A study material for M.Sc. Biochemistry (Semester: II) Students on the topic

(CC-8; Unit II)

Photo-Phosphorylation

The process of generation of life (Oxygen) and production of ATP from sunlight

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Phosphorylation is a biochemical process that involves the addition of phosphate to an organic compound. Examples include the addition of phosphate to Adenosine Di-phosphate (ADP) to form Adenosine Triphosphate (ATP).

Phosphorylation is of three types:

1. **Photo- Phosphorylation** – ATP formed through a series of sunlight-driven reactions in phototrophic organisms
2. **Oxidative Phosphorylation** - ATP formed through a series of redox reactions occurring during the final phase of the respiratory pathway
3. **Substrate Level Phosphorylation** – Transfer of Phosphate molecule directly from the substrate

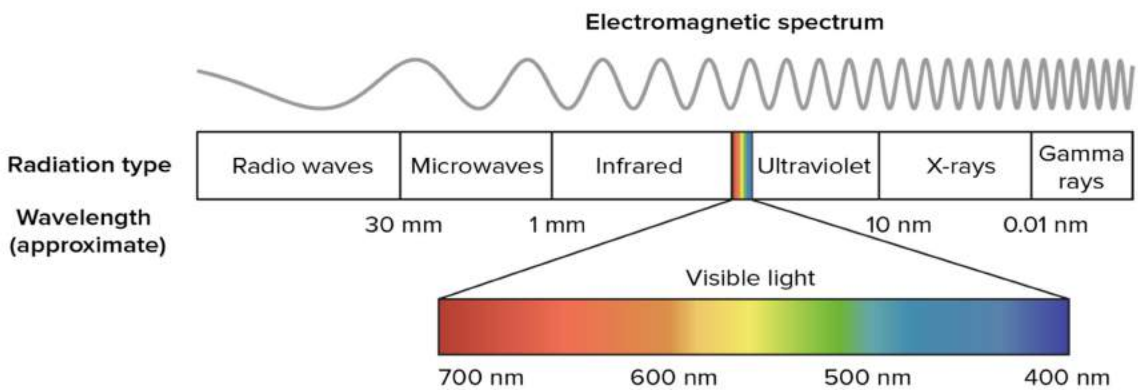
Sun is the only source of energy for all the living organisms on the earth. All the food chains ultimately depend on the Sun. But all the living organisms cannot convert this solar energy into chemical energy. This can be performed by only autotrophic organisms (such as green plants and algae etc.). These organisms are capable to do so because of the presence of a special cell organelle (Chloroplast) and due to the presence of light harvesting complex.

The process of conversion of Light energy into chemical energy is known as Photosynthesis. This process is divided into two stages:

1. **Light Dependent Reaction**: The process dependent on Sunlight. The products of this reaction are ATP and $NADPH+H^+$. The formation of ATP through this process with the help of Sunlight is known as **Photophosphorylation**. This reaction depends on the presence and absence of light as well as its several features (like: intensity, duration, wavelength etc.).
2. **Light Independent reaction**: The conversion of Carbon-di-oxide into glucose takes place in this phase. This **carbon fixation** needs the products of Light Dependent

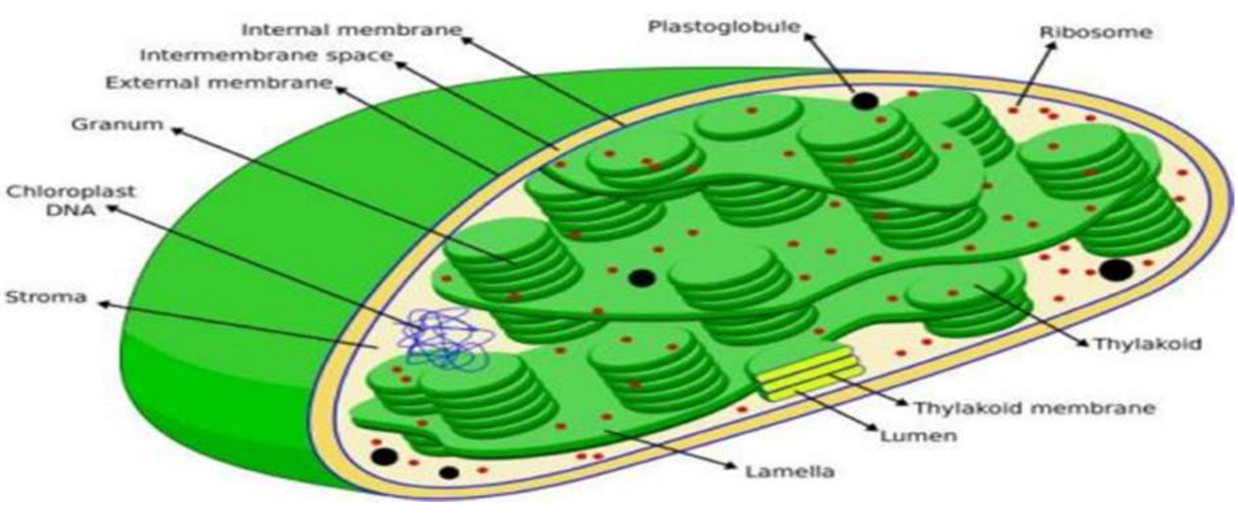
reactions (i.e. ATP and NADPH+H^+) hence although this reaction does not depend on the presence of light but depends on the product of Light Dependent reactions. Hence, once light becomes absent, it also stops.

All the wavelength of sunlight is not fit for photosynthesis although Sun has such a big spectrum of light:

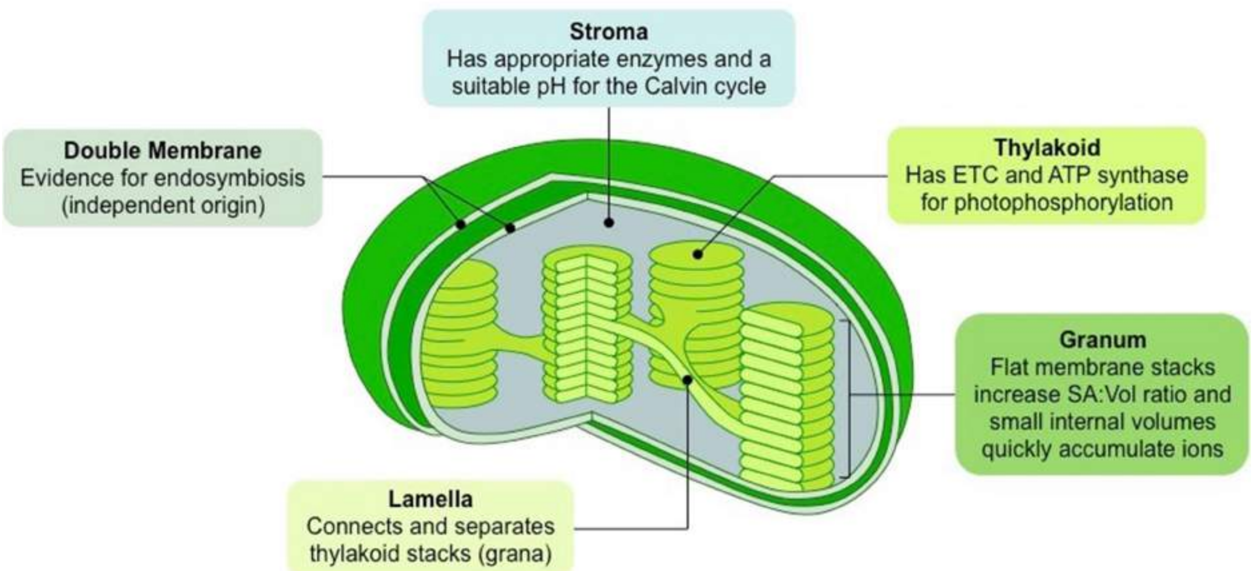


Only those light wavelengths are useful for photosynthesis which are absorbed by different available pigments, it means, different pigments have capacity to absorb different wavelength of light. They have different light absorption spectra. But generally all the light used in photosynthesis lies in visible range. Blue Light and Red light are considered better for this. But the blue light has more energy per quantum with respect to red.

Light has dual nature: wave nature as well as particle nature. When light falls on any surface, it transfers the energy of its particles (quanta or photon) to the electron of the absorbing surface and the absorbing surface emits the exited electron. This theory is based on Photo electric effect (proposed by the great physicist Albert Einstein, for which he got Nobel Memorial Prize in 1921). But for trapping the light, there is a need of light harvesting complex. It is found in Chloroplast (Green pigmented cell organelle):



Chloroplasts are semi-autonomous cell organelles. They are supposed to be free living photosynthetic bacteria, but during the course of evolution, it came with eukaryotic cell in symbiosis and then stayed with it (Endo-symbiotic Theory). They have their own DNA and their own protein machinery (70 s Ribosomes) beside two membrane outer boundary. The membrane of chloroplast contains several receptors which are similar to the receptors of bacteria, it has circular DNA and the ribosomes are also of 70 s type (similar to that of prokaryotes). One possible reason for its symbiotic association is that the Chloroplast DNA does not have the code for all the essential proteins required during photosynthesis and also for the survival of the chloroplast. Several membrane bound sub cellular organelles are present in the stroma of Chloroplast. They are termed as Thylakoids. They are present in pilled forms known as Grana. These grana are interconnected through membranous pipe line like structures known as Stroma lamellae or fret channels. The thylakoids are the sites of Light Dependent reactions or Photophosphorylation, while stroma is the site of Light Independent Reaction or Carbon Fixation.



The thylakoids are membrane bound sub cellular particles. They are the actual sites of Photophosphorylation. They contain several smaller globular structures known as Quantasomes. Quanta stand for small pockets of light energy. Quantasomes are actually the light harvesting complex. They have three regions. The outer region is Antenna Complex, middle region is core complex and the central region contains Central molecules.

a. Central molecule: Chlorophyll a mainly constitutes the central molecule. They act as reaction center.

b. Core complex: Other Chlorophyll molecules (such as Chlorophyll b molecule in higher plants and Chlorophyll c/d/e molecule in algae) along with several other proteins act as molecules of core complex.
c. Antenna Complex: Different other photosynthetic pigments (such as Carotenoids, Xanthophyll, Phycobillins etc.) are found in antenna complex

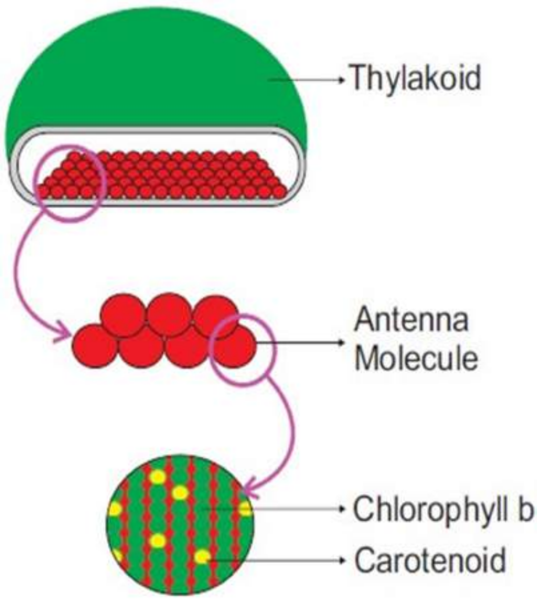
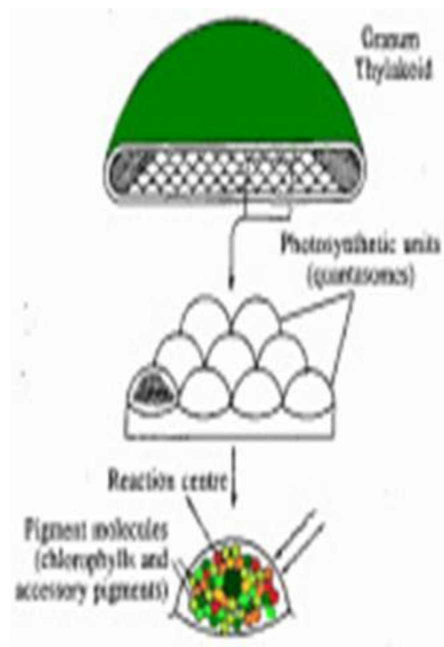


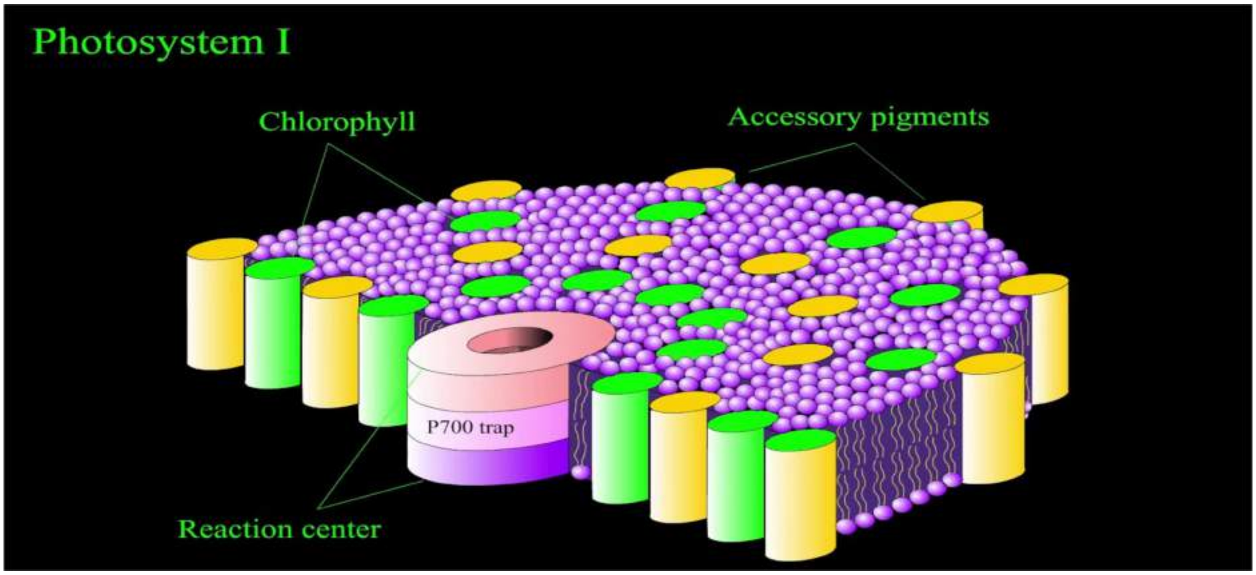
Figure 13.7: Quantasome



The pigments of Core complex and Antenna Complex have three important functions:

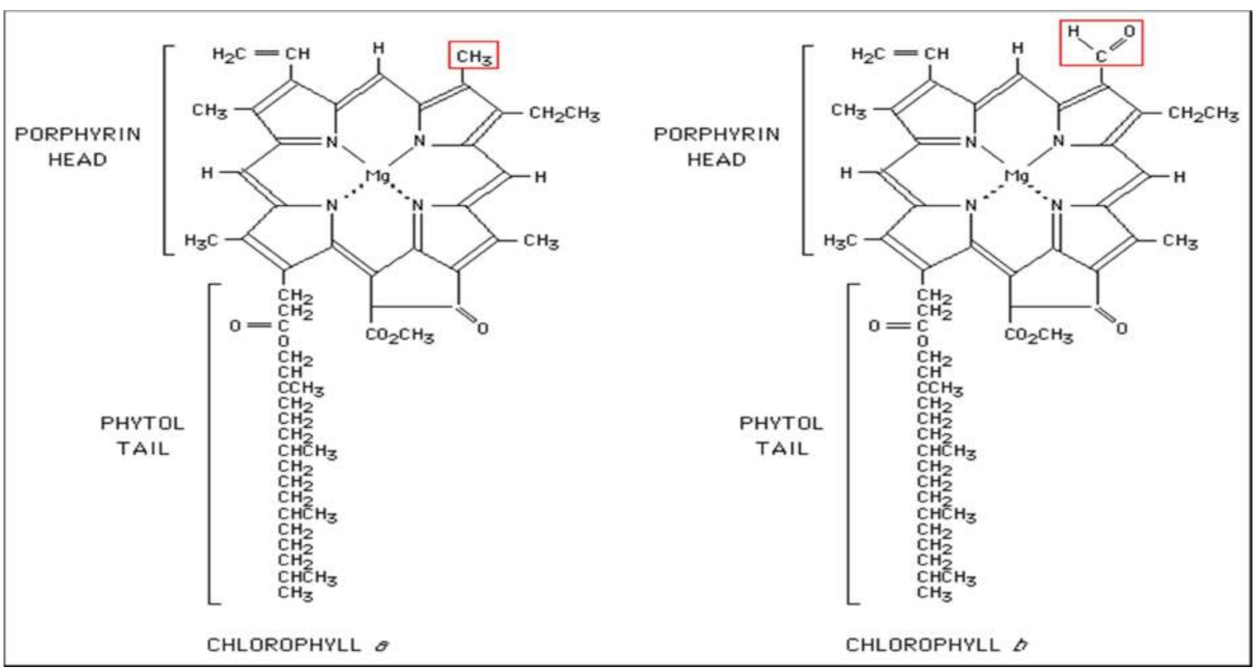
i) To increase the surface area of absorption of Sunlight, so that maximum sunlight can be gathered. They emit electrons too due to the incidence of sunlight on them. They transfer these electrons towards the reaction center.
ii) To protect chlorophyll and other pigments from destruction (photo-respiration).
iii) To provide adaptation towards sunlight for the pigments responsible for photosynthesis in odd situations (such as in low sunlight condition, in deep water etc.).

The arrangement of pigments in a quantasome is like this:



There is total nine type of Chlorophyll reported till date: Chlorophyll a (found in almost all photosynthetic organisms and acts as reaction center), Chlorophyll b (found in higher plants along with Chlorophyll a), Chlorophyll c, Chlorophyll d, Chlorophyll e (found in different algae in combination with Chlorophyll a); Bacterial Chlorophyll a and Bacterial Chlorophyll b are found in photosynthetic bacteria while Chlorobium (A special photosynthetic bacteria) have special chlorophyll, they are Chlorobium Chlorophyll 650 and Chlorobium Chlorophyll 660.

Chlorophyll molecules contain a Porphyrine ring and a phytol chain. There is no phytol chain in Chlorophyll c. The structure of chlorophyll molecule is like:

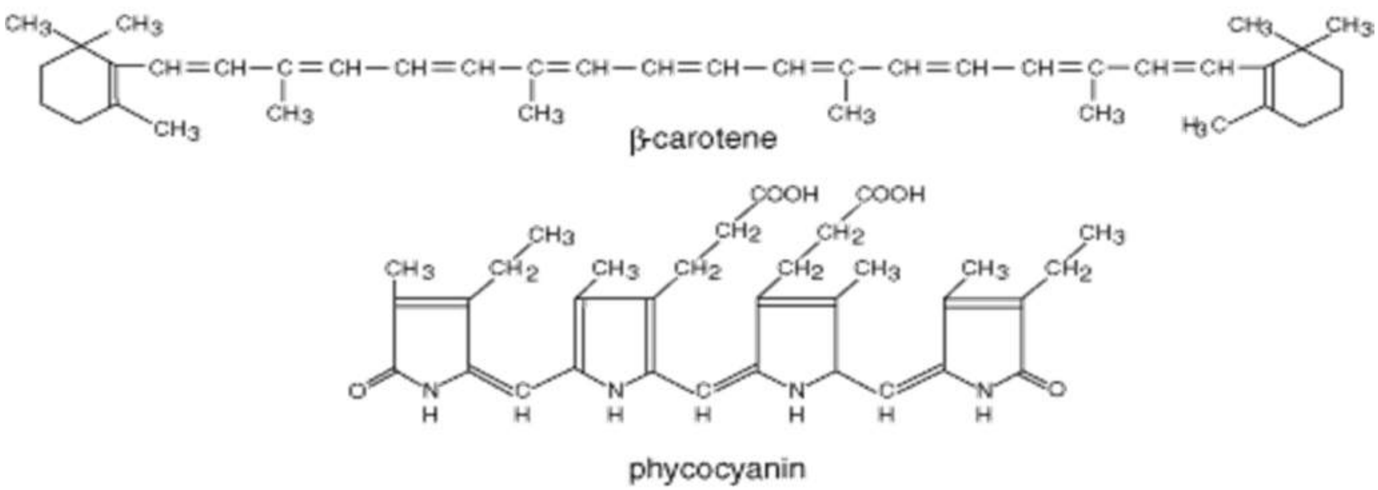


Carotenoids are lipid compounds distributed both in plants and animals. They are yellow to purple in colour. They are considered to be the derivative of Lycopene (a red pigment) having empirical formula $C_{40}H_{51}$, Lycopene is highly unsaturated straight chain hydrocarbons derived from Isoprene units.

Xanthophylls are oxygen derivative of carotene, containing 1-8 oxygen atoms. There are several types of Xanthophylls too (e.g. Lutein, Zeaxanthin, Violaxanthin, Cryptoxanthin, Neoxanthin etc.).

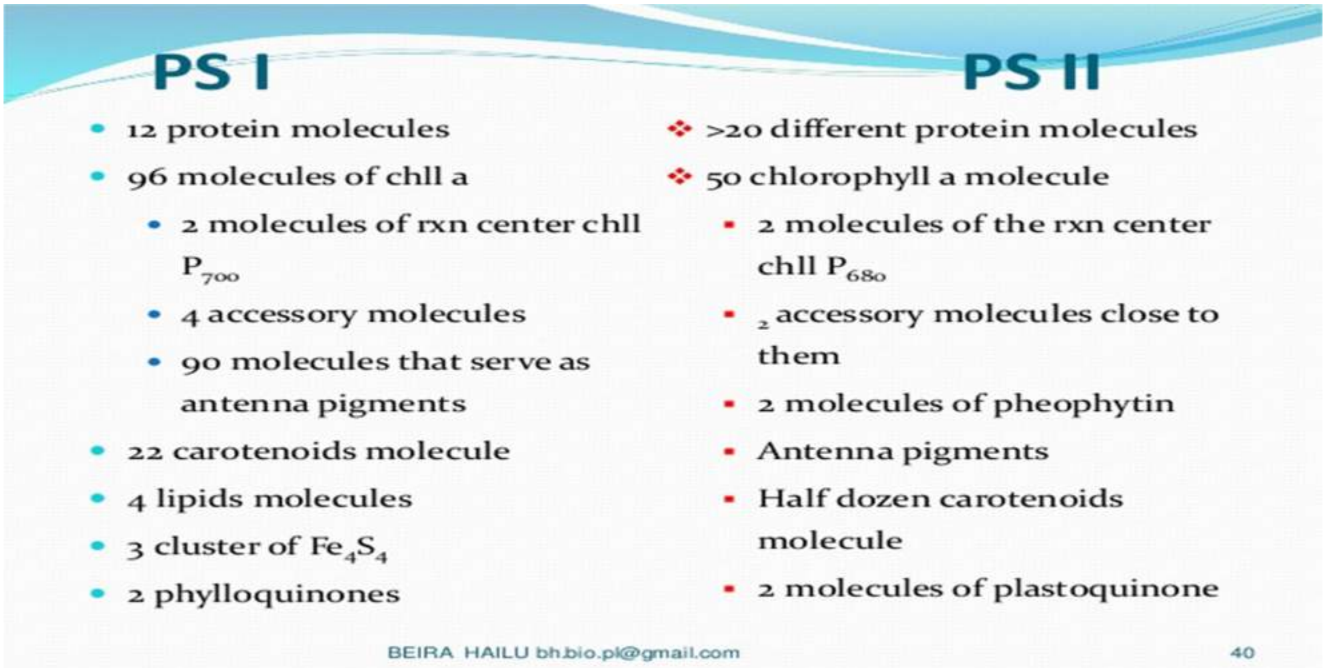
Phycobilins are protein linked pigments and they are water soluble. They are localized in small granules called Phycobilisomes attached to thylakoid. Like Chlorophyll, these pigments are open tetrapyrrole but do not contain Magnesium and phytol chain. There are two main types of Phycobilins; Phycocyanin (found in Blue Green Algae, i.e, Cyanobacteria) and Phycoerthyrin (found in red algae).

The structure of some accessory pigments is like:

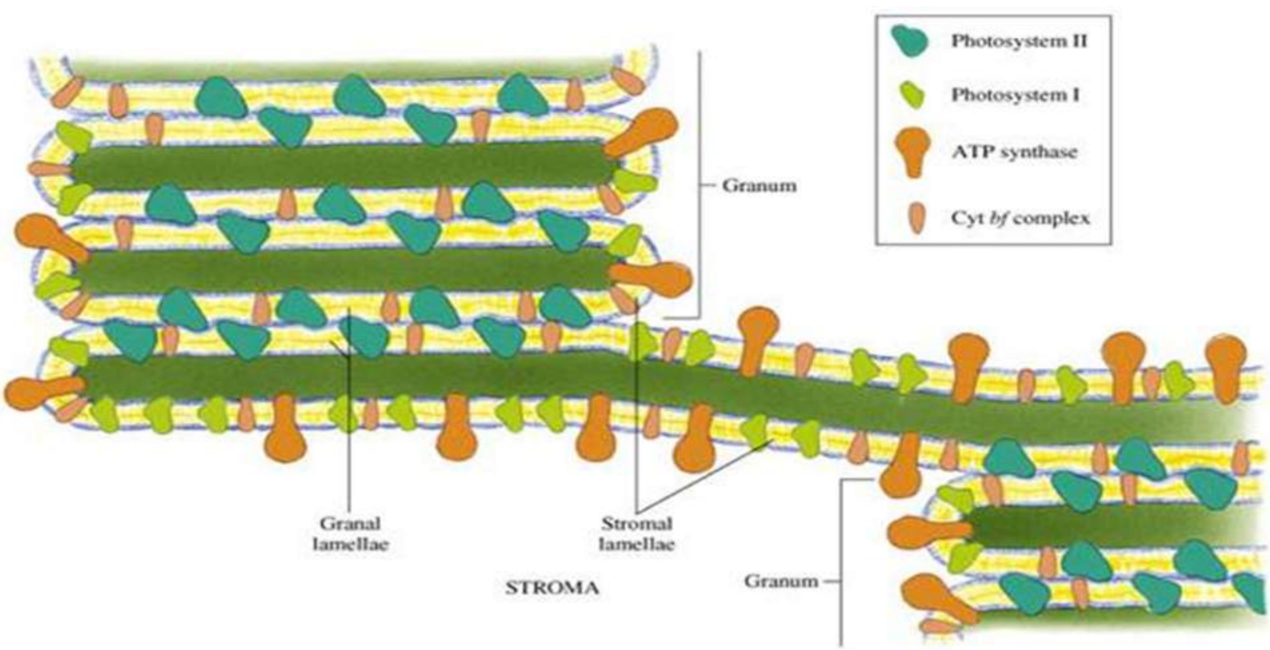


Formulas of a carotene and a phycobilin pigment. Compare the **cyclic** tetrapyrrole nucleus of chlorophyll with the **linear** tetrapyrrole nucleus of the phycobilin.

Now, these pigments (Chlorophyll and accessory pigments) constitute a system for photosynthesis known as Photosystem or we can say that The light-absorbing pigments of thylakoid or bacterial membranes are arranged in functional arrays called **photosystems.** The photosystem is of two types: Photosystem I and Photosystem II (named according to their discovery). The reaction centers (i.e. Chlorophyll a molecules) in these two photosystems have two different absorption spectra. Photosystem I (PS I) has P_{700} reaction center (means it absorbs and works better in the presence of the light having wavelength of 700 nm), while Photosystem II (PS II) has P_{680} as reaction center. Beside this, both the photosystems have several other differences too, listed in the table below:



These Photosystems are also uneven in their distribution pattern in the thylakoid membrane. PS I is found in non- oppressed regions of the thylakoid (it means in those regions only which are directly exposed outside and are not in contact with any other thylakoid membrane, such as terminal thylakoids in a granum) and as stroma lamellae has also no contact with other membrane hence they also have PS I, while PS II is found in the oppressed regions of the thylakoid membranes. The arrangement of these two Photosystems can be easily understood by this figure:



Now, we have to understand the mechanism of Photophosphorylation:

Photophosphorylation is also based on Chemio-osmotic Coupling Hypothesis of Peter Mitchell similarly as Oxidative Phosphorylation. Here a flow of electron takes place through different electron carriers embedded in the Thylakoid membrane and due to this electron flow, a difference of Protons (H^+) arises between Thylakoid Lumen and Chloroplast Stroma.

The flow of electron starts from the photosystems due to the absorption of Sunlight by them (because of Photo-electric effect or particle nature of light, as stated earlier). Now, due to the emittion of electrons from the photosystem particles, there creates an electron void in those particles. These electron voids are fulfilled by electrons emitted from the splitting of Water molecules. This photolysis of water molecule takes place with the help of a protein complex known as "Oxygen Evolving Complex". Here water splits with the help of Manganese Ion into two protons (H^+), two electrons (e^-) and oxygen molecule.

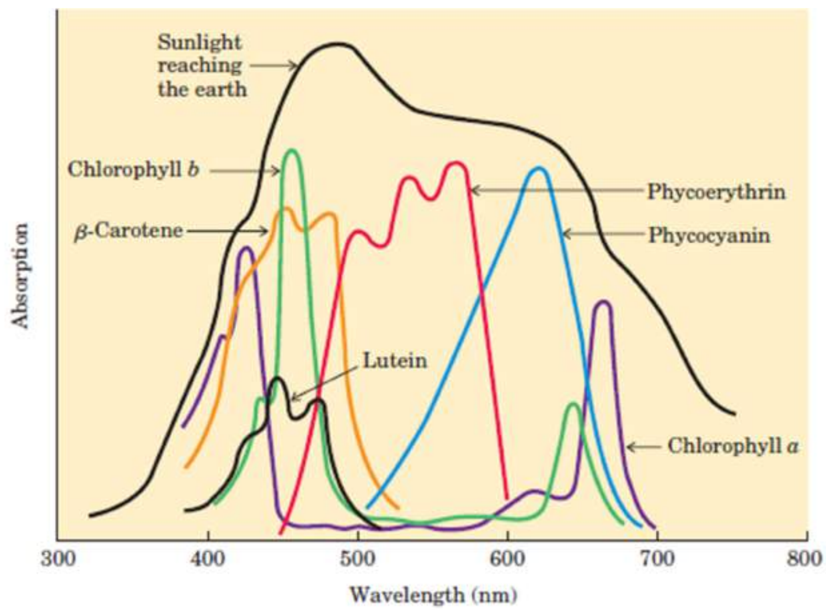


FIGURE 19-41 Absorption of visible light by photopigments. Plants are green because their pigments absorb light from the red and blue regions of the spectrum, leaving primarily green light to be reflected or transmitted. Compare the absorption spectra of the pigments with the spectrum of sunlight reaching the earth's surface; the combination of chlorophylls (*a* and *b*) and accessory pigments enables plants to harvest most of the energy available in sunlight.

The relative amounts of chlorophylls and accessory pigments are characteristic of a particular plant species. Variation in the proportions of these pigments is responsible for the range of colors of photosynthetic organisms, from the deep blue-green of spruce needles, to the greener green of maple leaves, to the red, brown, or purple color of some species of multicellular algae and the leaves of some foliage plants favored by gardeners.

The other pigment molecules in photosystem absorb light energy and transmit it rapidly and efficiently to the reaction center. When a photon is absorbed, an electron in the absorbing molecule (chromophore) is lifted to a higher energy level. This is an all-or-nothing event; to be absorbed, the photon must contain a quantity of energy (a quantum) that exactly matches the energy of the electronic transition. A molecule that has absorbed a photon is in an excited state, which is generally unstable. An electron lifted into a higher-energy orbital usually returns rapidly to its normal lower-energy orbital; the excited molecule decays to the stable ground state, giving up the absorbed quantum as light or heat or using it to do chemical work. Light emission accompanying decay of excited molecules (called fluorescence) is always at a longer wavelength (lower energy) than that of the absorbed light. An alternative mode of decay important in photosynthesis involves direct transfer of excitation energy from an excited molecule to a neighboring molecule. Just as the photon is a quantum of light energy, so the exciton is a quantum of energy passed from an excited molecule to another molecule in a process called exciton transfer.

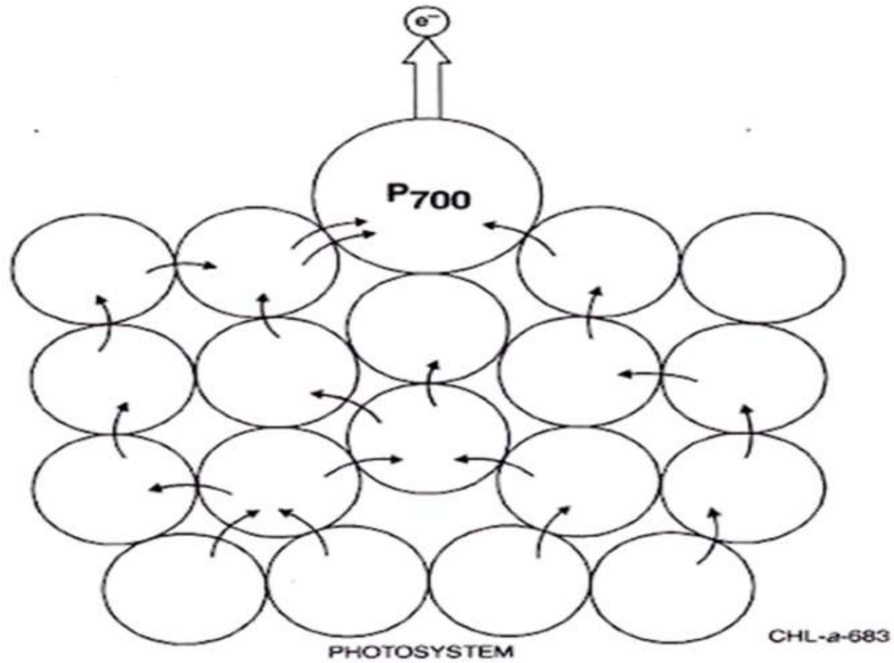


Fig. 13-8. Antenna chlorophyll molecules and the reaction centre. It is supposed that most of the chlorophyll molecules are light harvesting molecules. One chlorophyll molecule, however, is the reaction centre.

In this excited chlorophyll molecule, an electron is promoted to a high energy orbital. This electron then passes to a nearby electron acceptor that is part of the electron-transfer chain, leaving the reaction-center chlorophyll with a missing electron (an "electron hole,"). The electron acceptor acquires a negative charge in this transaction. The electron lost by the reaction-center chlorophyll is replaced by an electron from a neighboring electron-donor molecule, which thereby becomes positively charged. In this way, excitation by light causes electric charge separation and initiates an oxidation-reduction chain. This electron transfer starts from reaction center and end at $NADP^+$ and the electron hole created by it is fulfilled by electron emitted due to photolysis. Oxygen is evolved during this process as by product. To form one molecule of O2, which requires transfer of four electrons from two H_2O to two $NADP^+$, a total of eight photons must be absorbed, four by each photosystem. This oxygen is life for aerobic organisms. You can recall that this oxygen is utilized as final electron acceptor during Oxidative Phosphorylation.

Excitation of P_{680} in PSII produces P_{680}*, an excellent electron donor that, within picoseconds, transfers an electron to pheophytin, giving it a negative charge ($Pheo^-$). With the loss of its electron, P_{680}* is transformed into a radical cation, $P_{680.}$ Pheo very rapidly passes its extra electron to a protein-bound plastoquinone, PQ_A (or Q_A), which in turn passes its electron to another, more loosely bound plastoquinone, PQ_B (or Q_B). When PQ_B has acquired two electrons in two such transfers from PQ_A and two protons from the solvent water, it is in its fully reduced quinol form, PQ_BH_2.

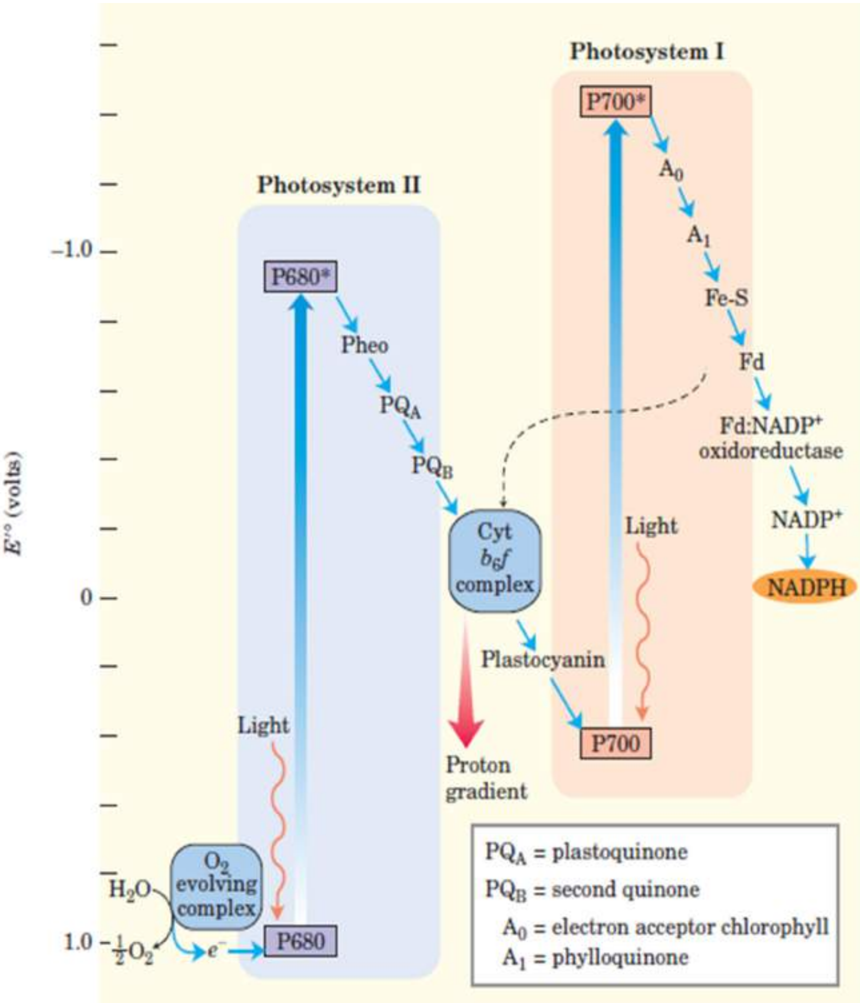
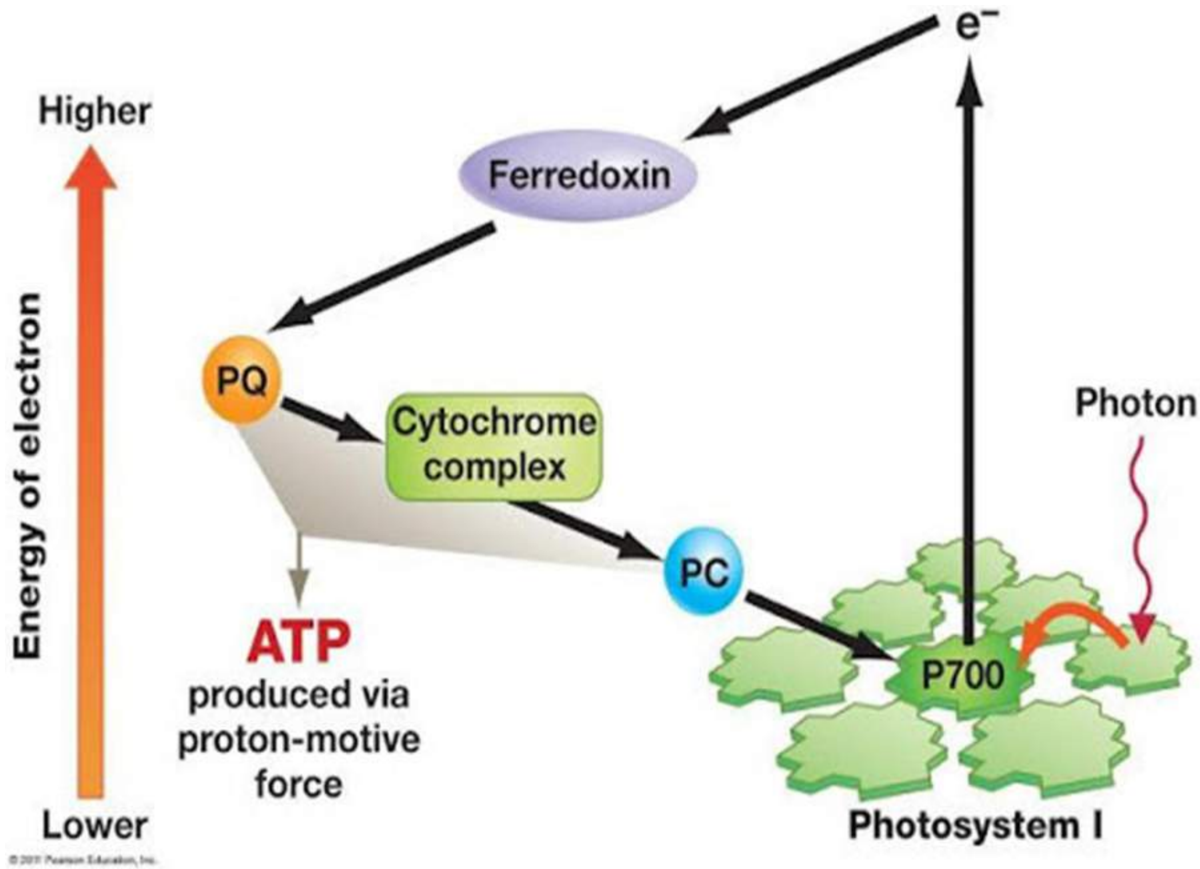


FIGURE 19–49 Integration of photosystems I and II in chloroplasts. This "Z scheme" shows the pathway of electron transfer from H_2O (lower left) to $NADP^+$ (far right) in noncyclic photosynthesis. The position on the vertical scale of each electron carrier reflects its standard reduction potential. To raise the energy of electrons derived from H_2O to the energy level required to reduce $NADP^+$ to NADPH, each electron must be "lifted" twice (heavy arrows) by photons absorbed in PSII and PSI. One photon is required per electron in each photosystem. After excitation, the high-energy electrons flow "downhill" through the carrier chains shown. Protons move across the thylakoid membrane during the water-splitting reaction and during electron transfer through the cytochrome b_6f complex, producing the proton gradient that is central to ATP formation. The dashed arrow is the path of cyclic electron transfer (discussed later in the text), which involves only PSI; electrons return via the cyclic pathway to PSI, instead of reducing $NADP^+$ to NADPH.

Eventually, the electrons in PQ_BH_2 pass through the cytochrome b_6 *f* complex. The electron initially removed from P_{680} is replaced with an electron obtained from the oxidation of water. The photochemical events that follow excitation of PSI at the reaction center P_{700} are formally similar to those in PSII. The excited reaction center P_{700}* loses an electron to an acceptor, A_0 (believed to be a special form of chlorophyll, functionally homologous to the pheophytin of PSII), creating A_0 and P_{700}; again, excitation results in charge separation at the photochemical reaction center. P_{700} is a strong oxidizing agent, which quickly acquires an electron from plastocyanin, a soluble Cu-containing electron-transfer protein. A_0 is an exceptionally strong reducing agent that passes its electron through a chain of carriers that leads to NADP. First, phylloquinone (A_1) accepts an electron and passes it to an iron-sulfur protein (through three Fe-S centers in PSI). From here, the electron moves to ferredoxin (Fd), another iron-sulfur protein loosely associated with the thylakoid membrane. The fourth electron carrier in the chain is the flavoprotein ferredoxin : NADP oxidoreductase**,** which transfers electrons from reduced ferredoxin (Fd_{red}) to NADP. This is Non-cyclic electron flow.



In some cases the synthesis of carbohydrate is curtailed due to limited supply of CO_2 or due to some other factors, then $NADPH+H^+$ starts accumulating in the stroma and no free $NADP^+$ is available to take electrons from the Electron Transport Chain. In these cases Ferredoxin transfers the electrons to Plastoquinone (PQ). Plastoquinone tranfers the electrons to Cytochrome b_6f complex. Then the electrons are transferred to Photosystem I through Plastocyanine. PS I is involved in this transport but there is no involvement of PS II. PSI transfers the electrons to Ferredoxin through different other electron carriers as stated earlier (A_0, A_1 and Fe-S centers). This kind of transport is called Cyclic Electron Transport and it causes Cyclic Photophosphorylation.

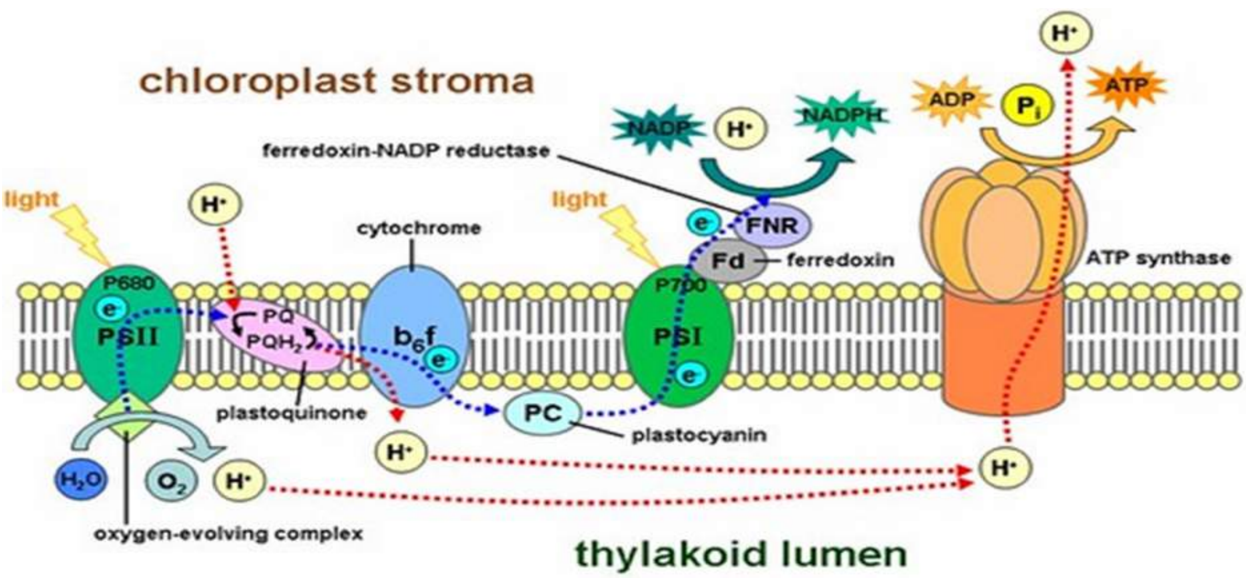
Plastoquinone is mobile electron carrier same as that of Ubiquinone (Q) of Oxidative Phosphorylation. The recycling of Q was taking place in Complex III of Mitochondrial Electron Transport while the recycling of this PQ takes place in Cytochrome b_6f Complex. Plastoquinone is a mobile electron carrier, it translocate protons from stroma side to Thylakoid lumen side and causes a proton gradient. Hence ATP is produced during Cyclic Photophosphorylation but $NADPH+H^+$ is not produced. There are several other differences too between cyclic and non-cyclic photophosphorylation:

S. No.	Non cyclic	Cyclic
1	PS-I and PS-II both are active	Only PS-I active
2	Photolysis of water takes place	Photolysis of water does not take place
3	Water is consumed	Water is not consumed
4	O_2 liberates	O_2 does not liberate
5	Source of electron is water	Source of electron is P-700
6	It is affected by DCMU (Dichlorophenyl Dimethyl Urea)	It is not affected by DCMU. Affected by paraquat (methyl viologen)
7	It is found in green plants	It is found in bacteria & green plants
8	NADPH+H⁺ is also formed with ATP	Only ATP is fomed.

Difference between non-cyclic and cyclic photophosphorylation

We have already studied Chemio-osmotic Coupling Hypothesis of Peter Mitchell in Oxidative Phosphorylation. The same theory acts here too. Due to the flow of electron flow a gradient of proton across the Thylakoid membrane is created. The concentration of proton in the Thylakoid lumen increases with respect to the concentration of proton in stroma due to three factors:

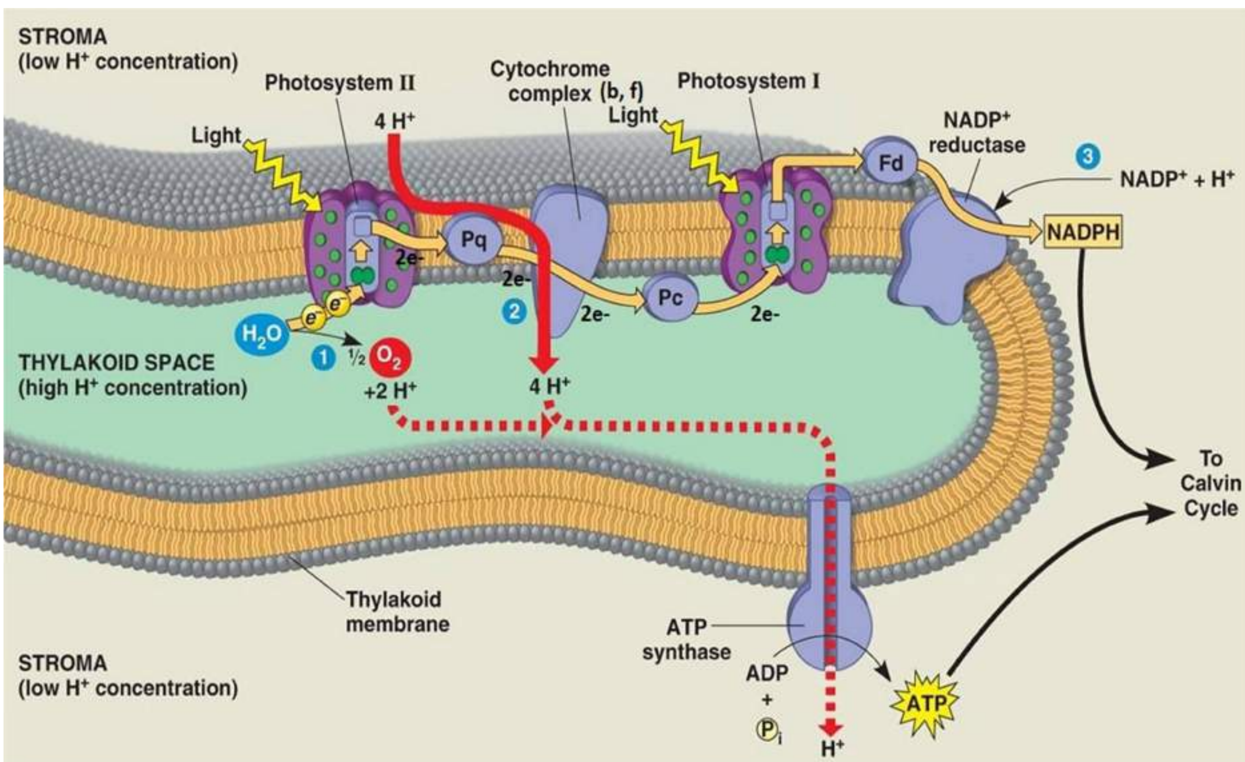
a. Photolysis of water takes place inside the thylakoid lumen and 2 protons are also released their along with 2 electrons (transferred to the electron transport chain) and Oxygen (liberated).
b. Two (2) protons are absorbed by Plastoqinone (PQ) after it gets 2 electrons from the electron transport chain to convert it into reduced form (PQH_2). It re oxidizes by transferring its electrons to Cytochrome b_6f Complex and transfers its protons towards lumen side. In this way, it creates a loss of 2 protons from stroma and a gain of 2 electrons to the lumen side.
c. 2 protons are consumed by $NADP^+$ when it converts into $NADPH+H^+$ after getting 2 electrons from the electron transport chain



Now, As the Thylakoid Membrane (TM) is completely impermeable for protons (H^+), hence the protons are accumulated in the lumen and they create an Electro-chemical gradient. There are three types of gradient created by protons transfer:

1. Electrical gradient: As protons have 1 positive charge, it creates a positive charged environment in lumen while the stroma side is negatively charge.
2. Chemical gradient: The presence of Proton (H^+) makes the environment acidic. Hence the lumen becomes more acidic as compared with the stroma side.
3. Osmotic gradient: As the protons are transferred from stroma to lumen, the solute particles in lumen increases and hence the solution of lumen becomes Hypertonic with respect to the solution of the stroma.

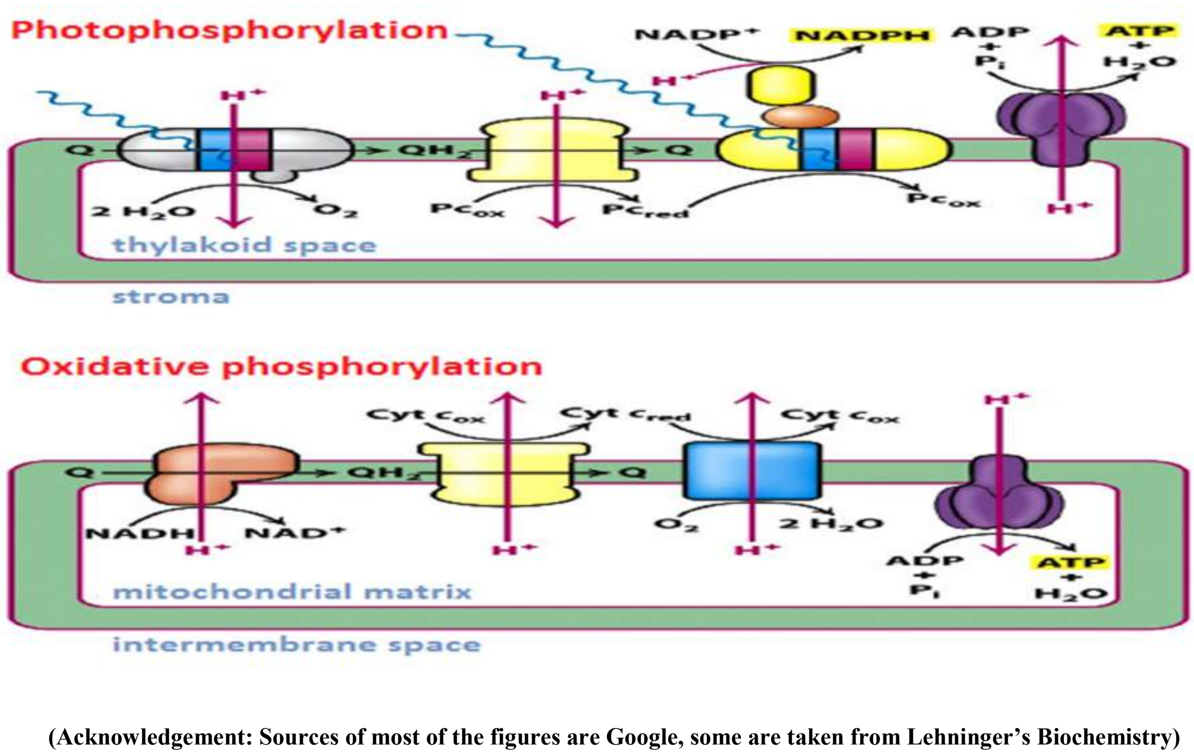
This gradient generates a force which is experienced by TM. But the protons have only one way to come out, i.e., ATP synthase complex embedded in the TM. Now, the proton motive force exerted by the flow of protons provides the required energy for the association of ADP and Pi to form ATP. The protons accumulated in lumen acts like water behind dam and the ATP synthase complex acts like turbine. As the water flows from the channels through the turbine, it rotates and generates electricity (Hydro-electric Power Plants), similarly the flow of proton through the ATP synthase complex make its rotation and during rotation, a conformational change takes place in the β- proteins of the ATP synthase complex. This conformational change converts ADP and Pi into ATP. This basic concept of Photo-phosphorylation is very similar to that of Oxidative Phosphorylation



Differences between Photo-phosphorylation and Oxidative Phosphorylation

Despite the similarity of basic concept of Phosphorylation, there are also several differences between Photo- Phosphorylation and Oxidative Phosphorylation. They can be enlisted in the following table:

Sl. No.	Attributes	Photo-Phosphorylation	Oxidative Phosphorylation
01	Site	Chloroplast	Mitochondria
02	Occurrence	In Photosynthetic Eukaryotes and Prokaryotes	In almost all Eukaryotes
03	Source of Energy	Sunlight	Catabolic Pathways
04	First Electron Donor	Water (H_2O)	$NADPH+H^+$/ $FADH^2$
05	Last Electron Acceptor	$NADP^+$	Oxygen
06	Oxygen	Produced	Utilized
07	Water	Utilized	Produced
08	Barrier	Thylakoid Membrane	Inner Mitochondrial membrane
09	Between	Within Chloroplast	Between Mitochondria and Cytoplasm



(Acknowledgement: Sources of most of the figures are Google, some are taken from Lehninger's Biochemistry)

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