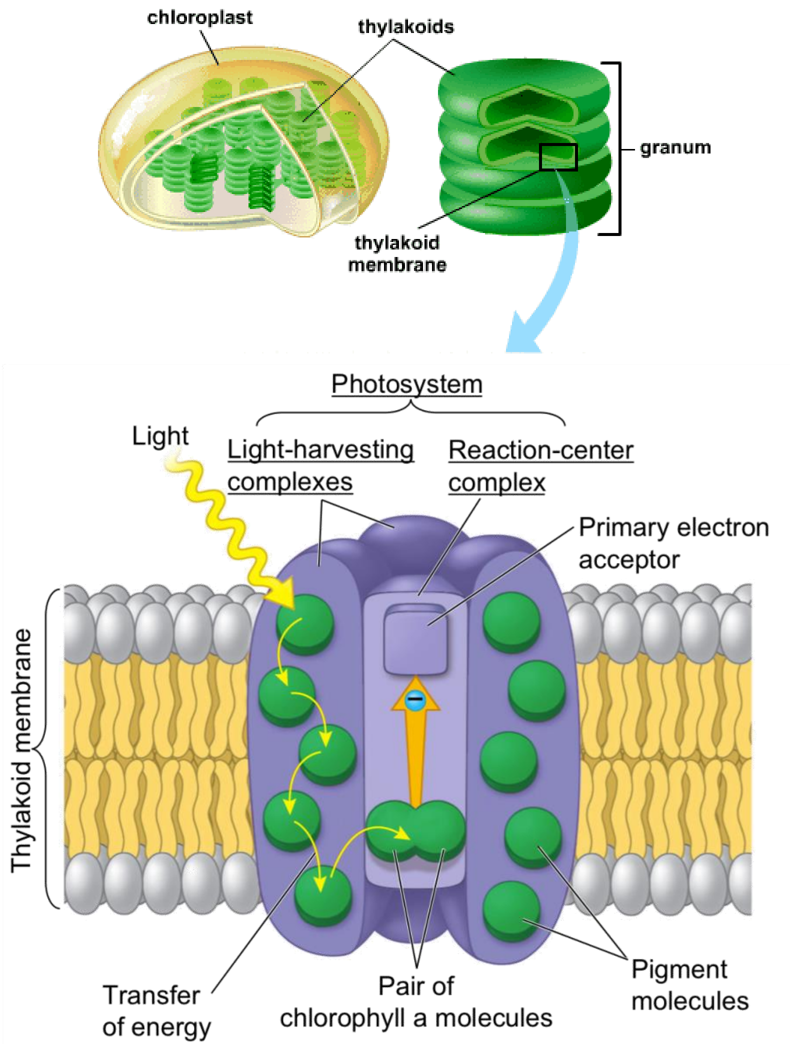


Excitation of electrons of photosynthetic pigments by light.

9. Photosystems

Notes to self

Chlorophyll molecules do not work in isolation. They cluster together to form a functional unit called a **photosystem**. Photosystems are located on the **thylakoid membrane** of the chloroplast.



How light is harvested by photosystem.

A photosystem is composed of a **reaction center** surrounded by a number of **light-harvesting complexes**.

- The reaction center is a protein complex that includes two **special chlorophyll *a*** molecules and a molecule called the **primary electron acceptor**.

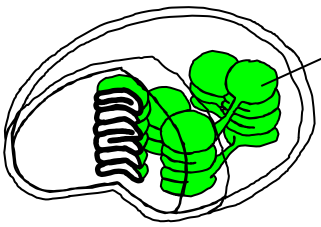
- Each light harvesting complex consists of photosynthetic pigment molecules (chlorophyll *a*, chlorophyll *b*, carotenoids) bound to particular proteins. The **variety of photosynthetic pigments allow light to be harvested over a wider range of wavelengths**.

Notes to self

The thylakoid membrane is populated by two types of photosystems, **photosystem II (PS II)** and **photosystem I (PS I)**.

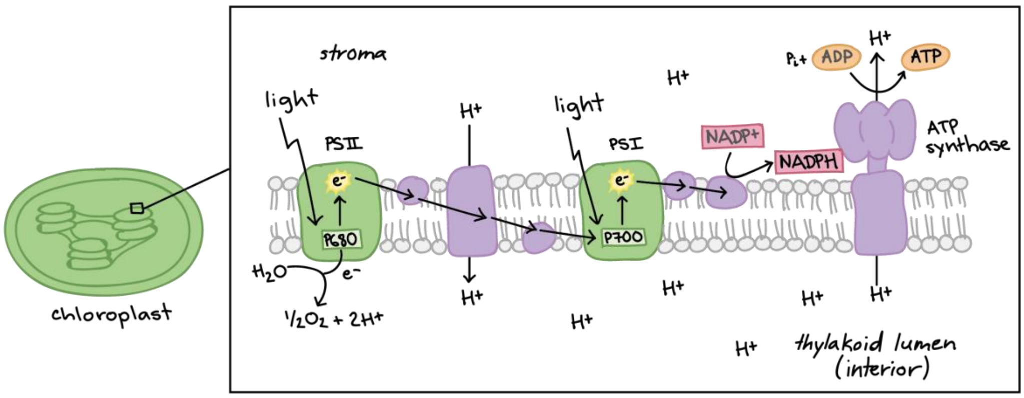
- PS II contains **P680** – which is the special chlorophyll *a* molecule in PS II's reaction center. It is so named because it most effectively absorbs light of wavelength 680nm.
- PS I contains **P700** – which is the special chlorophyll *a* molecule in PS I's reaction center. It is so named because it most effectively absorbs light of wavelength 700nm.
  - P700 and P680 are actually identical chlorophyll molecules. It is their association with different proteins in the thylakoid membrane that affects the electron distribution in the chlorophyll molecules and accounts for the slight differences in light-absorbing properties.
- PS II and PS I cooperate in the **light-dependent reactions** of photosynthesis. (They are named in the order of their discovery.)

10. Light Dependent Stage of Photosynthesis



Thylakoid membranes are the site of the light-dependent reactions of photosynthesis. Embedded in these membranes are:

1) **PS I** and **II**
2) **electron transport chains**
3) enzymes e.g. **ATP synthase**



There are two possible routes for the light-dependent reaction: **non-cyclic and cyclic**.

Non-cyclic light-dependent reaction is the **predominant** route.

a. Non-cyclic Light-dependent Reaction

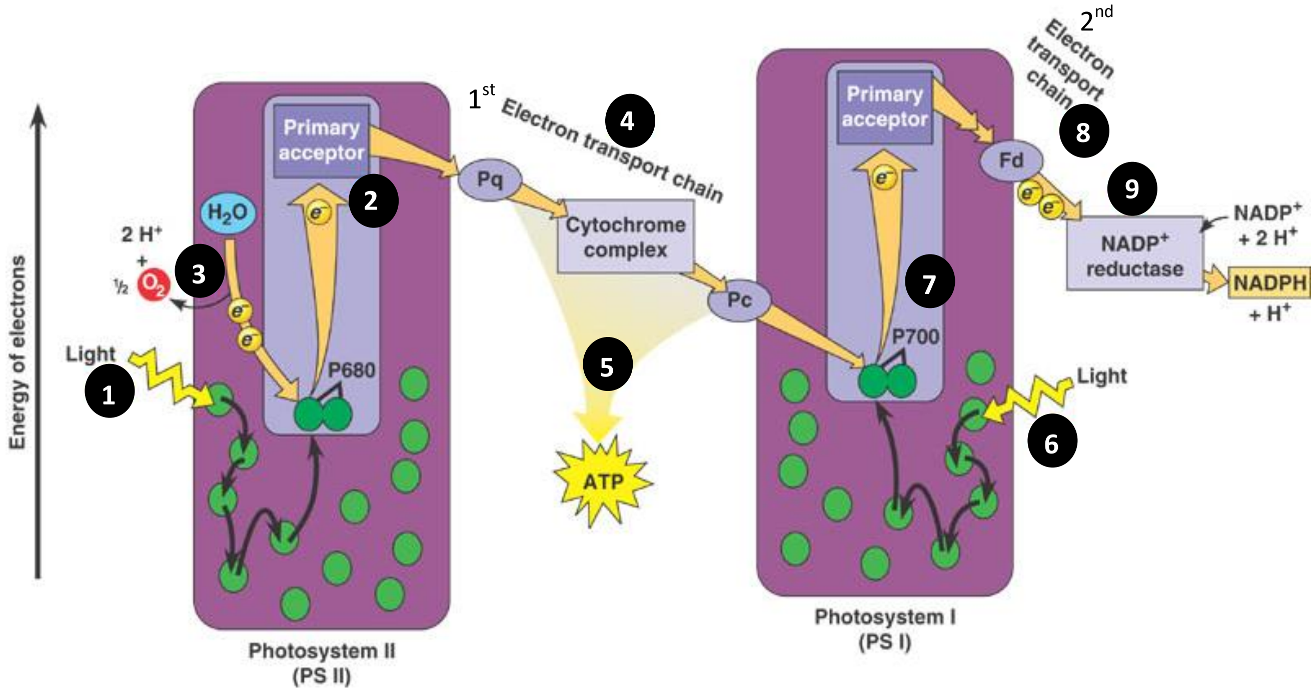
Notes to self

Involves both **PS II and PS I**.

In the non-cyclic light-dependent reaction, light drives the **synthesis of**:

1) **NADPH** (also known as reduced $NADP^+$)
   - $NADP^+$ stands for **n**icotinamide **a**denine **d**inucleotide **p**hosphate.
   - It is a **co-enzyme** and serves as a **high energy electron carrier**.
2) **ATP**

The key to this energy transformation is the flow of electrons through the photosystems and electron transport chains built into the thylakoid membrane.



Note: *You need not know the components of the 2 electron transport chains*

<u>How non-cyclic light-dependent reaction generates ATP and NADPH.</u>

<u>Photoactivation</u> *<u>at PS II</u>*

① A photon of light strikes a pigment molecule in a light harvesting complex. The energy captured is relayed to other pigment molecules via resonance transfer of energy, until it reaches one of the two P680 chlorophyll *a* molecules in the PS II reaction centre. **One of the P680 electrons is excited to a higher energy state**.

② This **electron is captured by** the **primary electron acceptor** in the reaction center and an electron "hole" is created in P680.

***Photolysis** of water*

Notes to self

3. An **enzyme splits a water molecule** into two electrons, two hydrogen ions, and an oxygen atom, in the presence of light.

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

The **electrons** are supplied one by one **to P680** molecules, each replacing an electron lost to the primary electron acceptor. The oxygen atom immediately combines with another oxygen atom, forming **O_2** which is released as a by-product. The **H^+ remains in the thylakoid space**. Therefore, water serves as an **electron donor**.

Electron Transport from PS II to PS I

4. Electron passes from the primary electron acceptor of **PS II to PS I** via an **electron transport chain**. The electron transport chain between PS II and PS I is made up of a series of **electron carriers** of **increasing electronegativity**. A series of **redox** (reduction-oxidation) reactions take place as electrons are transferred down the chain from one electron carrier to the next.

5. As the photoexcited electron travels down the electron transport chain, which consists of **electron carriers** of **progressively lower energy levels**, energy lost is **coupled to the formation of ATP**. This way of synthesizing ATP using light energy is called **photophosphorylation** (refer to section 10(c) for details).

***Photoactivation** at PS I*

6. Meanwhile, light energy has been relayed via pigment molecules to the PS I reaction centre via resonance. An **electron of** one of the two **P700** chlorophyll *a* molecules is **excited and then captured by the primary electron acceptor in PS I**, thus creating an electron "hole" in P700.

7. Electron (from step 4) that has reached the end of the first electron transport chain will fill the electron "hole" in P700.

Electron transport from PS I to $NADP^+$

8. Photoexcited electrons are passed from PS I's primary electron acceptor down a **second electron transport chain**. (Note: this electron transport chain is made up of different components compared with the first electron transport chain, and no ATP is formed here unlike the first.)

9. The electrons are finally transferred to $NADP^+$. This **reduction of $NADP^+$ to form NADPH** is catalyzed by the enzyme **$NADP^+$ reductase**.

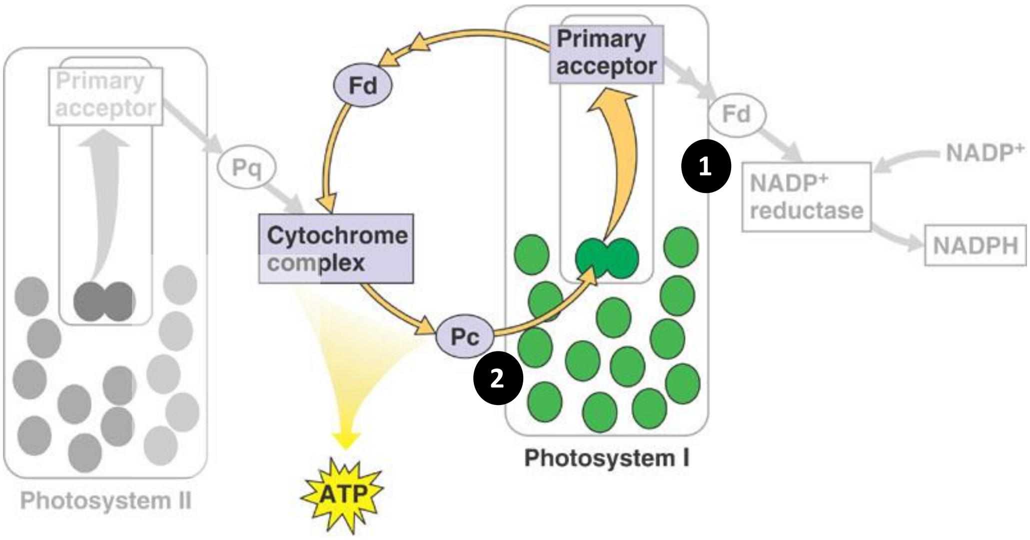
$$NADP^+ + 2e^- + H^+ \rightarrow NADPH$$

Hence, **$NADP^+$ is the final electron acceptor**. This reduction takes place in the **stroma**. Since the **high energy electrons are carried by NADPH**, it will provide the reducing power for the synthesis of sugar in the next step in photosynthesis, the **light-independent reaction** (i.e. **Calvin cycle**).

b. Cyclic Light-dependent Reaction

Notes to self

- Involves **PS I** only



Note: *You need not know the components of the electron transport chains.*

Cyclic light-dependent reaction.

(1) The photoexcited **electron from P700** is captured by the PS I's **primary electron acceptor** and then passed on to the **middle part of the first electron transport chain**. An electron "hole" in P700 is created.

(2) As the photoexcited electron travels down the electron transport chain, which consists of **electron carriers** of **progressively lower energy levels**, energy lost is **coupled to the formation of ATP**. This way of synthesizing ATP using light energy is called **photophosphorylation**. This electron eventually fills the electron "hole" left in P700, completing the cycle.

- **No NADPH is produced.**

  Instead of passing the excited electrons to the second electron transport chain as in the non-cyclic light-dependent reaction, they are transferred to the first electron transport chain.

- **No O_2 is produced** as there is **no photolysis of water**.
- While the **non-cyclic light-dependent reaction produces ATP and NADPH** in roughly equal quantities, **cyclic light-dependent reaction produces only ATP**. This is because the *next stage* of photosynthesis, the **Calvin cycle uses more ATP than NADPH**. Cyclic light-dependent reaction makes up this difference.

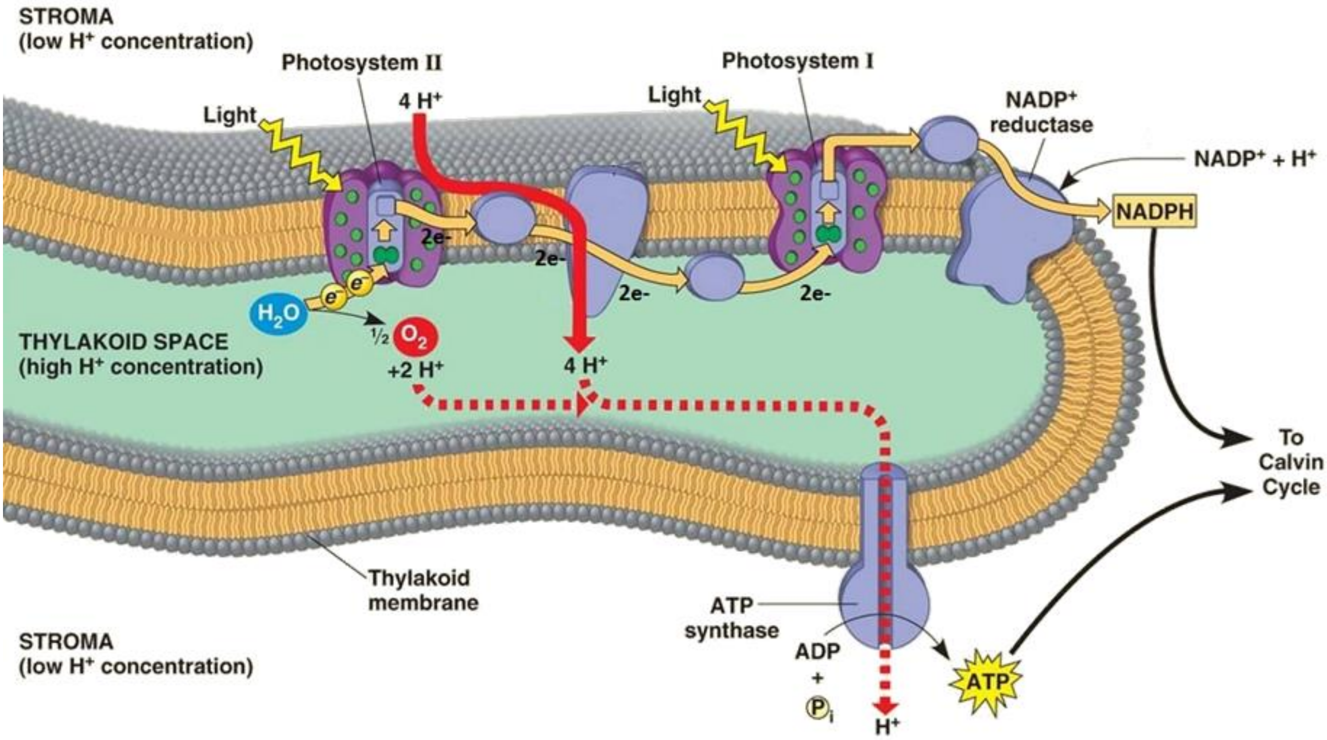
Q: In cyclic photophosphorylation, why doesn't the electron from PS I get passed to the 2nd ETC?

c. Synthesis of ATP via Photophosphorylation

Notes to self

It was mentioned earlier that as the excited electron travel down the electron transport chain which is made of electron carriers of progressively lower energy levels, energy lost is coupled to the formation of ATP. This process of ATP formation is called **photophosphorylation** and it involves the **use of a proton concentration gradient to drive the synthesis of ATP** from ADP and P_i (inorganic phosphate).

Definition of **photophosphorylation**:
The process of **synthesising ATP** by means of a **proton-motive force** generated across the thylakoid membrane of the chloroplast using **light energy** captured during the light-dependent reactions of photosynthesis.



Generation of ATP via photophosphorylation.

1. As electrons travel down a series of electron carriers that are progressively more electronegative, energy is released and used by certain electron transport chain proteins to **pump H^+ ions (protons)** from the **stroma** across the **thylakoid membrane** into the **thylakoid space**.

2. **H^+ (protons) accumulates in the thylakoid space** which serves as the H^+ (proton) reservoir. In this way, the electron transport chain transforms redox energy to a **proton-motive force**.

3. **Chemiosmosis** occurs when **H^+ (protons) diffuse** down the **proton concentration gradient** across the **thylakoid membrane** back into the **stroma** via **ATP synthase** (also called stalked particles).

4. **ADP** is phosphorylated to **ATP** in the process by ATP synthase.

5. The accumulation of **H^+ from the splitting of H_2O** (**photolysis**) in the thylakoid space further contributes to the electrochemical proton gradient across the thylakoid membrane.

6. The **uptake of H^+ ions (protons) from the stroma** when **$NADP^+$ is reduced to NADPH by $NADP^+$ reductase** also contributes to the proton gradient.

Q: ***What would happen to the pH of the thylakoid space and stroma if you shine light at chloroplasts? What would happen if the lights are turned off?***

Q: ***A poison disrupts the thylakoid membrane & renders it permeable to H^+ ions. Would ATP synthesis be affected?***

Q: ***If ATP can be generated through the light dependent reactions. Why do plant cells require both chloroplasts and mitochondria?***

Summary of the light-dependent reactions:

Notes to self

Type of light-dependent reaction	Components and/or reactions involved	Outcome
Non-cyclic light-dependent reaction (**non-cyclic photophosphorylation**)	PS II, PS I and both electron transport chains Reaction catalyzed by $NADP^+$ reductase: $NADP^+ + 2e^- + H^+ \rightarrow NADPH$	1. synthesises **ATP** through chemiosmosis 2. forms **NADPH** when electrons are finally transferred to $NADP^+$
	$H_2O \rightarrow 2e^- + 2H^+ + ½O_2$ (Splitting of water during photolysis)	3. generates electrons to be used to fill the electron "hole" in the reaction centre in PS II 4. produces O_2 as a by-product 5. generates H^+ (protons) which contributes to the higher H^+ concentration in thylakoid space
Cyclic light-dependent reaction (**cyclic photophosphorylation**)	PS I and the 1st electron transport chain	6. generates ATP through chemiosmosis

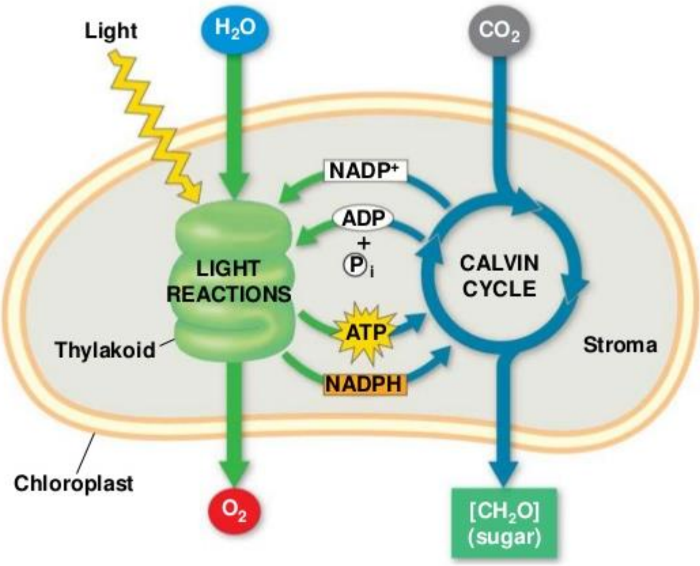
11. Light-independent Stage of Photosynthesis - Calvin Cycle

Notes to self

Location of Calvin cycle: **stroma** of chloroplast

The stroma contains the **enzymes** that catalyze the reactions in Calvin cycle of photosynthesis, e.g. **ribulose bisphosphate carboxylase** (**RuBisCO**).

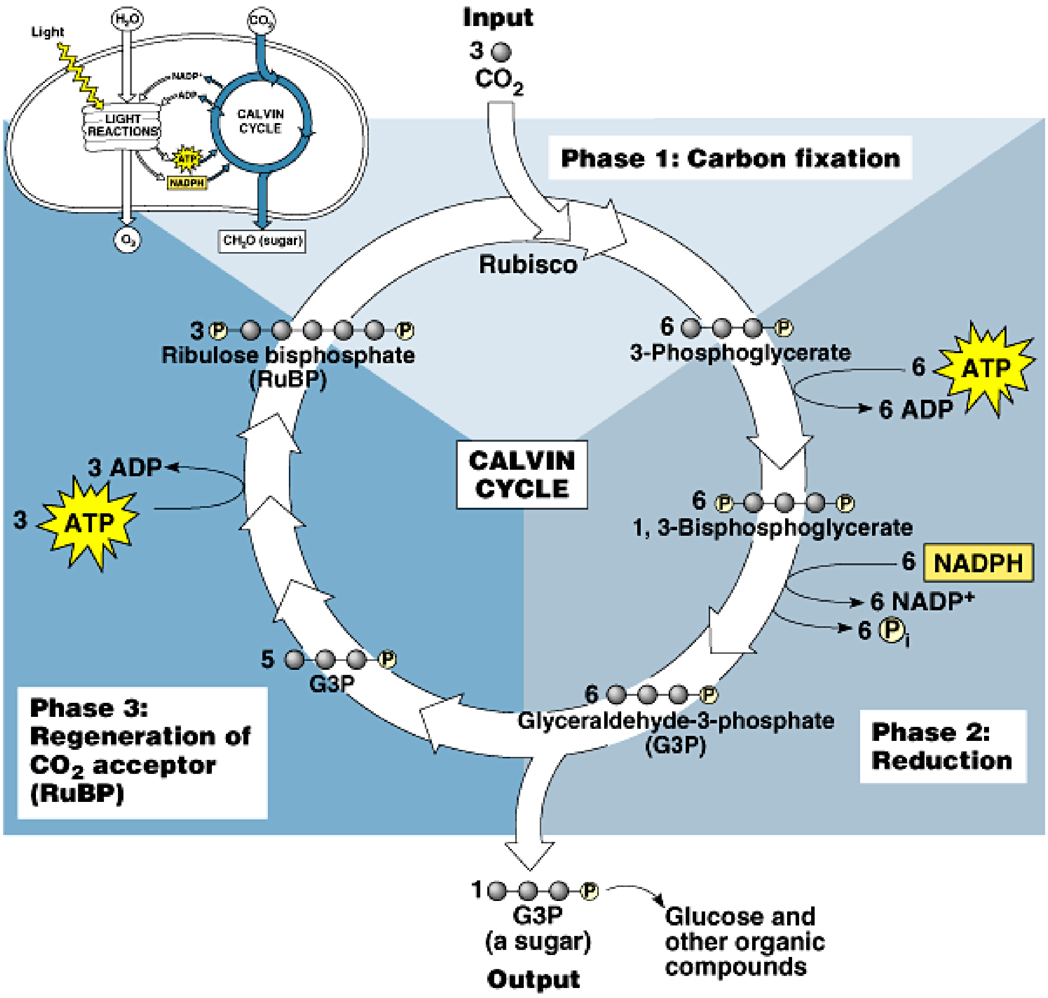
CO_2 and products of light dependent reaction (**NADPH** and **ATP**) are also required for Calvin cycle to occur.



Overview of cooperation between light-dependent and Calvin cycle (light-independent stage) of photosynthesis.

Calvin cycle is a pathway that reduces carbon dioxide to produce carbohydrates.

Notes to self



Calvin cycle is comprised of 3 phases:
i) Carbon fixation, ii) PGA reduction and iii) RuBP regeneration.

Phase 1 (Carbon fixation)

- This step involves carbon dioxide combining with **RuBP** (ribulose bisphosphate, a 5C sugar). This is catalyzed by **RuBP carboxylase/oxygenase (RuBisCO)**. RuBP is the **CO_2 acceptor**.
- The product of this reaction is an unstable 6C intermediate that will immediately split to form 2 molecules of **glycerate phosphate** (for every one molecule of CO_2).
- **Glycerate phosphate (GP)**, a 3C compound, is the first product of photosynthesis. This 3C-compound is also called **phosphoglycerate (PGA)**.

Notes to self

Phase 2 (Reduction of GP to G3P)

- This reaction requires the reducing power of **NADPH** and energy of **ATP** (both are products of non-cyclic light-dependent reaction).
- **GP is reduced (gains electrons)** to form a 3C compound, **glyceraldehyde-3-phosphate (G3P)**. G3P has 2 other names: phosphoglyceraldehyde (PGAL) and triose phosphate (TP).
- In fact, the **first sugar (3C sugar) formed in photosynthesis is G3P**. It is also the **end product** of Calvin cycle. It is a basic sugar **used to build up more complex carbohydrates** such as glucose, sucrose, starch or cellulose.

  For the net synthesis of 1 molecule of G3P for carbohydrate synthesis, 3 molecules of CO_2 have to be fixed.

Phase 3 (Regeneration of RuBP)

- G3P has to be used to regenerate **RuBP** which is the CO_2 acceptor.

  5 molecules of **G3P** (a 3C molecule; total 15C) used to regenerate **3 RuBP** (a 5C molecule; total 15C)
- 3 **ATP** from the light-dependent reaction is used in the regeneration step.

Q: ***Balance this cycle and determine how much of each reactant is needed to generate one glucose molecule (6C):***

CO_2 : 6 *ATP : 18* *NADPH : 12*

Q. More ATP is used in total than NADPH. How does the cell cope with this?

By cyclic photophosphorylation.

Q: What is/are the role(s) of NADPH and ATP in the light-independent reaction?

To allow light independent reaction to proceed so that G3P can be produced (by reducing GP), which in turn can be used to form glucose

In summary:

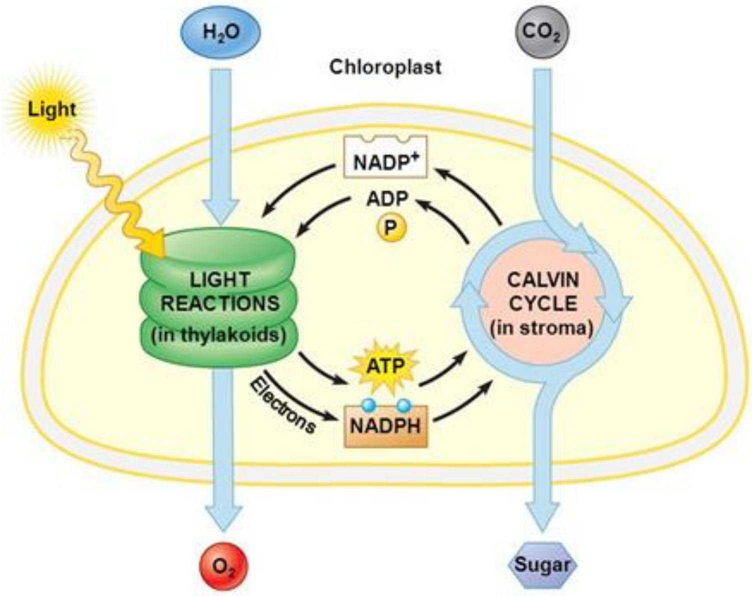
Notes to self

- Light-dependent reaction
  1. Light absorbed by chlorophyll drives a transfer of electrons and H^+ (protons) from water to $NADP^+$ which temporarily stores the energized electrons. $NADP^+$ is the final electron acceptor.
  2. Water is split (photolysis) in the process that gives off O_2 as a by-product.
  3. Generates ATP by chemiosmosis via the cyclic and non-cyclic photophosphorylation.
  4. Thus, light energy is converted to chemical energy in the form of NADPH which is a source of energized electrons ("reducing power") and ATP.

- Light-independent reaction
  1. Calvin cycle makes G3P from the products of light-dependent reaction, NADPH and ATP.
  2. Involves 3 stages:

     (i) carbon fixation (ii) PGA reduction and (iii) regeneration of RuBP.
  3. Regenerates $NADP^+$ and ADP for utilization in the light dependent reaction.

- Neither reaction alone can make sugar from CO_2. The chloroplast integrates the two stages of photosynthesis.

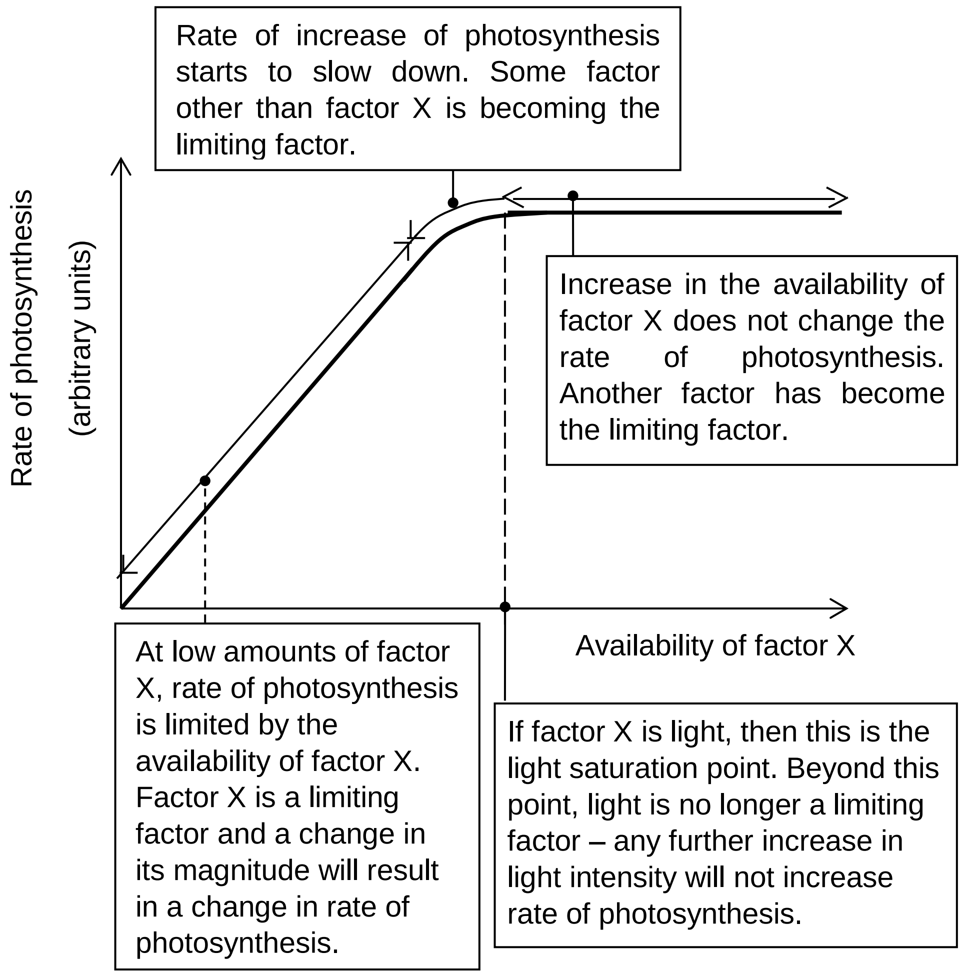


<u>Both light-dependent and light-independent reactions work together to make sugar.</u>

12. Limiting Factors in Photosynthesis

Notes to self

Limiting factor: the factor that limits the reaction rate in any physiological process governed by many variables. Any changes in the level of a limiting factor will change the rate of a reaction as long as other factors are in adequate supply. The main factors that can affect the rate of photosynthesis include light intensity, carbon dioxide concentration and temperature.



a) Light intensity

In low light intensities, the rate of photosynthesis increases linearly with increasing light intensity. Gradually, the rate of increase falls as other factors become limiting instead. Photosynthesis rate does not increase further once light saturation point is reached.

Illumination on a clear summer's day is about 100 000 lux whereas the light saturation point for photosynthesis is reached at about one-tenth of that, 10 000 lux. Therefore, light intensity is not normally a major limiting factor (except for shaded plants).

Very high light intensities may bleach chlorophyll. Plants that live in areas of high light intensities protect themselves with thick cuticles and hairy leaves.

Note: Apart from light intensity, **light wavelength** and **light duration** (photoperiod) also affect photosynthesis.

b) CO_2 concentration

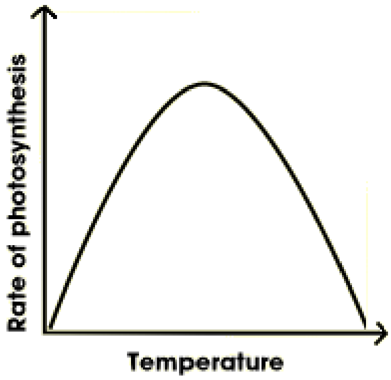
Notes to self

Concentration of carbon dioxide is a **major limiting factor**. The atmospheric concentration of CO_2 is between 0.03% - 0.04%, which is far below the optimum concentration of between 0.1- 0.5%.

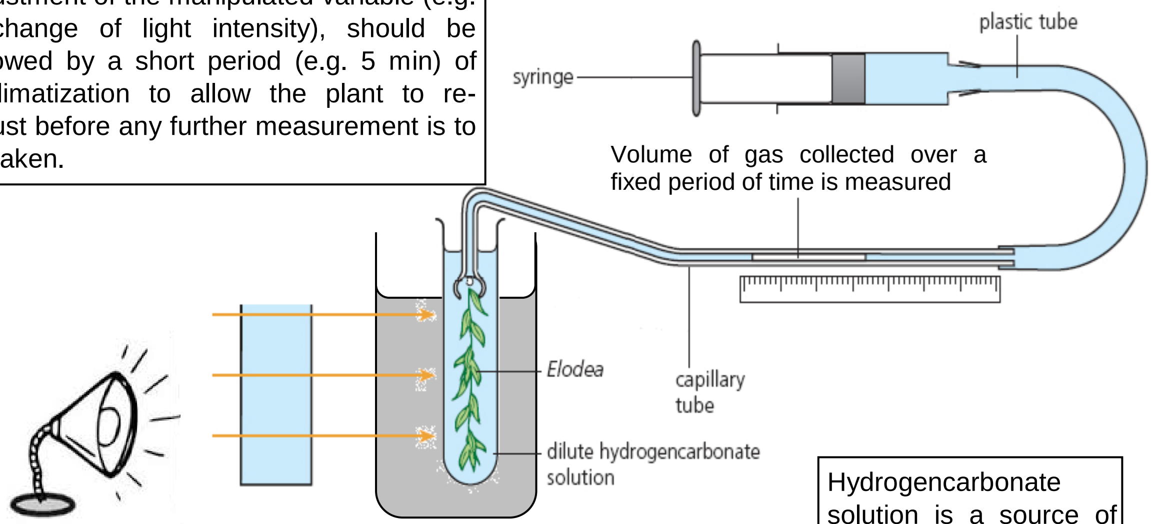
c) Temperature

Since both the light-dependent and to a larger extent, light independent reactions are **enzyme-controlled**, temperature will affect the rate of photosynthesis.

The rate of reaction doubles for every 10°C rise up to about 35°C. Beyond 40°C, the enzymes start to denature → rate of photosynthesis falls.



When photosynthesizing, *Elodea* releases O_2 from the cut end of the stem. Any adjustment of the manipulated variable (e.g. a change of light intensity), should be followed by a short period (e.g. 5 min) of acclimatization to allow the plant to re-adjust before any further measurement is to be taken.



Hydrogencarbonate solution is a source of CO_2. The concentration may be varied if $[CO_2]$ is to be the manipulated variable.

If light intensity is to be the manipulated variable then the light may be moved to a known distance from the plant.

A water screen can be used to prevent warming up of the water bath due to light source.

A water bath is used so that temperature can be the manipulated variable

Experimental setup to investigate the effect of light intensity or CO_2 concentration or temperature on rate of photosynthesis.

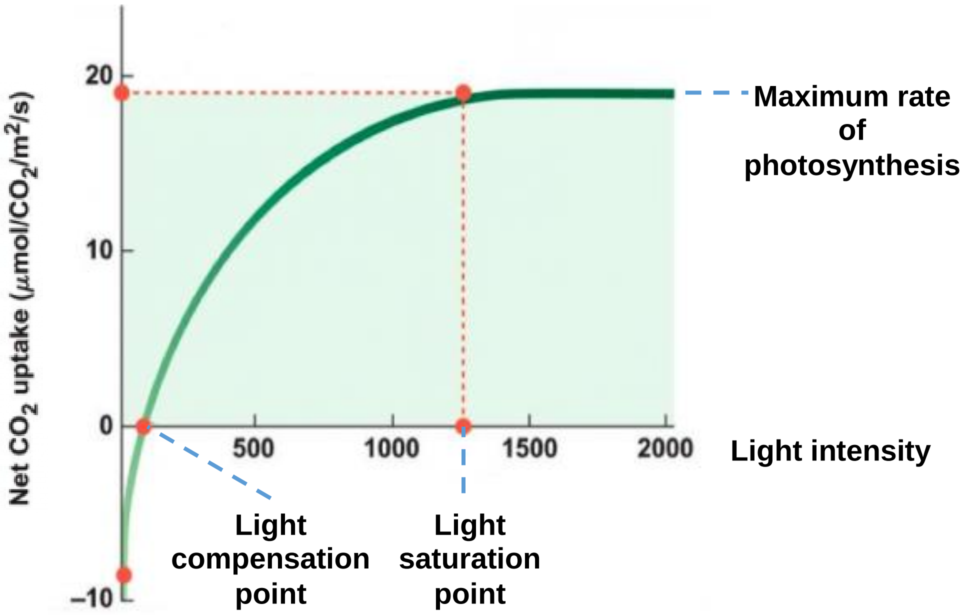
The bubbles of evolved gas are collected over a fixed duration of time. Rate of photosynthesis can then be calculated (rate of photosynthesis = amount of gas collected per unit time).

Notes to self

Light compensation point:

The light compensation point is a point when **photosynthetic rate equals to respiration rate**. As such, **the net assimilation of CO_2 is zero** and there is **no net change of O_2** as well at this point.

This is unique to plants as they photosynthesize (i.e. take in CO_2 and give out O_2), and respire (i.e. take in O_2 and give out CO_2) at the same time.



Graph showing light compensation point and light saturation point.

Effect of O_2 concentration on rate of photosynthesis:

Rubisco accepts **O_2 as a competitive inhibitor** when the CO_2:O_2 ratio is low.

This occurs on bright, hot, dry days. On such days, the **stomata of the leaf close** restricting water loss. This inevitably results in **reduced CO_2** entering the leaf and CO_2 concentration plummets, while **O_2 concentration builds up due to photosynthesis**.

Oxygenase function of Rubisco causes RuBP to be split into a 3C and 2C compound. The 2C (glycolate) compound is exported to the peroxisomes and mitochondria where it is broken down into CO_2. This is called **photorespiration** and it does not generate ATP.