Chloroplast

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Lesson: Chloroplast

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Introduction

The term plastids is derived from Greek word *"plastikas"* meaning formed or moulded. The term was first used by Schimper in 1885. Based on the presence and absence of pigments he classified plastids into two categories:

1. **Leucoplasts** (l*euco*=white): the plastids lack pigments are known as leucoplasts. They are mainly storage plastids and are further classified based on the nature of stored product.

   a) **Amyloplasts:** Store starch

   b) **Aleuroplasts/proteinoplast:** store protein

   c) **Elaioplasts/Oleosomes:** store fats

2. **Chromoplast** (*chroma*=color): These are colored plastids. They can be further classified based on the type of pigment.

   a) **Chloroplast:** these are green colored plastids and contain chlorophyll pigment.

   b) **Rhodoplast:** they contain pigments like fucoxanthin and are found in brown algae.

   c) **Phaeoplasts:** they contain pigments like phycoerythrin and are found in red algae.

Schimper suggested that different types of plastids can be interconverted e.g. chloroplast can be converted to chromoplast during fruit ripening. All plastids arise from undifferentiated organelles known as **proplastids** which are 0.5-1 µm in diameter. Depending on the cell's requirement, proplastids can be converted into various types of plastids. The development of the plastids depends on various external and internal signals. Chloroplasts develop only in the presence of light from the vesicles budding from the inner chloroplast membrane. An intermediate form known as **etioplast** is seen when the plants are kept in dark which is characterized by the array of membrane tubules that lack chlorophyll.

Presence

Chloroplasts are found in green plant parts like leaves and stems. These are absent from animals, fungi and bacteria.

Size

The size of chloroplast varies from 4 to 10 µm. The size may be controlled by genetic and several other factors. Polyploid cells have usually larger sized chloroplasts as compared to diploid cells, same is the case with the plants grown in shades which have larger sized chloroplast as compared to those grown in sunlight.

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Number

The number of chloroplast also varies from cell to cell. *Chlamydomonas* and *Spirogyra* have only one chloroplast, while higher plants have 20-40 chloroplast per cell. Chloroplasts are usually found near the nucleus or cell wall or may also be distributed evenly.

Ultrastructure of chloroplast

A chloroplast has a double membrane structure (Fig. 1) like mitochondria. The two membranes divide chloroplast into two distinct compartments: **periplastidial space** and **stroma or matrix**. However, unlike mitochondria, the chloroplast is also characterized by the presence of a third membrane system known as **thylakoid membrane**. This membrane system defines the third compartment of the chloroplast.

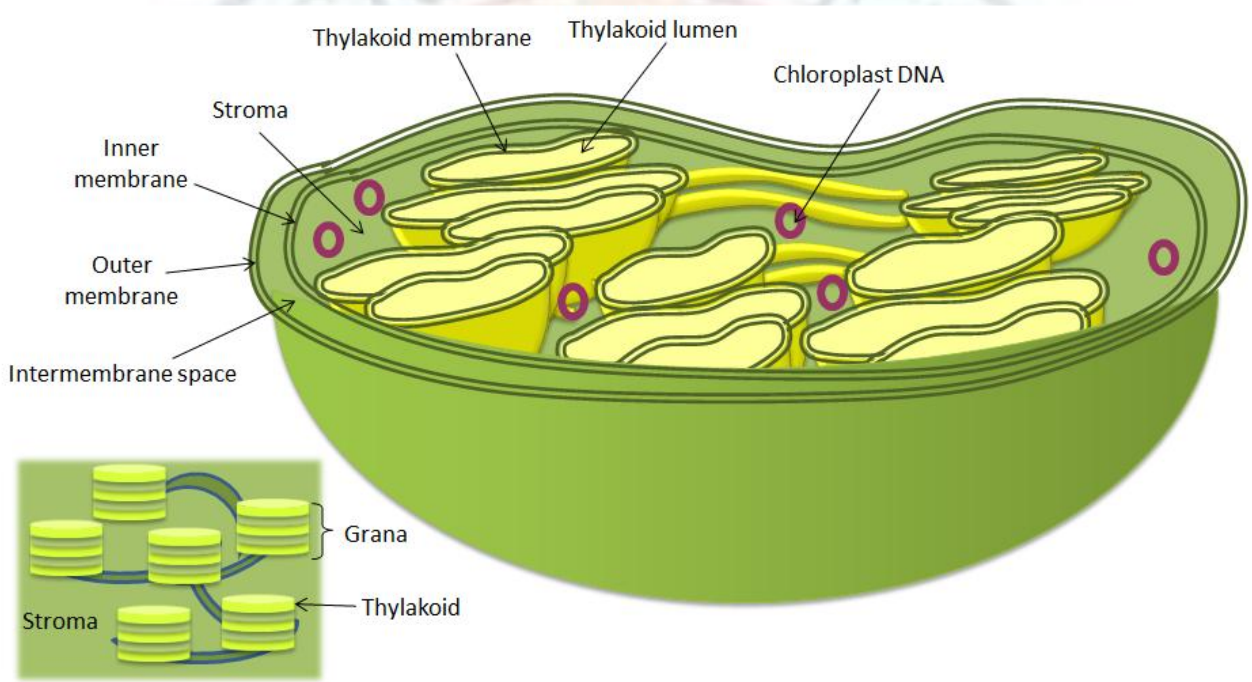

Fig. 1: Ultrastucture of chloroplast showing two membranes. The chloroplast diffes from mitochondria in having a third membrane system, the thylakoid membrane which contain green pigment chlorophyll. **Source: Author**

The outer chloroplast membrane has porin proteins (like found on the outer mitochondrial membrane) and therefore is freely permeable to small molecules. In contrast to this, the inner chloroplast membrane is highly impermeable (like inner mitochondrial membrane) and restricts the passage of molecules. The inner membrane contains various transporters through which the passage of molecules take place. The chloroplast stroma contains double strande, circular, naked DNA (**plastidome**), 70S ribosomes (**plastidoribosome**) and variety of metabolic enzymes. The stroma is the site for dark reaction that fixes CO_2 to produce carbohydrates. The stroma in green algae also contain **pyrenoids** which store starch.

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The stroma also contains third membrane system forming flattened discs stacked known as **thylakoids**. The thylakoids are stacked to form **grana**. The adjacent grana are connected by means of **stromal lamellae** or **frets**. Thylakoid membranes are rich in protein but contain relatively small amounts of phospholipid. Thylakoid membranes contain considerable amount of glycolipids and double bonds making it highly fluidic. Also thylakoid membrane is permeable to ions like Mg^{2+} and Cl^{-}. The thylakoids are functional unit of chloroplast as they contain photosynthetic pigments and are the sites of light reaction. The chloroplast like mitochondria are **semi-autonomous** organelles.

Origin of chloroplast

Just like mitochondria, the evolutionary origin of chloroplast has been described by **endosymbiotic theory** proposed by Lynn Margulis in 1970s. The chloroplast originated from a photosynthetic prokaryote like cyanobacteria that was engulfed by a large non-photosynthetic eukaryotic cell (Fig. 2).

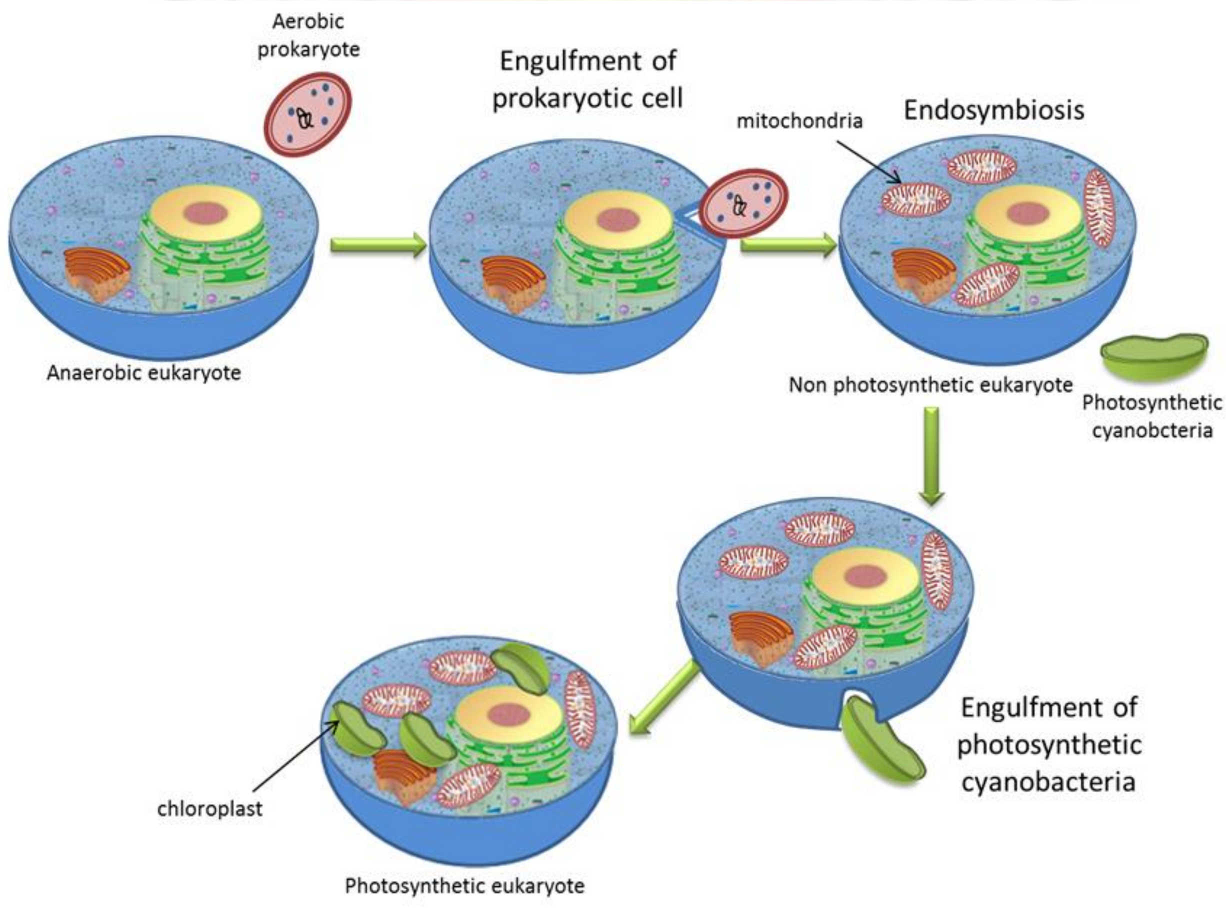

Fig. 2: The endosymbiotic origin of chloroplast from photosynthetic prokaryote like cyanobacteria as proposed by Lynn Margulis. **Source: Author**

Chloroplast

A genome based comparison of chloroplast DNA from different plants and genome sequence of many cyanobacteria (like *Synechocystis*) clearly supports the origin of chloroplast from the latter. There are also evidence to support the endosymbiotic gene transfer from the cyanobacteria to the host nucleus. The two membrane plastid is thought to be evolved from primary endosymbiosis while a secondary endosymbiosis is thought to be responsible for formation of thylakoid membrane system.

Chloroplast fission

The mechanism of fission in chloroplast resembles bacteria in many aspects. In bacteria FtsZ forms a contractile ring (also known as Z ring) responsible for fission. FtsZ are GTPases, related to tubulin proteins found in all eukaryotic cells. In case of bacteria there is a single FtsZ protein responsible for forming Z ring. In chloroplast homologue of bacterial FtsZ protein are found designated as FtsZ1 and FtsZ2 which are encoded by nuclear DNA. Both play important role in forming the contractile ring responsible for chloroplast fission. It has been proposed that FtsZ1 is localized inside the chloroplast like bacterial FtsZ protein while FtsZ2 functions on the cytosolic side resulting in constriction and fission of the chloroplast into two. Another hypothesis suggests that both the Z-ring is composed of both FtsZ1 and FtsZ2 forming copolymer (Fig. 3).

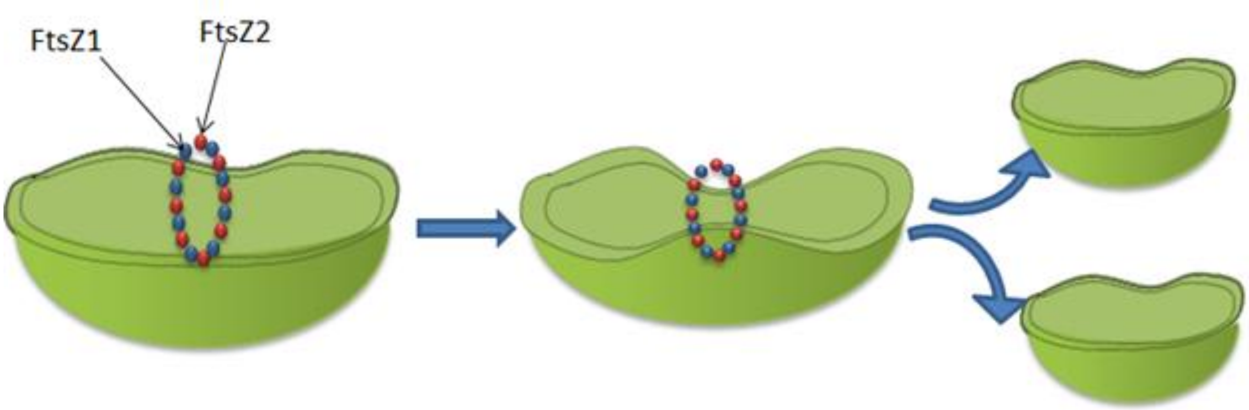

Fig. 3: Chloroplast division by fission. During fission a Z-ring is assembled which is composed of FtsZ1 and FtsZ2 homologs of bacterial FtsZ. **Source: Author**

Chloroplast DNA (cpDNA)

The presence of DNA in chloroplast was first reported by Ris and Plaut in 1962. Chloroplast contain double stranded, circular, naked DNA which is present in multiple copies like mitochondria. The cpDNA from many plants has been sequenced. The size of cpDNA ranges from 120-160kb and contains 60-200 genes, more as compared to mitochondria. The cpDNA contain genes for four rRNAs (4.5S rRNA, 5S rRNA, 16S rRNA and 23S rRNA), 30 tRNA, 21 ribosomal proteins, RNA polymerase, photosystem I, photosystem II, cytochrome b6f complex, ATP synthase and large subunit of ribulose1,5- bisphosphate carboxylase (Rubisco).

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Similarities between chloroplast and mitochondria

1. Both organelles are enclosed in double membrane
2. Both contain 70S ribosomes
3. Both contain double stranded, circular, naked DNA
4. Both are semi-autonomous organelles
5. Both are thought to be evolved by endosymbiosis
6. Both produce ATP
7. Both can replicate by fission

Differences between chloroplast and mitochondria

1. Inner membrane is folded into cristae in mitochondria while in chloroplast cristae are absent
2. Thylakoids are absent from mitochondria while they form the functional unit in chloroplast
3. Mitochondria lack pigments while chloroplast contain a variety of pigments
4. Mitochondria are catabolic while chloroplast are anabolic

Protein Import into chloroplast

Like mitochondria, chloroplast have their own genetic system and can synthesize their own proteins. Despite this, they are dependent on nuclear DNA for about 95% of their 3500 proteins. The proteins required by chloroplast are synthesized at the cytosolic ribosomes and are then imported into the chloroplast. However, unlike mitochondria protein import into chloroplast is slightly more complex due to presence of third membrane system-the thylakoid membrane. Many aspects of protein import into the chloroplast are not very well understood. We currently have little knowledge how proteins are imported into the outer and inner chloroplast membrane. But, the mechanism of protein import into the stroma and thylakoid is known in details.

Protein import into chloroplast stroma

The process of protein import into the stroma is similar to the protein import into the mitochondrial matrix. The proteins destined for chloroplast stroma (like small subunit of Rubisco and all other enzymes of Calvin cycle) have N-terminal **transit peptide** (stromal-import sequence) having 30-100 amino acids. Like mitochondria, proteins that are imported into chloroplast should be maintained in partially unfolded state mediated by Hsp70. But unlike mitochondria, the protein import into the stroma requires a **guidance complex.** The guidance complex directs the proteins to the translocases present on the outer chloroplast membrane known as **Toc (Translocase of the outer chloroplast membrane)**. The translocases are also present on the inner membrane and are known as **Tic (Translocase of the inner chloroplast membrane)**. Both Toc and Tic are multiprotein complexes but the composition of Tic complex is poorly understood.

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First the protein bound to the guidance complex interacts with Toc34 and Toc159 receptors and then is passed to the Toc75 import pore (Fig. 4). Toc34 hydrolyzes GTP which provides additional energy for protein translocation through the outer membrane and the protein is passed to the Tic complex present on the inner membrane. Another receptor Toc64 is also present on the outer chloroplast membrane and binds those proteins that have different targeting signals. The stromal side of Tic complex binds Hsp100 which hydrolyzes ATP and pulls the protein into stroma where **stromal processing peptidases (SPP)** cleave the transit peptide. Stromal Hsp70 are required for correct folding of the proteins.

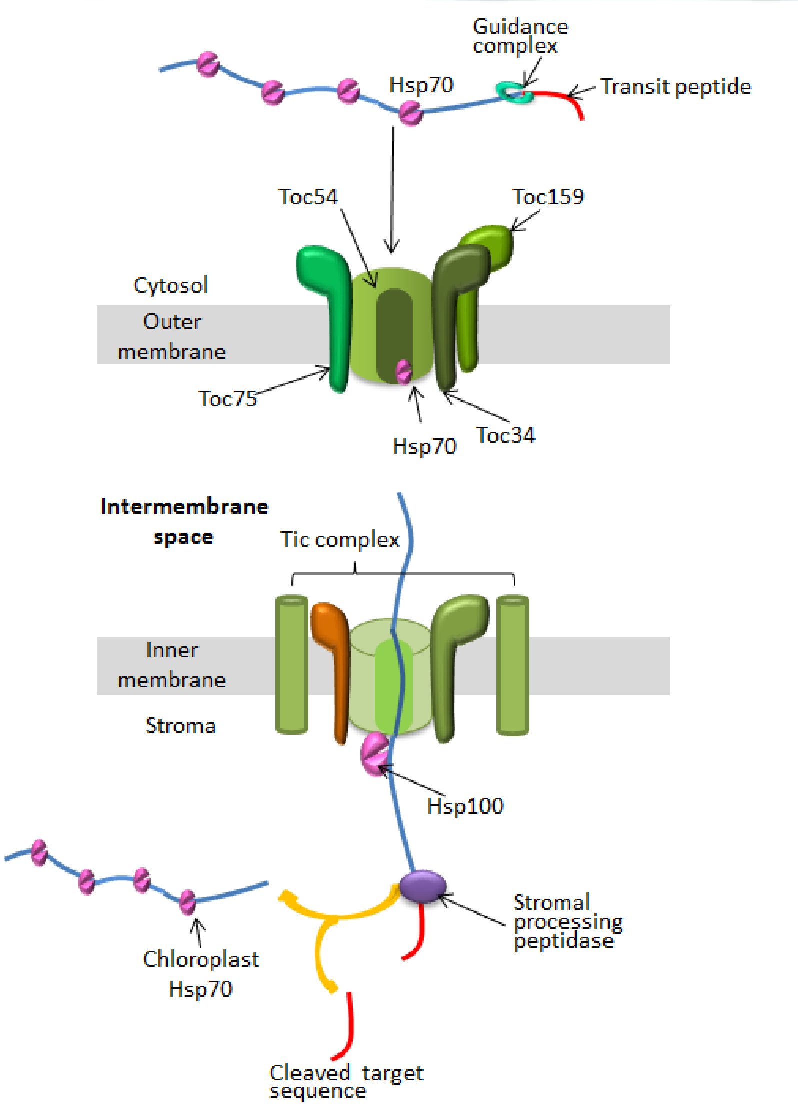

Fig. 4: Mechanism of protein import into the chloroplast stroma. For translocation into stroma the protein should have a transit peptide. A guidance complex binds with the protein and directs it to the translocons present on the outer membrane. **Source: Author**

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Protein import into thylakoid

Proteins destined for the thylakoid lumen (like plastocyanin) have two different targeting sequences:

1. N-terminal **transit peptide** that directs the protein to the stroma
2. Second import signal that translocates protein from the stroma to the thylakoid lumen

Once the protein is imported into the stroma, the stromal processing peptidases (SPP) cleave the transit peptide unmasking the second import signal known as **thylakoid signal sequence** or **thylakoid-targeting sequence**. There are three different pathways to import proteins into the thylakoid lumen:

1. The first pathway, also known as **Sec pathway** imports proteins maintained in partially unfolded state by chloroplast Hsp70. The thylakoid signal sequence is recognized by SecA which hydrolyses ATP to transport protein towards Sec translocon which imports protein into the thylakoid lumen (Fig. 5a). In the lumen, thylakoid signal sequence is cleaved by **thylakoid processing protease (TPP)** and the protein is refolded.

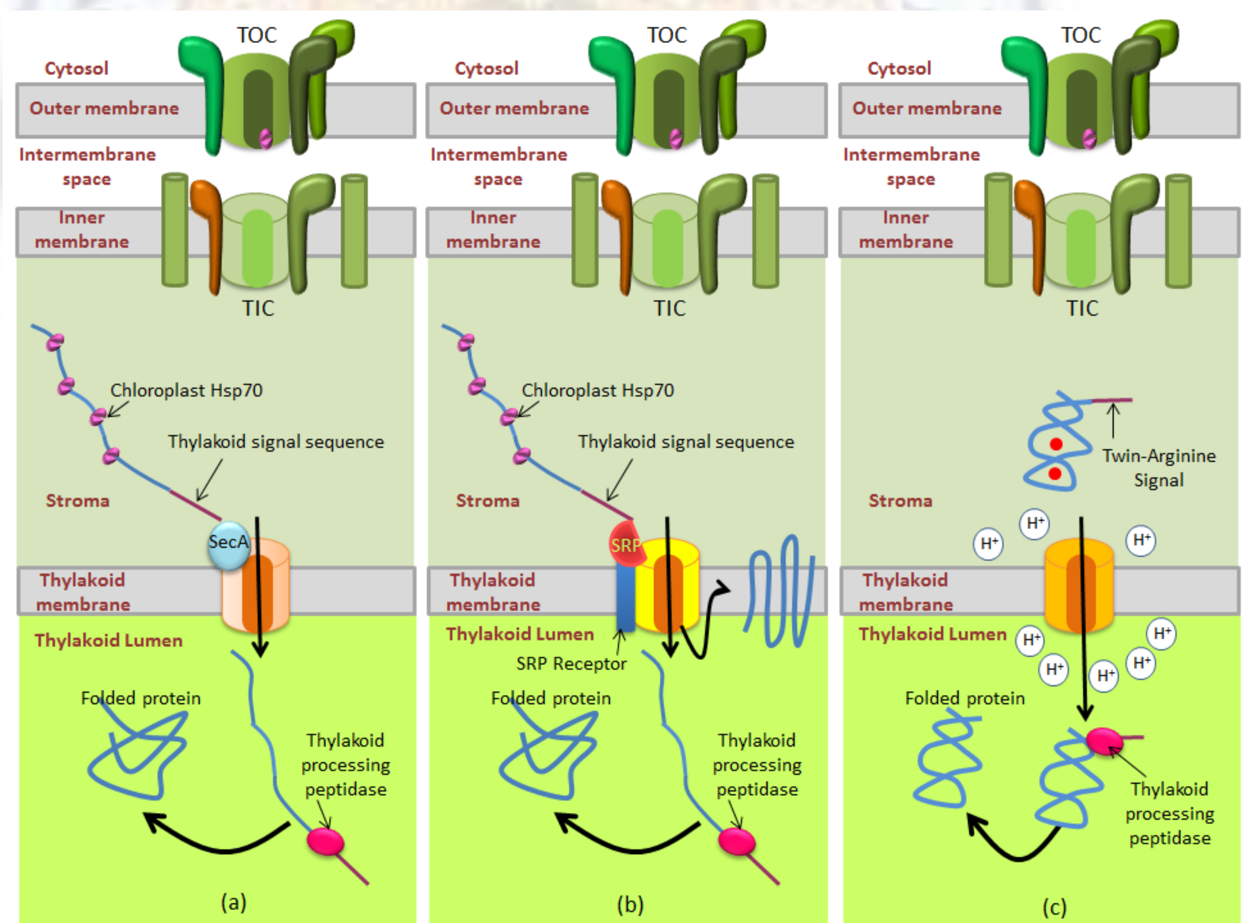

Fig. 5: Three pathways for imporingt proteins into the thylakoid: (a) the sec pathway (b) Signal Recognition Particle (SRP) dependent pathway and (c) twin-arginine translocation (TAT) pathway. See text for details. **Source: Author**

2. The second pathway, also known as **SRP (signal recognition particle)-dependent pathway** also imports unfolded proteins (Fig. 5b). SRP is found in the stroma which interacts with the SRP receptor present on the thylakoid membrane. The components of SRP-dependent pathway are homologous to the bacterial SRP transport proteins used for the translocation of unfolded proteins into the periplasmic space or the inner membrane. Once the protein is imported into the lumen, thylakoid signal sequence is cleaved by thylakoid processing protease (TPP) and the protein is refolded.
3. The third pathway, also known as **TAT (twin-arginine translocation) pathway** imports folded proteins and requires H^+ gradient established across the thylakoid membrane (5c). Therefore sometimes it is also referred to as **pH-dependent pathway**. The pathway is used to import those thylakoid proteins that have metal-containing cofactor. Such proteins first bind with the cofactor and undergoes folding into the stroma. Thylakoid-targeting sequence in these proteins contains two closely spaced arginine residues (hence the name of the pathway) which are essential for recognition. Similar mechanism of translocating folded proteins is also found in bacterial cells where similar arginine-containing sequence are required for protein translocation across inner membrane. Consistent with this finding, the protein subunits of translocon required for transport of such folded proteins are homologous to the proteins in the bacterial inner membrane.

   Proteins destined for thylakoid membrane require a protein related to the mitochondrial Oxa1 protein and SRP pathway.

Functions of chloroplast

Chloroplasts perform many important functions in the plant cells. They are responsible for the synthesis of almost all amino acids, fatty acids, purines, pyrimidines and carotenes. However, the most important function of chloroplast for which they are known is photosynthesis responsible for generation of O_2 and CO_2 fixation to produce carbohydrates. The association between chloroplast and photosynthesis was first identified by the German biologist T. Engelmann in 1881 while working with *Spirogyra*. The understanding of photosynthetic reaction came in the early 1930s. Prior to this it was thought that the O_2 evolved during photosynthesis is the result of photosplitting of CO_2. Cornelis van Neil a graduate student at Stanford University in 1931, while working with sulfur bacteria noticed that these organisms are capable of converting CO_2 to carbohydrate without releasing O_2:

$$\mathbf{CO_2 + 2H_2S \longrightarrow (CH_2O) + H_2O + 2S}$$

Using this finding, he proposed that the O_2 evolved during photosynthesis comes from H_2O and not CO_2.

$$\mathbf{6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2}$$

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The experimental evidences for the role of H_2O in the formation of O_2 were given by Samuel Ruben and Martin Kamen in 1941 while working at the University of California. These workers used labeled isotope of oxygen ^{18}O to demonstrate that the O_2 was not produced by splitting of CO_2 but from photospliting of H_2O.

The process of photosynthesis is essentially divided into two reactions:

1. **Light-dependent reaction or light reaction:** Light reaction takes place in the thylakoid membrane. The energy from sunlight is used to generate ATP and NADPH. O_2 is released during this reaction of photosynthesis.

2. **Light-independent reaction or dark reaction:** Dark reaction takes place in the stroma. The CO_2 fixation to produce carbohydrate using energy from ATP and NADPH takes place during dark reaction.

Light reaction

As already mentioned, light reaction takes place in the thylakoid membrane and is responsible for generation of ATP and NADPH. The thylakoid membrane contains light-absorbing pigments like chlorophyll and a number of membrane proteins with prosthetic groups attached to them. The light reaction can be understood in three steps:

a) Absorption of light by pigment molecules
b) Release of O_2, electron transfer and generation of proton motif force, and formation of NADPH
c) ATP production

In the first step light energy is absorbed by chlorophyll molecules found in the thylakoid membrane. Light travels in packets of energy known as photons quanta, the energy which is given by Planck's law:

$E = hv = hc/\lambda$

where h is Planck's constant (6.626×10^{-34} J.s
c is the speed of light in a vacuum (2.998×10^{8} m/s)
λ is the wavelength of light (for visible light it is 400-700 nm)

When the photon is absorbed by a molecule, the electrons get excited to higher energy state and the molecule is said to have moved from the **ground state** to **excited state** which is an unstable state and lasts only for 10^{-9} second. The electrons then return back to the ground state releasing the absorbed energy which appears in the form of either fluorescence or heat. The same phenomenon happens when a pigment molecule absorbs light photons. The most important pigment in plants is chlorophyll. The chlorophyll molecule consists of a porphyrin ring and a hydrophobic phytol tail. The porphyrin ring contains Mg^{2+} and functions in light absorption while the hydrophobic phytol tail keeps the pigment molecule embedded into the thylakoid membrane. Different types of chlorophyll molecules are found in different photosynthetic organisms and differ from each other in the side groups attached to porphyrin ring. Bacteriochlorophyll is present in green and purple bacteria while chlorophyll *a* is found to be present in all the photosynthetic organisms that evolve O_2 and is therefore universal, chlorophyll *b* is found in all higher plants and green algae, chlorophyll *c* is found in brown algae and diatoms; and chlorophyll *d* is found in red algae. Apart from chlorophyll, higher plants also contain accessory pigment molecules like carotenoids which serve many important functions like light absorption, collection of excess energy from excited chlorophylls and release it as heat which otherwise lead to production of singlet oxygen which is highly reactive and can damage biological molecules.

Reaction centers and photosystems

As mentioned, chlorophyll molecules absorb light energy and convert it to the chemical energy. Several hundred chlorophyll molecules act together and form large protein complexes known as **photosystems**. The photosystems consist of two components: a **reaction center** and an **antenna complex**. The antenna complex consists of many **light harvesting complexes** (LHCs) which absorb light and pass it to the reaction center. There are two types of photosystems: **photosystem I (PSI)** and **photosystem II (PSII).** The reaction center of photosystem II is P_{680} which is a chlorophyll *a* dimer also called **special-pair chlorophylls** (680 refers to the wavelength of light absorb by the chlorophyll molecule strongly). The reaction center of photosystem I is P_{700}. There is difference in the distribution of two photosystems in the thylakoid membranes. PSII is primarily located in the stacked grana while PSI is found in the unstacked regions. Both photosystems also differ in their function. PSII is involved in the splitting of H_2O to produce O_2, PSI plays important role in transferring the electrons to $NADP^+$ to produce NADPH.

The pigment molecules in the antenna complex of both PSI and PSII (chlorophylls and carotenoids) absorb energy of the photon and passed it to the respective reaction centers. The excited reaction centers release electron which is accepted by primary electron acceptor. This flow of electrons from photosystems may follow non-cyclic (linear) or cyclic route.

Non-cyclic (linear) electron flow

The non-cyclic electron flow also known as **non-cyclic photophosphorylation** involves both PSI and PSII. The PSII is a multiprotein complex consisting of more than 20 different polypeptides. Out of 20 different polypeptides, two D1 and D2 bind the reaction center P_{680} and other factors involved in electron transport. The first step is the absorption of light photon by the pigment molecules of light-harvesting complex II (LHCII) of the antenna complex II. The absorbed energy is passed from antenna pigments of LHCII to the chlorophyll molecules situated inner side of the antenna complex and finally the energy is passed to the reaction center P_{680}. The excited reaction center passes the electrons to the primary electron acceptor like **Pheophytin** which is a modified chlorophyll *a* molecule that lacks Mg^{2+}. The reaction center assumes a positive charge after losing electron ($P_{680}{}^+$) and acts as oxidizing agent (which can accept electrons). Pheophytin assumes a negative charge ($Pheo^-$) and acts as reducing agent (can donate electrons). The $Pheo^-$ transfers the electron to quinone (Q_A) which transfers it to the second quinone (Q_B). The PSII also consist of a complex of four Mn^{2+}, one Ca^{2+} and one to two Cl^- along with other proteins forming the **oxygen-evolving complex (OEC)** which binds two H_2O molecules. The OEC consists of three proteins of size and 17, 23 and 33 kDa forming a complex. The OEC during extracting H^+ and e^- from H_2O undergoes series of cyclic oxidation states also known as **S-states** which are denoted as S_0 to S_4 (the S-state hypothesis was given in 1970 by Pierre Joliot and Bessel Kok). The splitting of water in the presence of light is called photolysis which releases

O_2. The formation of one molecule of O_2 requires oxidation of two molecules of H_2O and 4 electrons.

The electrons released from photolysis of H_2O are transferred one at a time to P_{680}+ through a tyrosine residue 161 of the D1 polypeptide (Tyr_z) which functions as an intermediate electron carrier (earlier denoted as Z). Thus, the overall reaction is:

$$2H_2O \longleftrightarrow O_2 + 4H^+ + 4e^- \quad \text{------------- (1)}$$

From the pool of plastoquinone, the electron is passed to *cytochrome b_6f* complex which is similar to *cytochrome bc_1* complex found in mitochondrial electron transport chain as both translocate $4H^+$ per pair of electrons (Q cycle) (Fig. 6). The *Cytochrome b_6f* complex consists of iron-sulfur (Fe-S) clusters, cyt b6 and cyt f and passes electrons from plastoquinone to plastocyanin. Plastocyanin is a copper-containing, mobile peripheral membrane protein found on the lumen side of the thylakoid membrane which passes electron to PSI.

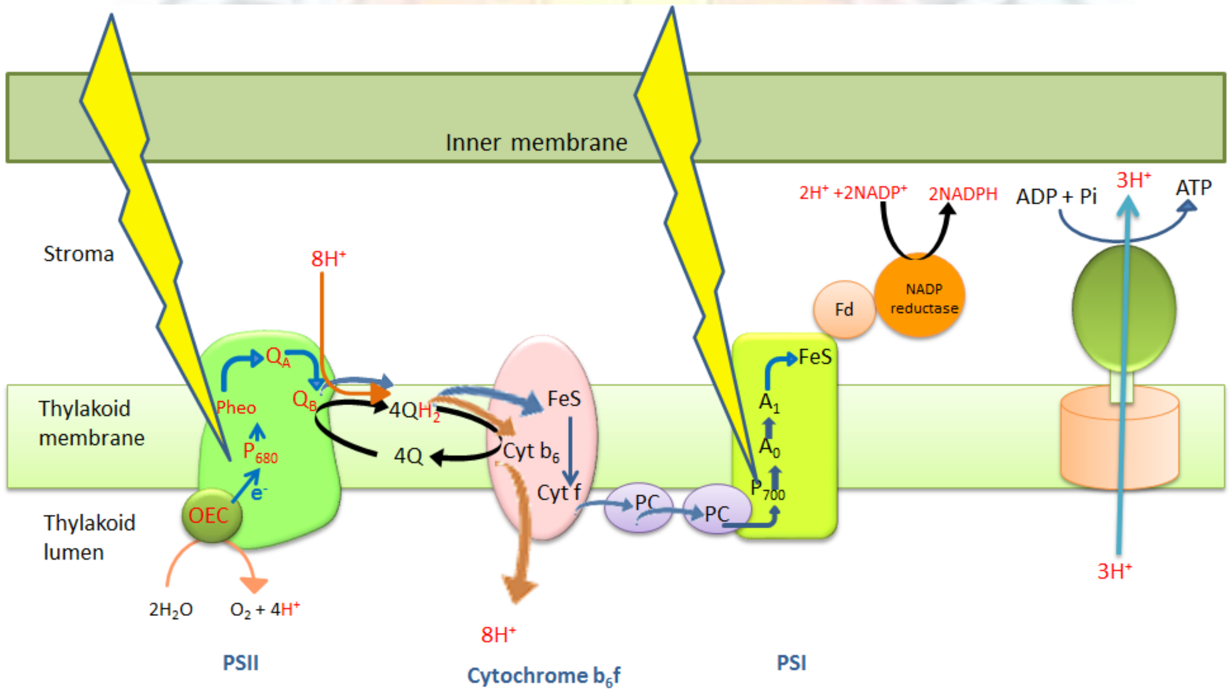

Fig. 6: Non-cyclic photophosphorylation. The non-cyclic photophosphorylation involves both PSI and PSII and generates ATP, NADPH and O_2. Blue arrows indicate flow of electrons while brown arrows indicate movement of H^+. **Source: Author**

PSI consists of a reaction center P_{700} and light harvesting complex I (LHCI) of antenna complex I. The pigments in the LHCI absorb energy from the photons and passed it to the PSI reaction center which is excited and releases electron. The electron is passed from the excited P_{700} to the primary acceptor: chlorophyll *a* molecule (A_0) and then to phylloquinone (A_1) and to three Fe-S clusters (F_X, F_B, and F_A). The electron is finally passed to an iron sulfur protein called ferredoxin. The enzyme ferredoxin-$NADP^+$ reductase catalyzes the reduction of $NADP^+$ to form NADPH. The reaction center P_{700}^+ is reduced by getting electron from plastocyanin. The reaction can be summarized as:

$$NADP + H^+ + 2e^- \longleftrightarrow 2NADPH \quad \text{-------------} \quad (2)$$

The overall reaction of non-cyclic electron transfer can thus be obtained combining (1) and (2):

$$2NADP + 2H_2O \longleftrightarrow 2NADPH + O_2 + 2H^+$$

The production of one molecule of O_2 thus requires four electrons from two molecules of water. The removal of one electron require one photon, so removal of four electron requires total four photons. Also to produce one NADPH two electrons are required. But due to presence of two photosystems the number of photons required is doubled (four photons are required by each photosystem). Thus eight photons are required to generate one molecule of O_2 and two molecules of NADPH. The subsequent pumping of H^+ generates proton motif force (pmf) which is harnessed to produce ATP. Thus the non-cyclic electron transport results in the generation of O_2, NADPH and ATP.

Do you know???

A number of herbicides that are used to control weeds act by binding to specific component of the electron transport chain found in the thylakoid membrane. The herbicides like s-triazenes bind to the PSII reaction center and block electron transport through PSII. Some herbicides like Paraquat affect the functioning of PSI by competing with ferredoxin for electrons.

Cyclic electron flow

In non-cyclic electron flow the electrons are passed from ferredoxin to $NADP^+$ to generate NADPH. The non-cyclic electron flow also produces ATP from the proton motif force generated due to subsequent pumping of H^+ to the thylakoid lumen. However, Daniel Arnon while working at the University of California, found that ATP synthesis can also take place even in the absence of CO_2 and $NADP^+$ suggesting that an alternative pathway does exist to produce ATP without producing NADPH. This alternative route is known as **cyclic electron flow** or **cyclic photophosphorylation** (Fig. 7).

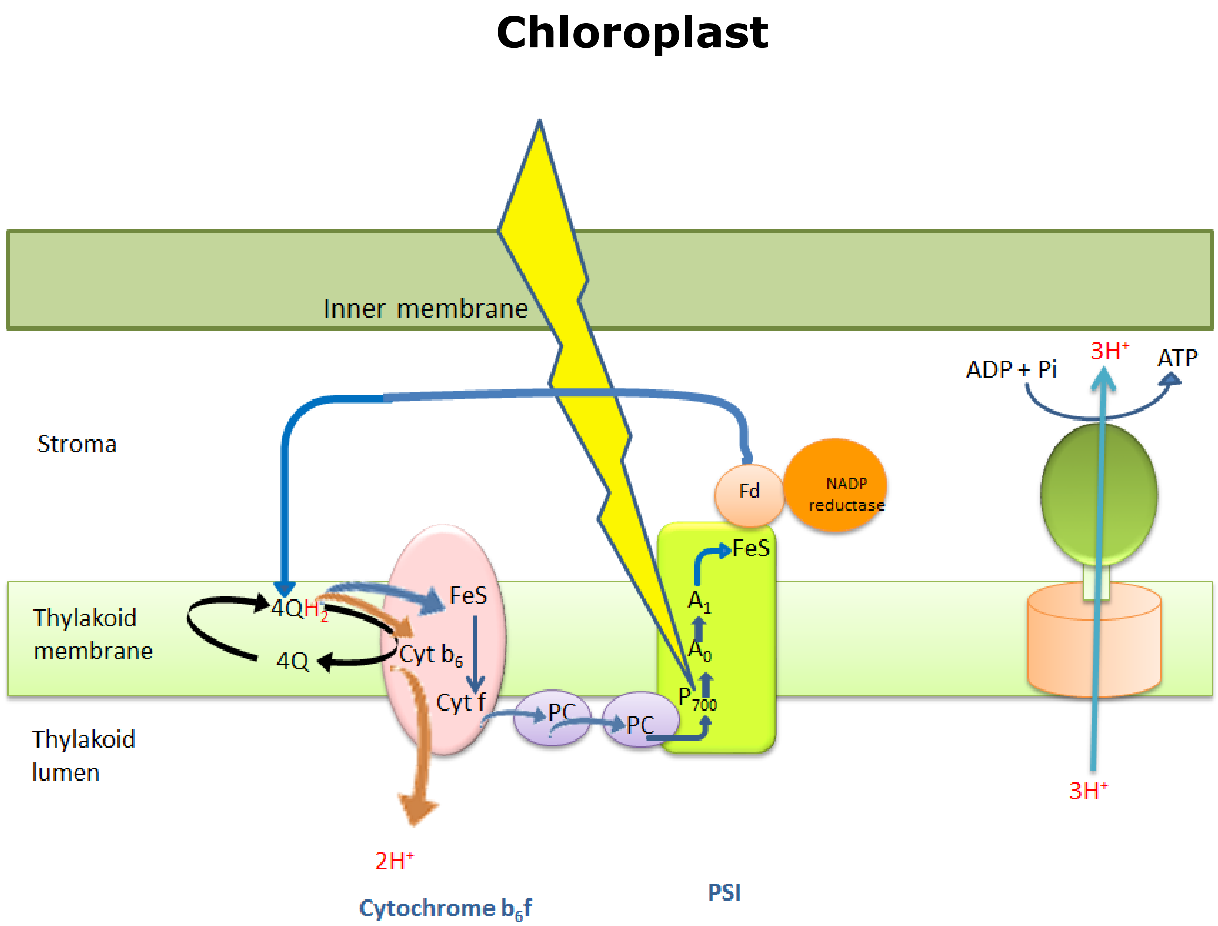

Fig. 7: Cyclic photophosphorylation. The cyclic photophosphorylation involves only PSII and generates ATP without NADPH production. **Source: Author**

Thus in cyclic photophosphorylation the electron from ferredoxin can be passed to plastoquinone passes the electrons to cytochrome b_6f complex and two H^+ to the thylakoid lumen. The electrons are returned back to PSI through plastocyanin. Thus, the flow is cyclic as the electrons extracted from PSI are returned back to PSI. The cyclic electron flow thus generates proton motif force responsible for ATP synthesis but no NADPH is produced.

ATP produced by non-cyclic and cyclic photophosphorylation

As already mentioned, in non-cyclic photophosphorylation eight photons are required to generate one molecule of O_2, two molecules of NADPH and $12H^+$ are translocated. It has been found that 1000-2000 fold differences in H^+ concentrations exist across the thylakoid membranes. It has been proposed that translocation of 3 H^+ through chloroplast ATP synthase (also known as CF_1-CF_0 complex) results in the generation of one ATP. So, total of 4 ATP are generated per O_2 molecule evolved or 4/8 or ½ ATP per photon absorbed. Non-cyclic photophosphorylation also results in generation of 2 NADPH. Each NADPH has free energy to generate 3 ATP molecules, so total of 6 ATP molecules are generated from 2 NADPH making a total of 10 (4+6) ATP per O_2 molecule evolved or 10/8=1.25 ATP equivalents per absorbed photon.

As compared to non-cyclic photophosphorylation, cyclic photophosphorylation seems to be more economical in terms of ATP produced only. In cyclic photophosphorylation four photons results in the translocation of 8 H^+ or 2 H^+ per photon resulting in the generation of 2/3 ATP per photon absorbed.

Do you know???
The chloroplast ATP synthase also known as CF_1-CF_0 complex is similar to the mitochondrial ATP synthase F_1-F_0 complex. The CF_1 like mitochondrial F_1 forms head domain with same subunit composition ($\alpha_3\beta_3\gamma\delta\varepsilon$) while CF_0 like F_0 is a transmembrane protein containing H^+ translocation channel. Both generate ATP in a similar fashion using the proton motif force. However, unlike mitochondria the electrochemical H^+ gradient is only the function of pH gradient as translocation of H^+ across thylakoid membrane also results in the subsequent movement of Cl^- and Mg^{2+} ions which eliminates membrane potential ($\Delta\Psi$) that plays an important role in mitochondrial ATP synthesis.

Dark reaction

The process by which CO_2 is fixed to produce carbohydrates is known as dark reaction which takes place in the stroma of the chloroplast. The pathway by which the production of carbohydrates take place was elucidated by Melvin Calvin, Andrew Benson and James Bassham while working at University of California. The extraordinary contribution by Melvin Calvin can be judged by the fact that dark reaction is also known as **Calvin cycle or Calvin-Benson cycle**. These workers used radioactive isotope of carbon ^{14}C and found that after brief exposure of algae to $^{14}CO_2$ the first stable product formed is 3-phosphoglycerate which is a three carbon molecule (Fig. 8). Since the first stable product identified was a three carbon molecule, it is sometimes refer to as **C3 pathway** and plants utilizing this pathway are known as **C3 plants**.

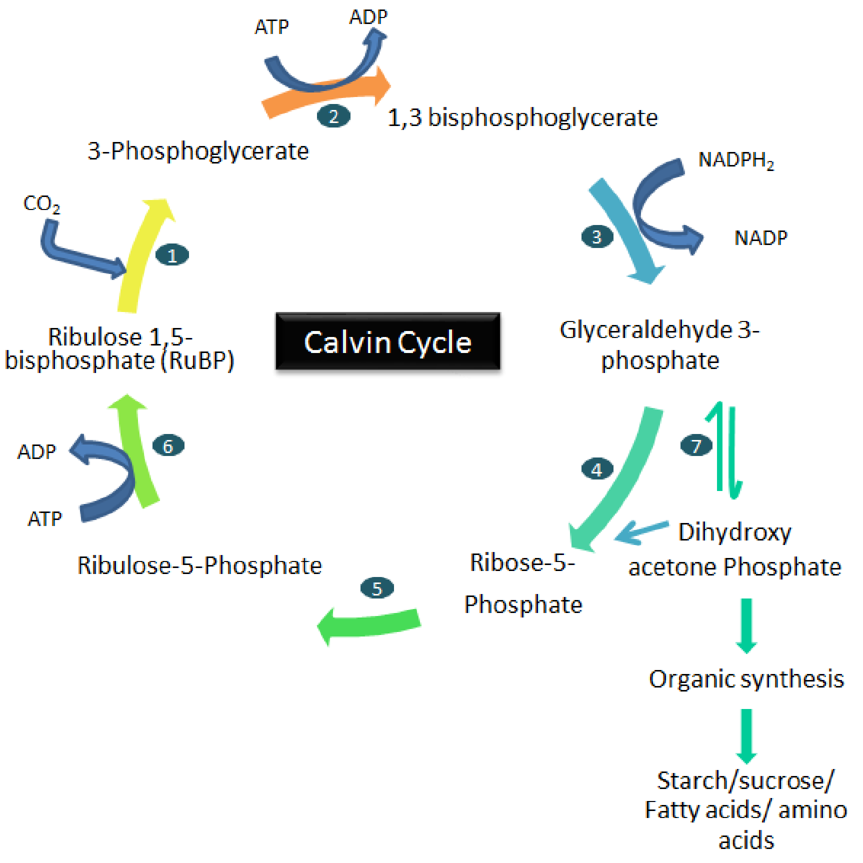

Fig. 8: Calvin cycle or dark reaction. The Calvin cycle also known as C3 cycle takes place in the stroma and utilize ATP and NADPH generated during light reaction. Six CO_2 molecules react with 6 RuBP to produce 12 molecules of phosphoglycerate. Overall process requires 18 ATP, 12 NADPH to produce 12 molecules of glyceraldehyde 3-phosphate (GAP). Out of 12, 2 GAP are used for the production of sucrose in the cytosol and remaining 10 molecules are converted to 6 molecules of RuBP. The enzymes involved in the calvin cycle are- 1: ribulose 1,5-bisphosphate carboxylase or Rubisco; 2: phosphoglycerate kinase; 3: glyceraldehyde 3-phosphate dehydrogenase; 4: transketolase; 5: ribose phosphate isomerase; 6: phosphoribulose-kinase. **Source: Author**

The key enzyme in Calvin cycle is ribulose 1,5-bisphosphate carboxylase or Rubisco which catalyzes the conversion of ribulose 1,5-bisphosphate, a five-carbon sugar to two molecules of 3-phosphoglycerate by adding CO_2. The pathway is complex and involves number of different enzymes.

Do you know???

Rubisco is a large large multisubunit enzyme composed of eight identical large and eight identical small subunits with molecular weight ~500kDa. Rubisco though a key enzyme in plants, has turnover number of 3 i.e. it can fix only 3 molecules of CO_2 per second. This low turnover number is compensated by increasing the number of Rubisco molecules catalyzing the reaction. It has been found that more than 50% of the protein in leaves is Rubisco and therefore it is the most abundant protein found on this planet.

The Calvin cycle can be divided into three stages or phases:

(a) **carboxylation** of ribulose 1,5-bisphosphate (RuBP) to form 3-phosphoglycerate (PGA)
(b) **Glycolytic reversal** involves reduction of PGA to produce glyceraldehyde 3-phosphate (GAP) using the NADPH and ATP (produced during light reaction)
(c) **Regeneration of RuBP**

The stoichiometry of Calvin cycle can be summarized as:

$$6CO_2 + 18ATP + 12NADPH \longrightarrow 12GAP + 18ADP + 16P_i + 12NADP^+$$

It can be seen that dark reaction is energy consuming process where the production of 2 molecules of Glyceraldehyde 3-phosphate (GAP) requires 18 ATP and 12 NADPH molecules. Glyceraldehyde 3-phosphate (GAP) is the key product formed during dark reaction. It can be transported to the cytosol in exchange for phosphate ions (Fig. 9) or stay at the chloroplast stroma. At cytosol it is converted to sucrose (a major transport sugar in plants) in a series of reaction which is then transported into the phloem. In the chloroplast, it is converted to starch which is stored as starch granules.

Do you know???

Many enzymes of Calvin cycle are activated only in light when the light reaction generates ATP and NADPH. A stromal protein thioredoxin which can exist either as reduced or oxidized form controls the activation of Calvin cycle enzymes. In dark thioredoxin contains a disulfide bond (S-S), which in light is reduced to sulfhydryl group (-SH) by getting electrons from PSI through ferredoxin. The reduced thioredoxin then reduces activates selected Calvin cycle enzymes by reducing disulfide bonds to sulfhydryl groups. However, in dark the thioredoxin is again oxidized thus Calvin cycle enzymes are no longer activated. Therefore, the name dark reaction is actually a misnomer as during dark, there cannot be any CO_2 fixation as enzymes are not active in dark.

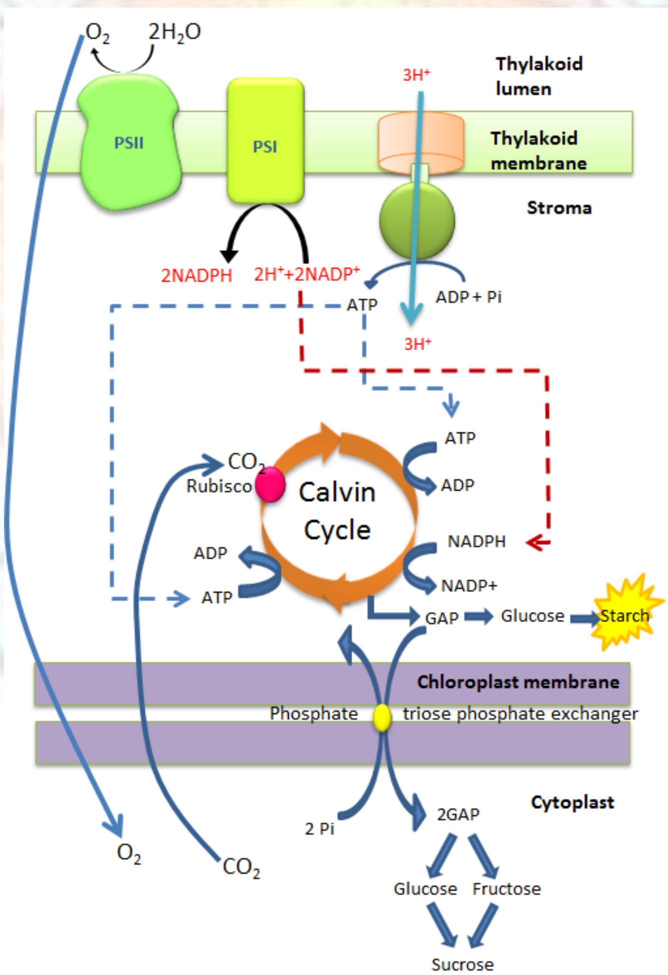

Fig. 9: The figure summarizing the overall process of photosynthesis. **Source: Author**

Photorespiration and C4 cycle

Rubisco which is the key enzyme responsible for fixation of CO_2 can also act as oxygenase in a process known as **photorespiration** also known as C2 cycle. This seems to be an unavoidable process because of the catalytic activity of Rubisco with mild differences in the preference for CO_2 and O_2. In this process Rubisco adds O_2 to ribulose 1,5-bisphosphate forming 3-PGA and phosphoglycolate which is subsequently converted to glycolate in the chloroplast stroma. Glycolate is transported to the peroxisome where it is converted to glyoxylate and then to glycine (Fig. 10). The glycine formed in peroxisomes is transported to mitochondria and is converted to serine releasing CO_2. In this manner the fixed CO_2 is released. The process is waste of not only previously fixed CO_2 but also involves use of ATP and O_2. It has been estimated that up to 50% of fixed CO_2 can be lost due to photorespiration by crop plants growing under high light intensity.

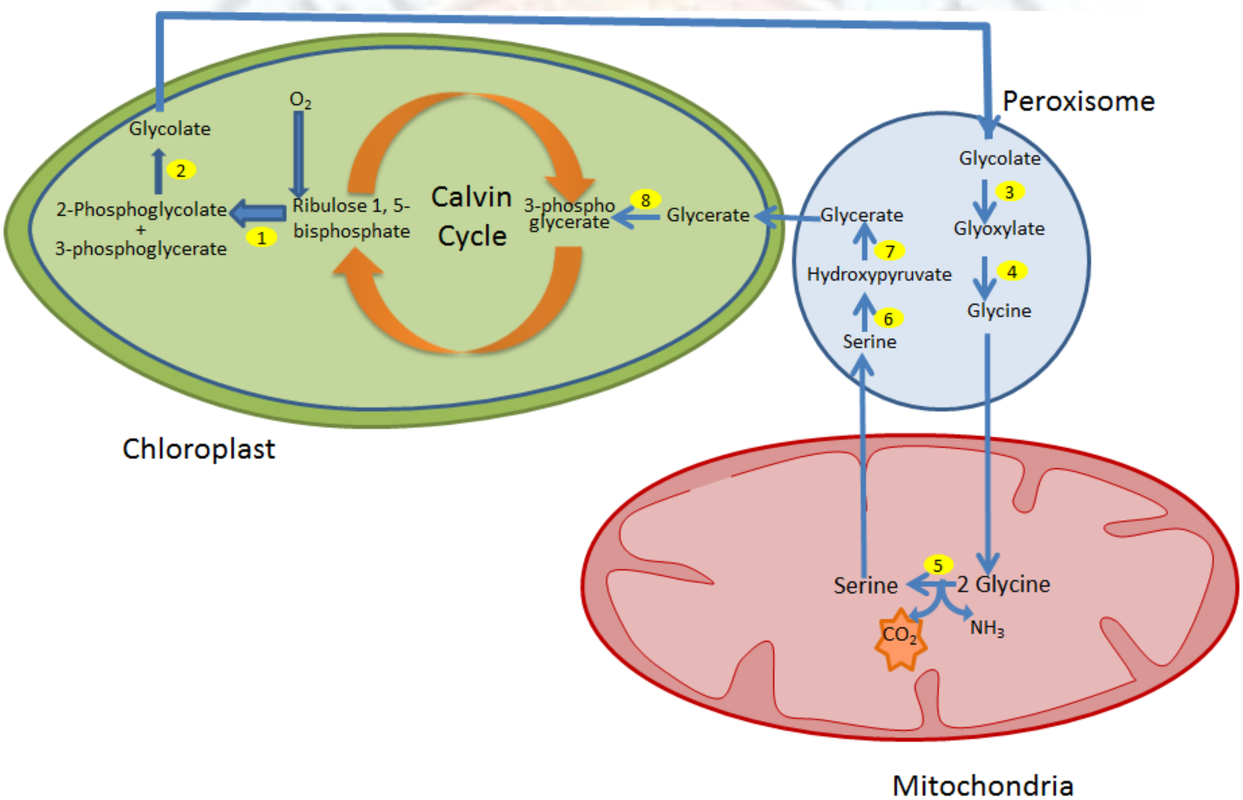

Fig. 10: Photorespiration or C2 cycle. The pathway involves three organelles: chloroplast, peroxisomes and mitochondria. The 2-phosphoglycolate formed due to oxygenase activity of Rubisco is metabolized in these organelles. There is lost of already fix CO2 and therefore the process is not beneficial for plants. The C2 cycle involves following enzymes- 1: Rubisco; 2: phosphoglycolate phosphatase; 3: glycolate oxidase; 4: serine:glyoxylate aminotransferase; 5: glycine decarboxylase, serine hydroxymethyl transferase; 6: serine:glyoxylate aminotransferase; 7: hydroxypyruvate reductase; 8: glycerate kinase. **Source: Author**

Chloroplast

Photorespiration is more prominent in a hot dry environments where in order to prevent water loss plants close their stomata. This results in decrease in the concentration of CO_2 promoting oxygenase activity of Rubisco. Therefore, the crop plants that grow in hot dry environmental conditions like sugarcane, sorghum, crabgrass and corn have developed mechanisms to avoid this loss of fixed CO_2. These crop plants have evolved a two-step mechanism which is known as **C4 pathway** as the first stable compounds formed using radioactive ^{14}C (reported by Hugo Kortschak in 1965) were found to be a four Carbon compounds like oxaloacetate and malate. The complete C4 cycle was elucidated in 1960s by Marshall Hatch and Rodger Slack and therefore it is also known as **Hatch and Slack pathway**. The plants that employ C4 cycle also known as **C4 plants** continue photosynthesis at even at very low CO_2 concentration and have characteristic leaf anatomy. The leaves consists of single layer of **bundle-sheath cells** surrounded by **mesophyll cells**. The mesophyll cells lack Rubisco in their chloroplast and fix CO_2 using enzyme PEP (phosphoenolpyruvate) carboxylase (Fig. 11) forming oxaloacetate. The enzyme PEP carboxylase is found exclusively in C4 plants. The oxaloacetate is converted to malate which is transported to bundle-sheath cells where it is decarboxylate to produce CO_2 and pyruvate. In this way the CO_2 is concentrated which enters into the Calvin cycle. The bundle-sheath cells have CO_2 concentration as high as 100 times that of mesophyll cells.

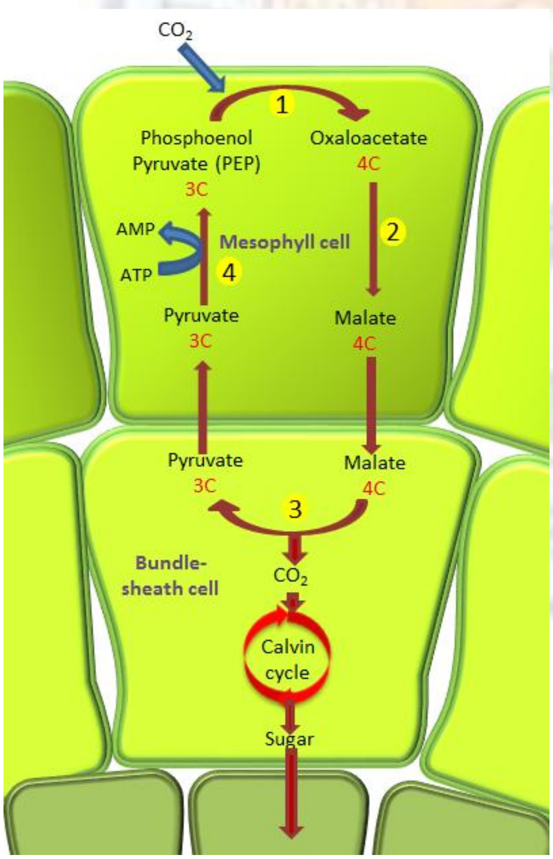

Fig. 11: The C4 pathway. The plants that carry out C4 cycle have mesophyll cells surrounding the bundle sheath cells. The CO2 is concentrated into the bundle-sheath cells and is used for Calvin cycle. **Source: Author**

Do you know???

The carbohydrate production in C3 plants stops when the CO_2 concentration falls to about 50 ppm. However C4 plants continue carbohydrate production till CO_2 concentration is 1-2 ppm. This is because of the enzyme PEP carboxylase which is not affected by O_2.

Summary

The term plastids was first used by Schimper in 1885 which is derived from Greek word *"plastikas"* meaning formed or moulded. Leucoplasts are the plastids lack pigments and are mainly storage while Chromoplast are colored plastids. Green colored plastids are known as chloroplast. Leucoplasts are further classified based on the nature of stored product. All plastids arise from undifferentiated organelles known as proplastids. A chloroplast has two membranes which divide it into two distinct compartments: periplastidial space and stroma or matrix. A third membrane system known as thylakoid membrane is also present. The outer chloroplast membrane has porin proteins therefore is freely permeable to small molecules while the inner membrane is highly impermeable and restricts the passage of molecules. The stroma contains double strande, circular, naked DNA, 70S ribosomes, variety of metabolic enzymes and flattened discs known as thylakoids which are stacked to form grana. The chloroplast are semi-autonomous organelles and thought to have evolved from a photosynthetic prokaryote like cyanobacteria. Like mitochondria, chloroplast undergoes fission. The size of cpDNA ranges from 120-160kb and contains more genes as compared to mitochondria.

Chloroplasts have their own genetic system and can synthesize their own proteins. Still they are dependent on nuclear DNA for about 95% of their 3500 proteins. Many aspects of protein import into the chloroplast are not very well understood. The proteins destined for chloroplast stroma have N-terminal transit peptide having 30-100 amino acids. The protein import into the matrix requires a guidance complex which directs the proteins to Toc (Translocase of the outer chloroplast membrane) from where it is passed to Tic complex. The Hsp100 present on the stromal side of Tic complex pulls the protein into stroma where transit peptide is cleaved by stromal processing peptidases. Three different pathways exist to which import proteins into the thylakoid lumen. Out of these three, two pathways: Sec pathway and SRP (signal recognition particle)-dependent pathway import partially folded

proteins while TAT (twin-arginine translocation) pathway imports folded and requires H^+ gradient established across the thylakoid membrane.

Chloroplasts are responsible for the synthesis of almost all amino acids, fatty acids, purines, pyrimidines and carotenes. The most important function of chloroplast is photosynthesis which is essentially divided into two reactions: Light reaction which takes place in the thylakoid membrane and generates ATP, NADPH and O_2 and Dark reaction which takes place in the stroma and produce carbohydrates from CO_2 fixation. Chlorophyll molecules absorb light energy and convert it to the chemical energy. Several hundred chlorophyll molecules act together and form large protein complexes known as photosystems. The photosystems consist of two components: a reaction center and an antenna complex. Two types of photosystems exist: photosystem I (PSI) and photosystem II (PSII). The reaction center of photosystem I is P_{680} while the reaction center of photosystem II is P_{700}. PSII is involved in the splitting of H_2O to produce O_2, PSI plays important role in transferring the electrons to $NADP^+$ to produce NADPH. The first step is the absorption of light photon by the pigment molecules of light-harvesting complex of the antenna complex. The absorb energy of photon is passed to the respective reaction centers. The excited reaction centers release electron which is accepted by primary electron acceptor. This flow of electrons from photosystems may follow non-cyclic (linear) or cyclic route. The non-cyclic electron flow also known as non-cyclic photophosphorylation involves both PSI and PSII while cyclic photophosphorylation involves only PSI. The non-cyclic photophosphorylation generates 4 ATP per O_2 molecule evolved or ½ ATP per photon absorbed while cyclic photophosphorylation results in generation of 2/3 ATP per photon absorbed.

The process of CO_2 fixation also known as dark reaction takes place in the stroma of chloroplast. The dark reaction is known as Calvin cycle or Calvin-Benson cycle. The key enzyme in Calvin cycle is ribulose 1,5-bisphosphate carboxylase or Rubisco which catalyzes the conversion of ribulose 1,5-bisphosphate to two molecules of 3-phosphoglycerate by adding CO_2. 3-phosphoglycerate is converted to Glyceraldehyde 3-phosphate which is converted to sucrose in cytosol and to starch in chloroplast.

Rubisco can also act as oxygenase in a process known as photorespiration where it adds O_2 to ribulose 1,5-bisphosphate forming 3-PGA and phosphoglycolate. This process leads to the loss of fixed and uses ATP and O_2. Photorespiration is more prominent in a hot dry environmental conditions and therefore, the crop plants that grow in such environments have developed a two-step mechanism known as C4 pathway also known as Hatch and Slack pathway. These plants have characteristic leaf anatomy consisting of single layer of bundle-sheath cells surrounded by mesophyll cells. The CO_2 is concentrated into the bundle-sheath cells which subsequently enters into the Calvin cycle.

Exercise/ Practice

A. Multiple choice questions:

1. The plastids lack pigments are known

   a) etioplast b) chromoplast c) leucoplasts d) proplastids

2. All plastids arise from undifferentiated organelles known as

a) etioplast b) chromoplast c) leucoplasts d) proplastids

3. The third membrane system of chloroplast is known as

a) inner membrane b) thylakoid membrane c) outer membrane d) periplastidial space

4. Mitochondria and chloroplast differ in

a) 70S ribosomes b) pigments c) circular DNA d) semi-autonomous organelles

5. The proteins destined for chloroplast stroma have N-terminal sequence) having 30-100 amino acids

a) stop transfer sequences b) leader peptide c) signal sequence d) transit peptide

6. Protein import into thylakoid does not require

a) Sec pathway b) signal sequence c) SRP dependent pathway d) TAT pathway

7. Chloroplasts are involved in these functions except

a) ATP synthesis b) amino acids synthesis c) fatty acid oxidation d) carotenes synthesis

8. Light reaction is responsible for the synthesis of

a) sucrose b) NADPH c) fructose d) amino acids

9. Light reaction takes place in

a) thylakoid membrane b) thylakoid lumen c) stroma d) cytosol

10. Copper-containing, mobile peripheral membrane protein found on the lumen side of the thylakoid membrane

a) Fe-S clusters b) Plastoquinone c) Plastocyanin d) phylloquinone

11. Dark reaction takes place in

a) thylakoid membrane b) thylakoid lumen c) stroma d) cytosol

12. The number of photons required to generate one molecule of O_2 is

a) 8 b) 4 c) 16 d) 2

13. Cyclic photophosphorylation leads to the production of

a) sucrose b) NADPH c) fructose d) ATP

14. Bundle-sheath cells surrounded by mesophyll cells is the characteristic feature of

a) C3 plants b) C4 plants c) cacti d) both C3 and C4 plants

15. The activation of Calvin cycle enzymes is controlled by

a) thioredoxin b) O_2 c) CO_2 d) malate

Chloroplast

B. Fill in the blanks:

1. An intermediate form seen when the plants are kept in dark is known as ________________
2. The contractile ring formed during chloroplast fission is known as________________
3. The thylakoid protein import mechanisms that import unfolded proteins are____________ and ____________
4. The thylakoid protein import mechanism that imports folded proteins is ____________
5. Protein translocation by twin-arginine translocation pathway requires ________________ across the thylakoid membrane.
6. The chlorophyll molecule consists of two parts: ____________ and____________.
7. Chlorophyll *c* is found in ____________
8. Key large protein complexes formed by several hundred chlorophyll molecules required for light reactions ____________
9. The photosystems consist of two components: _______________ and _______________
10. The reaction center of photosystem II is___________ and reaction center of photosystem I is _______________
11. Non-cyclic photophosphorylation results in the generation of ______ ATP and__________ NADPH. 4, 2
12. Rubisco CO_2 can also act as oxygenase in a process known as______________
13. Crop plants growing in hot dry environmental conditions have developed ______________ to avoid photorespiration.
14. The most abundant protein found on earth is _______________
15. Chlorophyll *d* is found in _______________

C. True/False

1. Green colored plastids that contain chlorophyll pigment are known as chloroplast.
2. Polyploid cells have larger sized chloroplasts as compared to diploid cells.

3. Non-cyclic photophosphorylation is responsible for production of ATP without generating NADPH.
4. The mechanism of fission in chloroplast resembles bacteria.
5. Oxygen-evolving complex is found closely associated with PSI.
6. The electrons from H_2O are transferred to P_{680}+ through a tyrosine residue which functions as an intermediate electron carrier.
7. Dark reaction is also known as Hatch and Slack pathway.
8. Rubisco a key enzyme in dark reaction has high turnover number.
9. Production of 2 molecules of Glyceraldehyde 3-phosphate requires 18 ATP and 12 NADPH molecules.
10. In C4 plants, mesophyll cells have higher CO_2 concentration as compared to bundle-sheath cells.

D. Expand the following

1. cpDNA
2. Toc
3. Tic
4. SPP
5. TPP
6. SRP
7. TAT
8. LHC
9. PSI
10. OEC
11. RuBP
12. PGA
13. GAP
14. PEP carboxylase

E. Match the following scientist with their contributions:

Chloroplast

A

1. Schimper
2. Lynn Margulis
3. Pierre Joliot and Bessel Kok
4. Melvin Calvin
5. Marshall Hatch and Rodger Slack
6. T. Engelmann
7. Cornelis van Neil

B

a. Dark reaction
b. first indication of role of H_2O in O_2 release
c. Plastids
d. C4 cycle
e. Experimental evidence for O_2 production by photospliting of H_2O
f. S-state hypothesis
g. Association between chloroplast and photosynthesis
h. Endosymbiotic theory

Glossary

Aleuroplasts/proteinoplast: Leucoplasts which store protein

Amyloplasts: Leucoplasts which store starch

Chloroplast: green colored plastids which contain chlorophyll pigment

Chromoplast: colored plastids

Dark reaction: reaction takeing place in the stroma, CO_2 is fixed to produce carbohydrate using energy from ATP and NADPH

Elaioplasts/Oleosomes: Leucoplasts which store fats

Etioplast: an intermediate form of seen when the plants are kept in dark, characterized by the array of membrane tubules that lack chlorophyll

Leucoplasts: white colored plastids which lack pigments

Light reaction: Reaction taking place in the thylakoid membrane, used to generate ATP, NADPH and O_2

Plastidome: double stranded, circular DNA molecule found in chloroplast

Plastidoribosome: 70S ribosomes found in chloroplast

Proplastids: undifferentiated organelles from which all other plastids arise, 0.5-1 μm in diameter

Pyrenoids: starch storing granules found in the stroma of green algae

Chloroplast

S-states: series of cyclic oxidation states by Oxygen Evolving Complex during extracting H^+ and e^- from H_2O

Transit peptide: N-terminal stromal-import sequence having 30-100 amino acids required for protein translocation into the stroma

References/ Bibliography/ Further Reading

Allen, J. F. 2002. Photosynthesis of ATP—electrons, proton pumps, rotors, and poise. Cell 110:273–276.

Allen, J.F. and Forsberg, J. 2001. Molecular recognition in thylakoid structure and function. Trends Plant Sci. 6: 317-326.

Barber, J. and Anderson, B. 1994. Revealing the blueprint of photosynthesis. Nature 370: 31-34.

Buchanan, B. B. 1991. Regulation of CO2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status, and future development. Arch. Biochem. Biophys. 288:1–9.

Golbeck, J. H. 1993. Shared thematic elements in photochemical reaction centers. Proc. Nat'l. Acad. Sci. USA 90: 1642–1646.

Hankamer, B., J. Barber, and E. Boekema. 1997. Structure and membrane organization of photosystem II from green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 48:641–672.

Heathcote, P., P. Fyfe, and M. Jones. 2002. Reaction centres: the structure and evolution of biological solar power. Trends Biochem. Sci. 27:79–87.

Horton, P., A. Ruban, and R. Walters. 1996. Regulation of light harvesting in green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47:655–684.

Jarvis, P. and Robinson, C. 2004. Mechanism of protein import and routing in chloroplast. Curr. Biol. 14: R1064-R1077.

Jarvis, P. and Soll, J. 2002. Toc, Tic and chloroplast protein import. Biochim. Biophys. Acta 1590: 177-189.

Penner-Hahn, J. 1998. Structural characterization of the Mn site in the photosynthetic oxygen-evolving complex. Struc. Bond. 90:1–36.

Portis, A. 1992. Regulation of ribulose 1,5-bisphosphate carboxylase/oxygenase activity. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43:415–437.

Prince, R. 1996. Photosynthesis: the Z-scheme revisited. Trends Biochem. Sci. 21:121–122.

Rawsthorne, S. 1992. Towards an understanding of C3-C4 photosynthesis. Essays Biochem. 27:135–146.

Rokka, A., I. Zhang, and E.-M. Aro. 2001. Rubisco activase: an enzyme with a temperature-dependent dual function? Plant J. 25:463–472.

Sage, R., and J. Colemana. 2001. Effects of low atmospheric CO_2 on plants: more than a thing of the past. Trends Plant Sci. 6:18–24.

Schneider, G., Y. Lindqvist, and C. I. Branden. 1992. Rubisco: structure and mechanism. Ann. Rev. Biophys. Biomol. Struc. 21:119–153.

Wollman, F. A. 2001. State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J. 20:3623–3630.

Wolosiuk, R. A., M. A. Ballicora, and K. Hagelin. 1993. The reductive pentose phosphate cycle for photosynthetic CO_2 assimilation: enzyme modulation. FASEB J. 7:622–637.

Answers

A. Multiple choice questions

1. c) leucoplasts
2. d) proplastids
3. b) thylakoid membrane
4. b) pigments
5. d) transit peptide
6. b) signal sequence
7. c) fatty acid oxidation
8. b) NADPH
9. a) thylakoid membrane
10. c) Plastocyanin
11. c) stroma
12. a) 8
13. d) ATP
14. b) C4 plants
15. a) thioredoxin

B. Fill in the blanks:

1. etioplast
2. Z ring
3. Sec pathway, SRP-dependent pathway

4. twin-arginine translocation pathway
5. H^+ gradient
6. Porphyrin ring, phytol tail
7. brown algae and diatoms
8. photosystems
9. reaction center, antenna complex
10. P_{680}, P_{700}
11. 4, 2
12. photorespiration
13. C4 pathway
14. Rubisco
15. red algae

C. True/False

1. True
2. True
3. False
4. True
5. False
6. True
7. False
8. False
9. True
10. True

D. Expand the following

1. chloroplast DNA
2. Translocase of the outer chloroplast membrane
3. Translocase of the inner chloroplast membrane
4. stromal processing peptidases
5. thylakoid processing protease
6. signal recognition particle
7. twin-arginine translocation
8. light harvesting complexes

9. photosystem
10. oxygen-evolving complex
11. ribulose 1,5-bisphosphate
12. 3-phosphoglycerate
13. Glyceraldehyde 3-phosphate
14. phosphoenolpyruvate carboxylase

E. Match the following

1. c.
2. h.
3. f.
4. a.
5. d.
6. g.
7. b.
8. e.