

# Chloroplast



**Lesson: Chloroplast**

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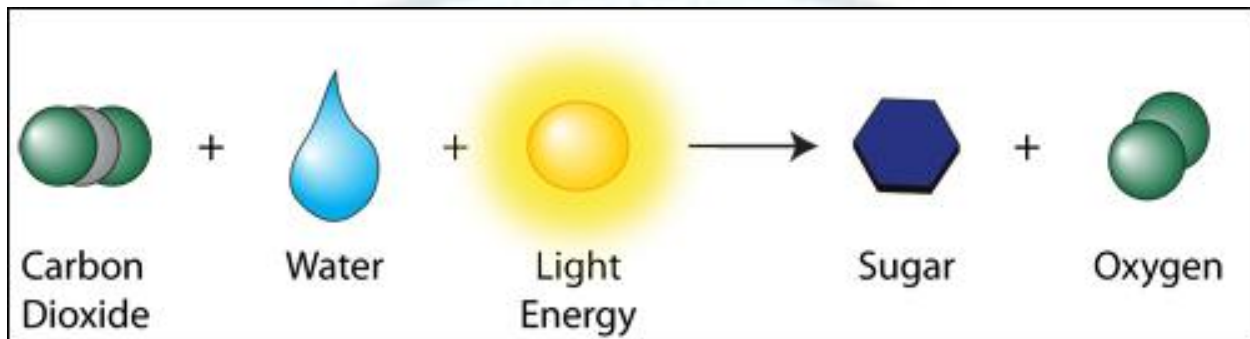
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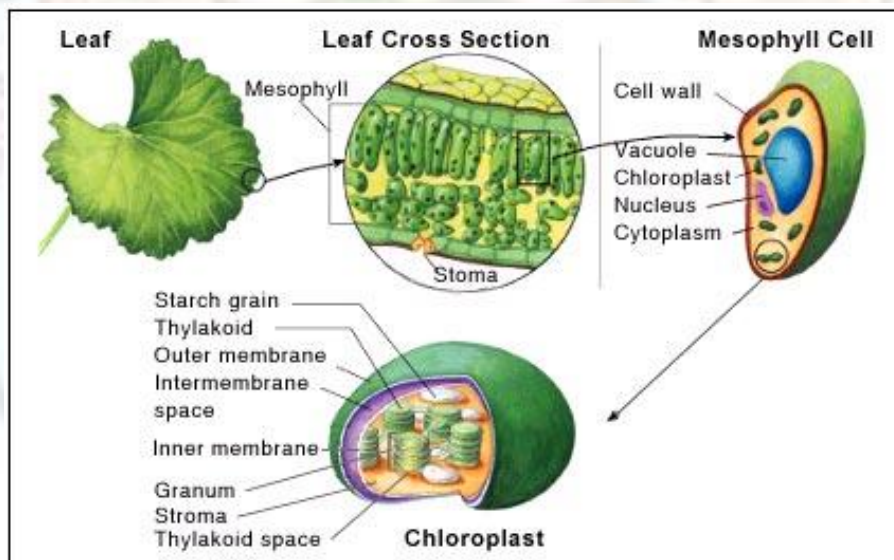
## Introduction

Chloroplasts are organelles involved in photosynthesis, the process of formation of carbohydrates from carbon-di-oxide and water in the presence of sunlight by the plants.



**Figure:** Summarizing the photosynthetic reaction

Source: [http://www.hartnell.edu/tutorials/biology/images/photosynthesis\\_equation\\_pic.jpg](http://www.hartnell.edu/tutorials/biology/images/photosynthesis_equation_pic.jpg)



Source: [http://www.southtexascollege.edu/moderh/1408webpage\\_files/CH%207\\_files/image005.jpg](http://www.southtexascollege.edu/moderh/1408webpage_files/CH%207_files/image005.jpg)

## Chloroplast



**Video:** Structure and function of chloroplasts

Source: <http://www.britannica.com/EBchecked/topic/113761/chloroplast>

Chloroplasts were first described as 'chlorophyllkornern' (chlorophyll granules) by German botanist Hugo von Mohl in 1837, though because of their large size they were seen long back by Nehemiah Grew who described them in 1682 as green precipitates! It was A.F.W. Schimper who in 1883 introduced the term 'plastids' as a substitute for chlorophyll granule. In the same year A. Mayer described 'grana' as dense dot like structures embedded in the 'stroma' of these plastids. The terms grana and stroma are still in use but chloroplast has replaced the term plastids for the green organelles of leaves. T.Engelmann a German biologist in 1881 identified the chloroplasts as the sites where photosynthesis occurs. He observed that outside the cells of green alga *Spirogyra* just close to the large ribbon shaped chloroplasts, bacteria would collect probably to utilize the oxygen being liberated during photosynthesis in the chloroplasts (bacterial chemotaxis).

For details visit: <http://link.springer.com/content/pdf/10.1007%2Fs11120-004-6313-8.pdf>

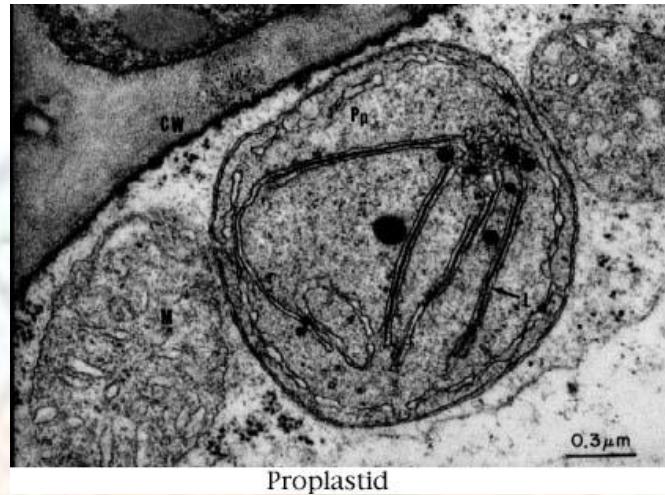
In addition to photosynthesis plastids are also involved in:

- Synthesis of amino acids, fatty acids and lipid components of their membranes.
- The important step of conversion of nitrite ( $\text{NO}_2^-$ ) to ammonia ( $\text{NH}_3$ ) occurs in the chloroplast providing the plant with the nitrogen for the synthesis of amino acids and nucleotides.

A typical plant cell may have 20-40 chloroplasts. There are many alga which have single chloroplast per cell that occupies most of the cell (remember cup shaped chloroplast of *Chlamydomonas*).

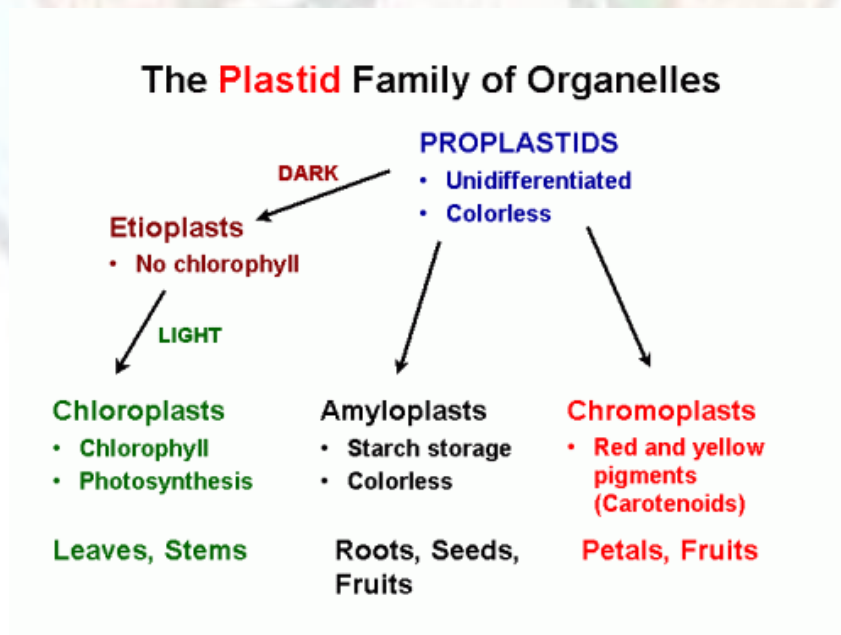
## Chloroplast

Chloroplasts are organelles that belong to the plastid family. This group of organelles which are present in various plant cells differ in their color and function. In young meristems small pro-plastids develop which mature according to the requirement of the cell and also as dictated by the nuclear genome.



**Figure:** A proplastid

Source: [http://www.skidmore.edu/academics/biology/plant\\_bio/photos/photos/cellbio/Proplastid.jpg](http://www.skidmore.edu/academics/biology/plant_bio/photos/photos/cellbio/Proplastid.jpg)

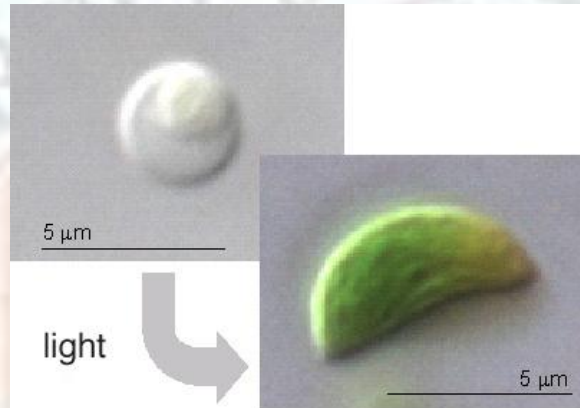


## Chloroplast

Source: [http://plantcellbiology.masters.grkraj.org/html/Plant\\_Cellular\\_Structures10-Plastids\\_files/image001.gif](http://plantcellbiology.masters.grkraj.org/html/Plant_Cellular_Structures10-Plastids_files/image001.gif)

For example:

- **Etioplasts-** these develop in leaves grown in dark. These have a yellow chlorophyll precursor and semi crystalline membranes. When exposed to light these develop to form chloroplasts.



Source: [http://www2.unine.ch/files/content/sites/physiologievegetale/files/shared/images/Toc/etioplastes\\_HR.jpg](http://www2.unine.ch/files/content/sites/physiologievegetale/files/shared/images/Toc/etioplastes_HR.jpg)

- **Leucoplasts-** these are white colored, non -photosynthetic and develop in epidermal and internal tissues. A common type of leucoplast is the amyloplast which accumulates polysaccharides and has a principal function of storage for e.g. as in potatoes.



**Figure:** A potato cell containing amyloplast.

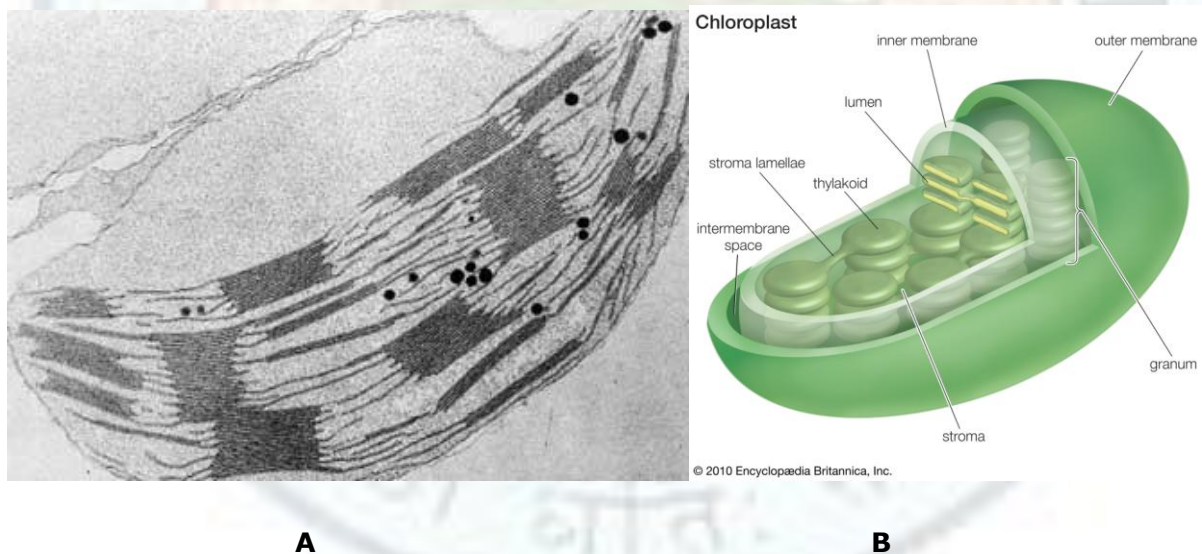
Source: [http://en.wikipedia.org/wiki/File:Potato\\_-\\_Amyloplasts.jpg](http://en.wikipedia.org/wiki/File:Potato_-_Amyloplasts.jpg)

## Structure and function

Chloroplasts are large organelles, generally lens shaped in higher plants, of around 2-4  $\mu\text{m}$  wide 5-10 $\mu\text{m}$  in long, bound by a double membrane called chloroplast envelope. In addition a third internal membrane called as the thylakoid membrane is also present. The thylakoid membrane forms a network of flattened sac like structures called thylakoids often stacked to form grana (singular granum). The three membranes divide the chloroplast into three compartments: The intermembrane space between the two outer membranes, the stroma and the thylakoid lumen.

Chloroplast structure can be understood under three sub-heads:

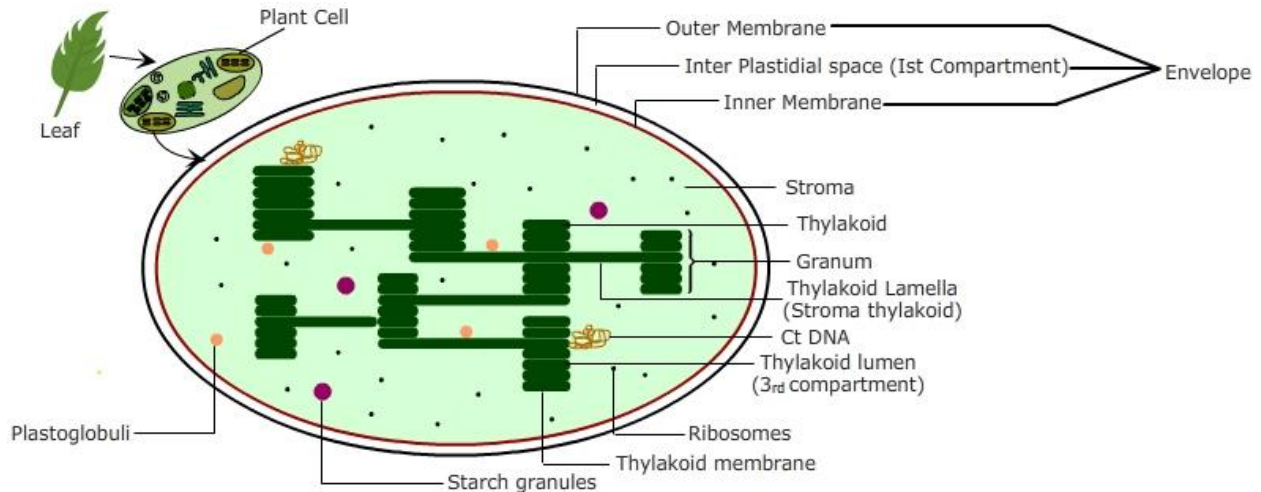
- 1) Envelope
- 2) Thylakoids
- 3) Stroma



**Figure:**A. Electron micrograph; B. Pictorial image of chloroplast

Source: [www.doctortee.com/dsu/tiftickjian/cse-img/botany/plant-anat/cell/chloroplast-tem.jpg](http://www.doctortee.com/dsu/tiftickjian/cse-img/botany/plant-anat/cell/chloroplast-tem.jpg)(Displayed with permission), <http://media-2.web.britannica.com/eb-media//76/53076-050-9830E88F.jpg>

## Chloroplast



**Figure:** Chloroplast structure

Source: Author

- 1) **Envelope:** Just like nucleus and mitochondria, chloroplasts are bound by a double membrane envelope called outer membrane and inner membrane. Both the membranes are made up of phospholipid bilayer. The space in between the membranes is inter-membrane space. The outer membrane of the chloroplast envelope contains 'porins' and so is freely permeable to small molecules. Whereas the inner membrane is impermeable to ions and metabolites and they need specific membrane transporters (integral membrane proteins) to enter the chloroplast. The outer membrane has very less protein content about 30% and have unusually have very little phospholipid. These have a high percentage of galactose containing glycolipids, which have several double bonds in their fatty acids making the membranes highly fluid. This facilitates the lateral diffusion of the protein complexes in the membrane.

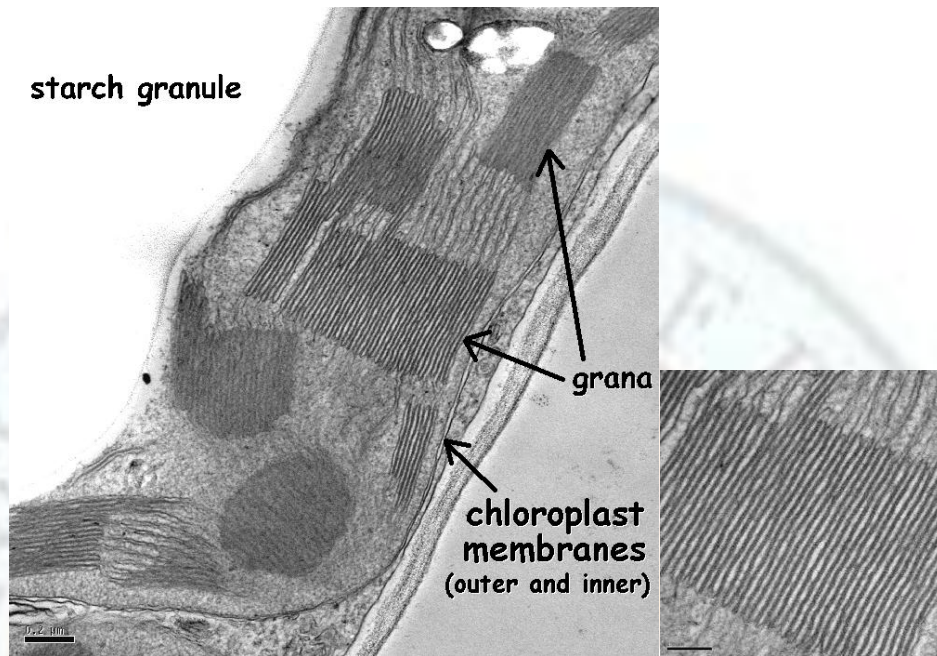
**Unlike the mitochondria, the inner membrane of the chloroplast is not involved in either electron transport or photosynthesis. Instead the thylakoid membrane contains the electron transport system. The protons are pumped across into the lumen of the thylakoids creating a proton gradient that drives the ATP synthesis as protons cross back into the stroma.**

- 2) **Thylakoids:** The internal membrane system (separate from the double membrane envelope) is organized into disc shaped/flattened membranous structures with empty



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lumen called thylakoids that are frequently arranged in stacks. Each stack is called granum (plural: grana). Adjacent grana are connected by stromal thylakoid (thylakoids in direct contact with the stroma).



**Figure :** Electron micrograph of the chloroplast showing the stacked grana.

Source: [http://www.lifesci.sussex.ac.uk/home/Julian\\_Thorpe/tem57.jpg](http://www.lifesci.sussex.ac.uk/home/Julian_Thorpe/tem57.jpg) (Displayed with permission)

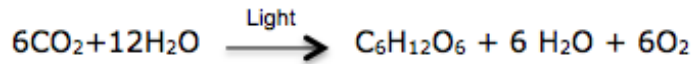
The thylakoid membrane contains the light absorbing pigments, the chain of electron carriers (ETC) and the ATP synthesizing machinery (in this sense it is analogous to the inner membrane of mitochondria). The space inside the thylakoid is the lumen. The protons are pumped across this membrane from the stroma to the thylakoid lumen. This results in formation of an electrochemical gradient which generates ATP when protons are transported back to the stroma.

- 3) **Stroma:** The space enclosed by the inner membrane of the chloroplast is called stroma. Stromal matrix is rich in metabolic enzymes and has small double stranded circular DNA molecules, ribosomes (70-S) and plastoglobuli (lipid granules). Dark reaction or the C-3 cycle (Calvin cycle) takes place in stroma. Enzymes for amino acid synthesis and fatty acid synthesis are also present in stroma.

## Photosynthesis

## Chloroplast

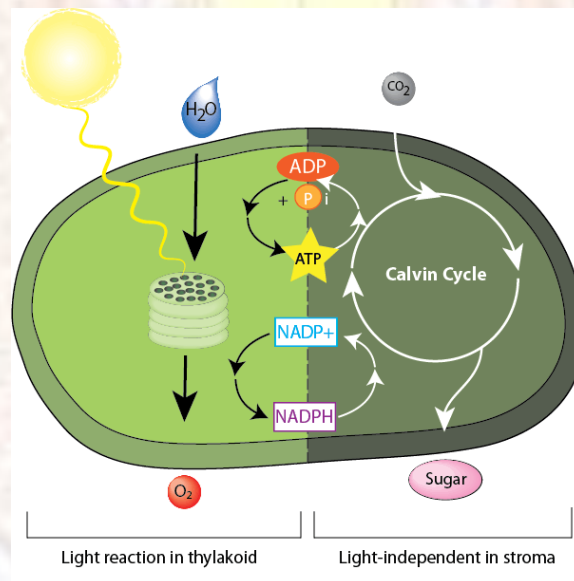
Photosynthesis is the main source of metabolic energy. Chloroplasts capture energy from sunlight and use it to fix carbon and convert it into carbohydrates.



The reactions that occur during photosynthesis can be divided into two stages:

- The light dependent reactions- During light reactions energy from the sunlight drives the synthesis of ATP and NADPH coupled with the splitting of the water molecule and generation of O<sub>2</sub>.
- The light independent reactions (commonly called dark reactions) – the synthesis of carbohydrates using CO<sub>2</sub> and the energy stored in the ATP and NADPH.

These reactions occur in different compartments of the chloroplast, the thylakoid membrane and stroma respectively.



**Figure:**

Source: [http://setarosite.org/images/biobook\\_images/photosynthesis\\_light\\_dark.gif](http://setarosite.org/images/biobook_images/photosynthesis_light_dark.gif)

Image: [http://canmedia.mcgrawhill.ca/istudy3/books/0070741751/images/figures/bro41751\\_0803L\\_lg.jpg](http://canmedia.mcgrawhill.ca/istudy3/books/0070741751/images/figures/bro41751_0803L_lg.jpg)

## Light reaction

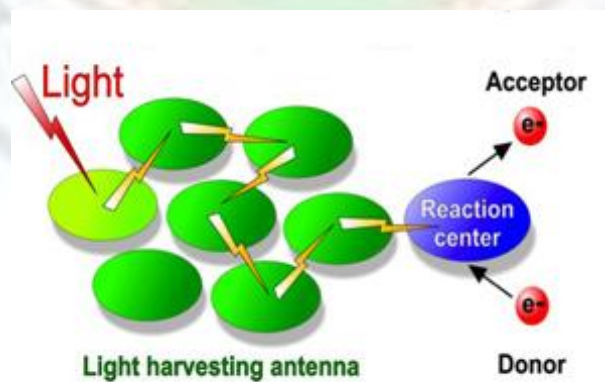
ENERGY AND ELECTRON  
TRANSFER IN  
PHOTOSYNTHETIC  
ORGANISMS:

PLANTS, CYANOBACTERIA,  
GREEN SULFUR BACTERIA, AND  
PURPLE BACTERIA

**Video:** Energy and electron transfer in photosynthesis

Source: <http://multimedia.mcb.harvard.edu/media.html>

Sunlight is captured by photosynthetic pigments the most abundant of which are the – chlorophylls. The photosynthetic pigments are arranged into photocenters with each photocenter containing hundreds of antenna pigment molecules which absorb the photons and transfer energy to the reaction center chlorophyll.



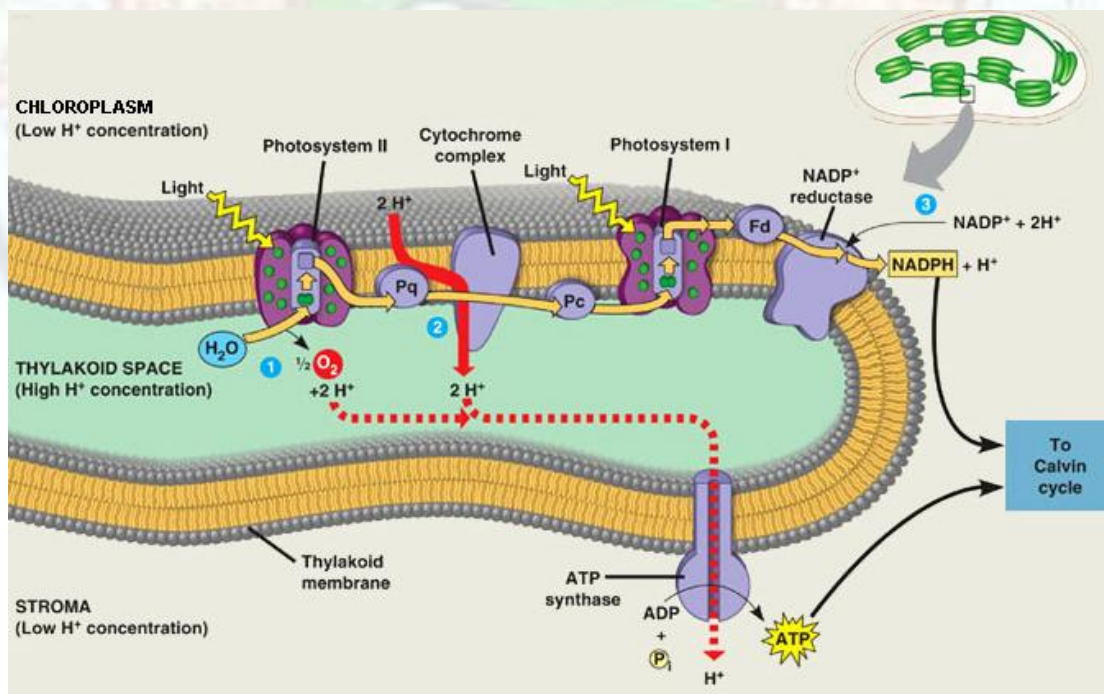
## Chloroplast

Source: [http://news.wustl.edu/news/PublishingImages/antenna\\_300.jpg](http://news.wustl.edu/news/PublishingImages/antenna_300.jpg)

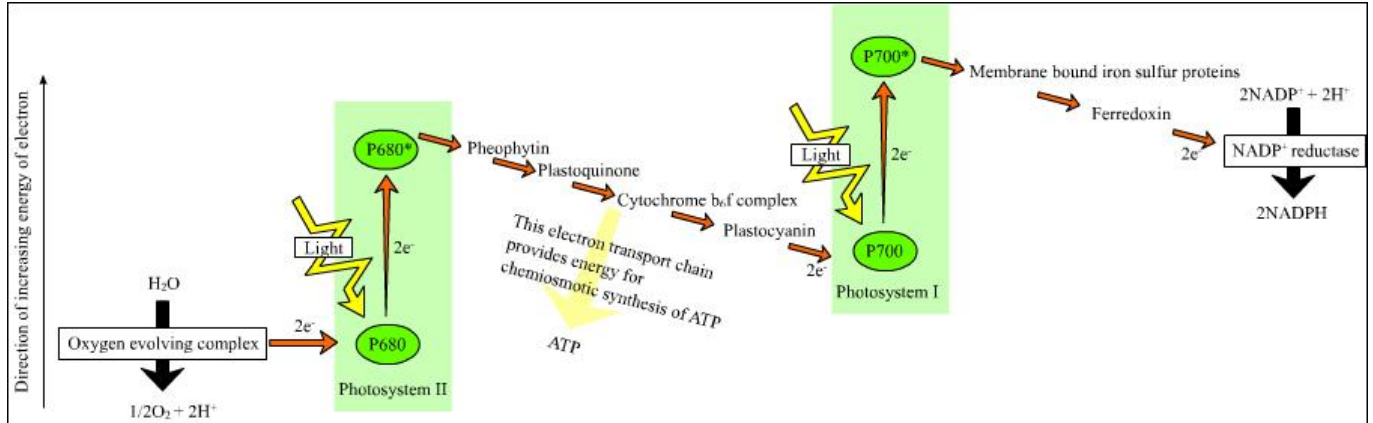
For details visit: [http://plantphys.info/plant\\_physiology/light.shtml](http://plantphys.info/plant_physiology/light.shtml)

The energy derived from the sunlight excites an electron (derived from the splitting of water molecule and the generation of  $O_2$ ) of the chlorophyll thus converting the energy of the sunlight to potential chemical energy. This electron then moves along an electron transport chain (a series of electron carrier proteins located in the thylakoid membrane). Four protein complexes located in the thylakoid membrane function as electron carriers and are also involved in the synthesis of ATP and NADPH.

Energy initially harvested by PSII (photosystem II) is used to split a water molecule within the thylakoid lumen. Electrons are then carried by plastoquinone to the cytochrome b<sub>f</sub> complex where they are transferred to low energy state and protons are pumped into the thylakoid lumen. Electrons are transferred to photosystem I by plastocyanin(PC). At PSI the sunlight absorbed is again used to generate high-energy electrons. The photosystem I uses the high energy electrons to reduce  $NADP^+$  to NADPH.



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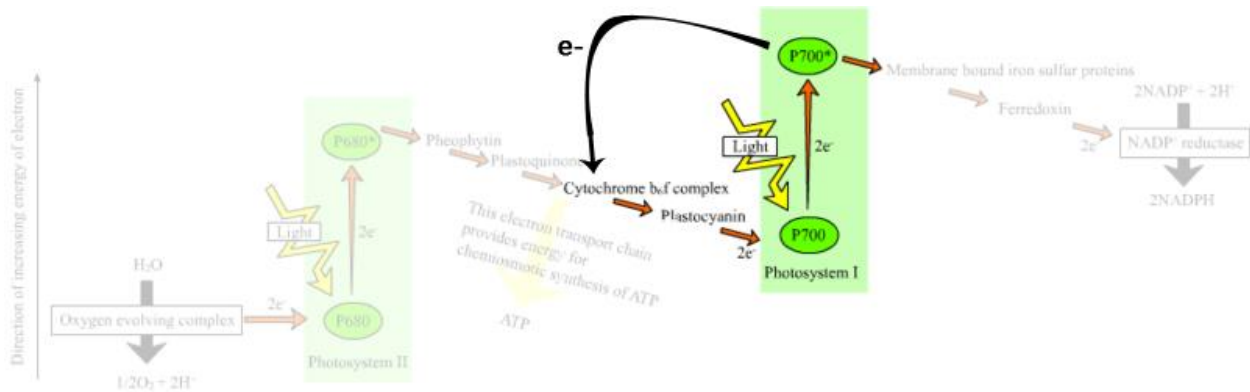
**Figure:** Structure of thylakoid membrane showing components of light-reaction and photophosphorylation. Light is captured by the photosystems. Photolysis of water takes place. Electron travels from PSII to PSI and NADPH is synthesised from NADP. Proton gradient is generated across the thylakoid membrane which drives ATP formation in the stroma.

Source: <http://apbiologywiki.wikispaces.com/file/view/chemi.jpg/330240152/chemi.jpg>,  
<http://www.hartnell.edu/tutorials/biology/images/Z-scheme.jpg>

Finally, the high energy electrons that move through the electron transport chain of light reaction and the  $H^+$  convert the  $NADP^+$  to NADPH. The movement of electron through the photosystem I and II generates both ATP and NADPH which are then used in the Calvin cycle to convert  $CO_2$  to carbohydrates.

Another electron transport pathway that operates to produce ATP is called the **cyclic electron flow**. However unlike the non-cyclic pathway no NADPH is generated. The light energy is harvested at the photosystem I and the high energy electrons generated are transferred to cytochrome b<sub>6</sub> complex coupled to which protons are transferred across the thylakoid membrane establishing a proton gradient and thus synthesizing ATP. Plastocyanin returns these electrons to photosystem I thus completing the cycle. Thus PS I participates in both cyclic and non cyclic pathways forming ATP and NADPH depending on the requirement of the cell.

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Source: <http://figures.boundless.com/4ff32b97246b709a9cd78b10/full/64356908-3ba1-4493-a989-87372aac8235.png>

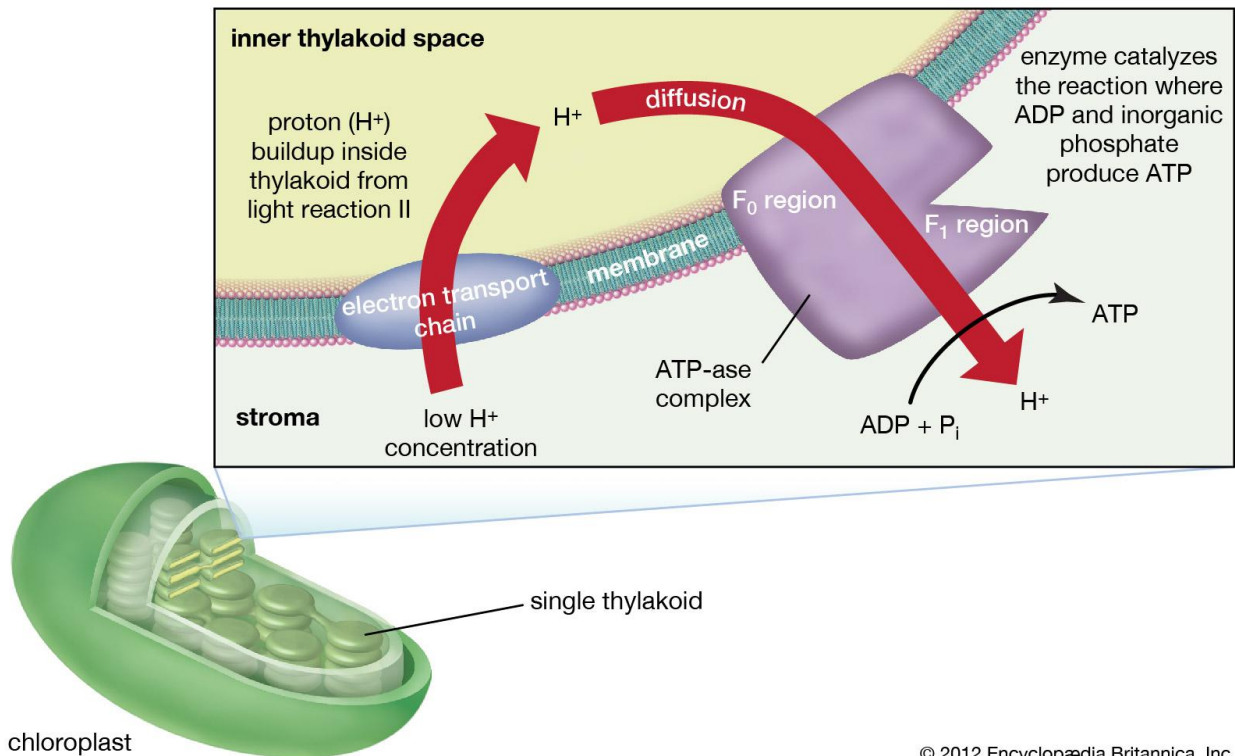
### Synthesis of ATP

The electron transfer process is accompanied with the movement of  $H^+$  across the thylakoid membrane. The resulting electrochemical proton gradient drives the ATP synthesis in the stroma and is known as **photophosphorylation**. ATP formation both in chloroplast and mitochondria is guided by chemiosmosis. **Chemiosmosis** is an energy-coupling mechanism that uses energy stored in the form of a hydrogen ion gradient across a membrane to drive cellular work (generating ATP). During chemiosmosis and electron transport chain assembled in a membrane pumps protons across the membrane as electrons are passed through a series of carriers in the electron transport chain thus establishing a proton gradient. An ATP synthase complex is built into the same membrane. The movement of the protons down the gradient as they pass through the ATP synthase complex provides the energy by which ATP is regenerated from ADP and phosphate. This chemiosmotic theory was proposed by Peter Mitchell in 1961 for which he received the Nobel prize in 1978.

Two protons are transported at the Photosystem II and two to four protons are transported at the Cyt b<sub>6</sub> complex. Since one molecule of ATP is synthesized for four protons transported movement of a pair of electrons through the non-cyclic pathway yields 1 to 1.5 ATP. In contrast the number of ATP synthesized by the cyclic pathway is considerably less with 0.5 to 1 ATP molecules synthesized per pair of electrons transported.

## Chloroplast

### Chemiosmosis in chloroplasts



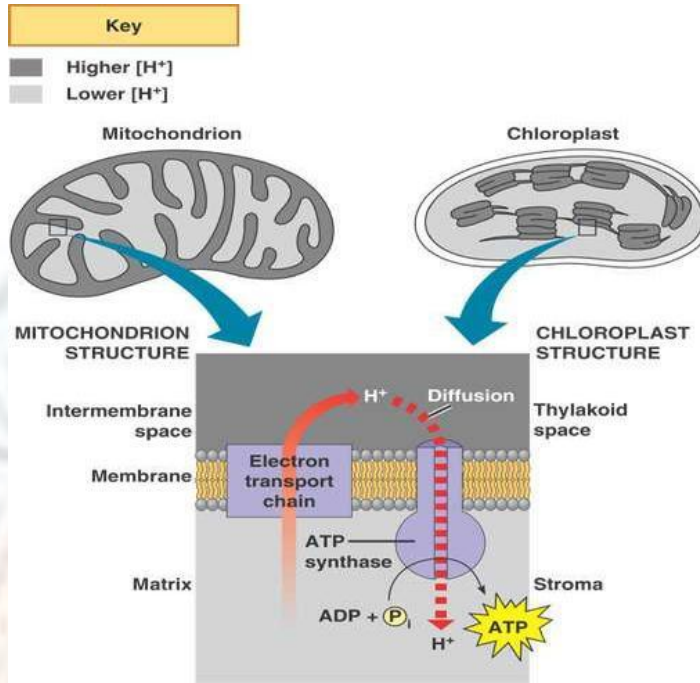
**Figure:** The electrochemical proton gradient drives the synthesis of ATP.

Source: <http://media-2.web.britannica.com/eb-media//42/157842-050-9B26AA4F.jpg>

#### Similarities between chloroplast and mitochondria-ATP synthesis

- Chemiosmosis takes place in both mitochondria and chloroplasts. They both generate ATP by chemiosmosis
- In both organelles, electron transport chains pump protons ( $H^+$ ) across a membrane from a region of low  $H^+$  concentration to one of high  $H^+$  concentration
- Both move  $H^+$  from the liquid (stroma and matrix) to a membrane enclosed space
- The protons then diffuse back across a membrane through ATP synthase, driving the synthesis of ATP
- Some of the electron carriers, including cytochromes, are very similar in chloroplasts and mitochondria
- The ATP synthase complexes of the two are also very similar

**Difference between chloroplast and mitochondria – ATP Synthesis**



[http://bio1151.nicerweb.com/Locked/media/ch10/10\\_16ChemiosmosisCompared\\_L.jpg](http://bio1151.nicerweb.com/Locked/media/ch10/10_16ChemiosmosisCompared_L.jpg)

However, there are key differences in this process between these organelles. Here is a chart comparing them both:

**Chemiosmosis in Mitochondria (oxidative phosphorylation)**

**Chemiosmosis in Chloroplasts (photophosphorylation)**

**Source of electrons:** extracted from organic molecules which are oxidized

**Source of electrons:** water molecules

Use chemiosmosis to transfer chemical energy from food molecules to ATP (uses energy stored in NADH or FADH<sub>2</sub>)

Do not need molecules from food to make ATP, their photosystems capture light energy and use it to drive the electrons from water to the top of the transport chain



## Chloroplast

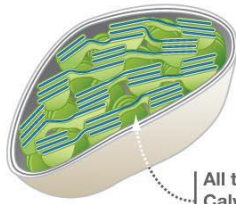
	Chloroplasts transform light energy into chemical energy in ATP (uses light energy)
The inner membrane of the mitochondria pumps protons from the mitochondrial matrix out to the intermembrane space, which then serves as a reservoir of hydrogen ions	The thylakoid membrane of chloroplasts pumps protons from the stroma into the thylakoid space which functions as the H <sup>+</sup> reservoir
Protons diffuse down their concentration gradient from the intermembrane space through ATP synthase to the matrix, driving ATP synthase	ATP is synthesized as the hydrogen ions diffuse from the thylakoid space back to the stroma through ATP synthase complexes. Thus ATP is formed in the stroma, where it is used to help drive sugar synthesis during the Calvin Cycle

### Dark reactions or the carbon fixation reactions

The ATP (energy source) and NADPH (reducing power), produced during light reaction, drive the conversion of CO<sub>2</sub> to carbohydrate (sucrose) and other organic molecules like amino acids and fatty acids. These reactions occur partly in the chloroplast stroma and partly in the cytosol. Dark reaction or the Calvin cycle converts CO<sub>2</sub> to carbohydrates. This reaction occurs in three phases. Phase one is **carboxylation** where one molecule of CO<sub>2</sub> enters the Calvin cycle by joining with a 5-carbon molecule ribulose 1,5-bisphosphate RuBP. This reaction is catalyzed by ribulose 1,5-bisphosphate carboxylase, perhaps the most abundant protein on earth! The product a 6-carbon compound immediately breaks down to two molecules of 3-carbon compound, 3-phosphoglycerate. Next phase is **Reduction** where 3-phosphoglycerate is reduced to glyceraldehyde 3-phosphate using ATP and NADPH. Lastly, **regeneration of RuBP** occurs by converting some of the 3-phosphoglycerate to RuBP. Later, 3-phosphoglyceraldehyde is exported to cytoplasm where it is converted to sucrose.

# Chloroplast

(a) The Calvin cycle has three phases.

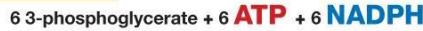


All three phases of the Calvin cycle take place in the stroma of chloroplasts

**1. Fixation**



**2. Reduction**

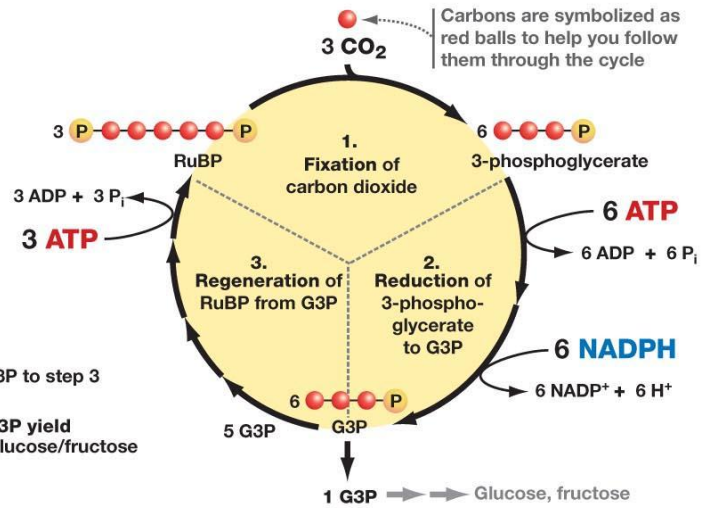


**3. Regeneration**



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(b) The reaction occurs in a cycle.

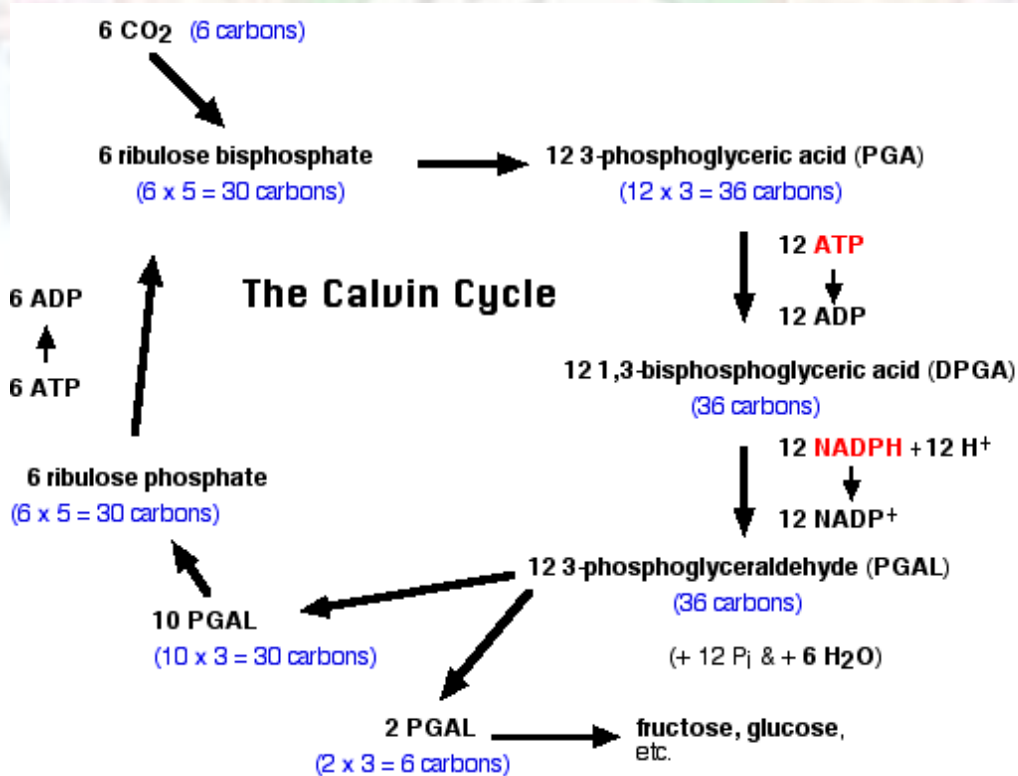


## Abbreviations

RuBP: ribulose 1,5-bisphosphate

G3P: Glyceraldehyde 3-phosphate

Source: [http://www.uic.edu/classes/bios/bios100/lectures/10\\_18\\_calvin\\_cycle-L.jpg](http://www.uic.edu/classes/bios/bios100/lectures/10_18_calvin_cycle-L.jpg)



Source: <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/CalvinCycle.png>

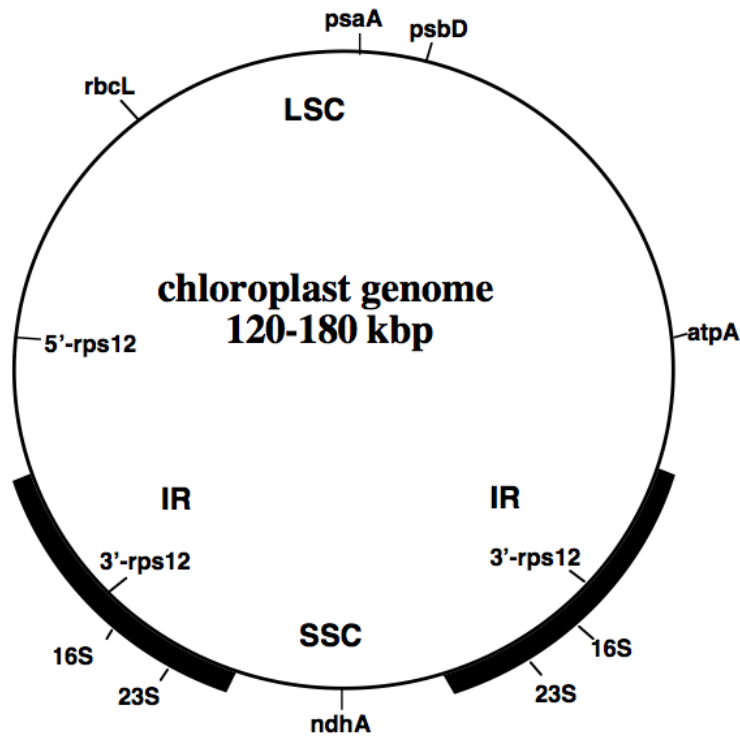
### **Chloroplast genome**

We know that apart from nucleus, DNA is also found in mitochondria and chloroplast. The chloroplasts, like mitochondria, are semi- autonomous organelles that have their own genetic systems. Chloroplast genomes of a number of organisms have been sequenced and have provided a detailed information regarding the number of genes and the products encoded by them.

The important features of chloroplast genome include:

- Circular DNA molecules present in several copies per organelle
- Size varies from 120-160 kb
- Approximately 150 genes (depending on the organism) that can be grouped in two categories: those involved in gene expression and those involved in photosynthesis.
- Two inverted repeats (IRs) 10-28 Kb in length divide the genome into a large single copy region (LSC) and a small single copy region (SSC)

## Chloroplast



Source: [www.uky.edu/~aghunt00/PLS620/notes/7chloroplast.pdf](http://www.uky.edu/~aghunt00/PLS620/notes/7chloroplast.pdf)

The chloroplast genes code both for RNAs and proteins. Along with mRNAs, both t-RNAs and rRNAs are encoded by the chloroplast genome. However a high percentage of the 2000-3500 polypeptides of the chloroplasts are encoded by the nuclear genes. For the smooth functioning of the organelle, the nuclear and the chloroplast genomes thus work synergistically. For example, some of the subunits of the RNA polymerase and the enzyme Rubisco found in the chloroplast are encoded by chloroplast genome itself while rest of the subunits are encoded by the nucleus and then imported inside the chloroplast.

**Table:** A few points of comparison amongst genomes found in organelles, prokaryotes and eukaryotes.

SNo	Attribute	Prokaryotes	Mitochondria	Chloroplast	Eukaryotes
1	Size of DNA	0.5 mbp-10mbp	10-200kb	120-160kb	2.9mbp-4000mbp
2	Code	Universal genetic code	Unique mitochondri	Universal genetic code	Universal genetic

## Chloroplast

			al genetic code		code
3	tRNAs	>30	22	30	>30
4	Introns	Absent	Present	Present	Present
5	Ribosomes	70S	70S	70S	80S
6	Division of cell/organelle	Binary Fission	Binary Fission	Binary Fission	Mitosis

Protein translation in chloroplast is strikingly similar to protein translation in prokaryotes. For example, both in prokaryotes and chloroplast, the protein synthesis starts with N-formyl Met, ribosomes are sensitive to antibiotics chloramphenicol and tetracycline; bacterial RNA polymerase can transcribe chloroplast DNA, chloroplast mRNA can be translated in bacteria.

In the wake of recent advances in chloroplast genome sequencing and maternal inheritance of chloroplast genes, it is increasingly being used for genetic engineering of plants. Additionally, chloroplast DNA is also present in multiple copies (50-100 copies) per cell resulting in high production of foreign protein engineered in the plant.

### **Marker Enzymes**

Some enzymes are located exclusively in one particular organelle. These enzymes can be used to establish the presence of the organelle in the isolated fraction of the cell. Such enzymes are called marker enzymes. For example, if in a fraction of cell activity of the enzyme succinate dehydrogenase is ascertained, the presence of mitochondria is confirmed. The marker enzymes also confirm the biochemical purity of the isolated organelle. Marker enzymes can also be used to judge the biochemical purity of the fractionated organelle. If the activity of other marker enzymes is also found in the fractionated organelle it is due to contamination with other organelles. The specific activity of the target marker enzyme reveals the degree of enrichment of the desired organelle. Marker enzymes for chloroplast are RUBISCO and NADP-glyceraldehyde-3-phosphate dehydrogenase. Both these enzymes participate in the Calvin cycle and are located in the stroma of the chloroplast.

If it interests you, you may look for marker enzymes and their function in various other organelles as well.

## **Semiautonomous nature**

Some organelles enjoy some amount of autonomy or independence like in growth, division, production of some enzymes, they are known as semiautonomous organelles. Mitochondria and chloroplast fall in this category. They are able to do this because they have their own DNA/ genome. Both the organelles are also known to have evolved from free living prokaryotes, according to the endosymbiotic theory. Endosymbiotic theory was proposed by Lynn Margulis in 1960s. She proposed that primitive anaerobic bacteria engulfed another aerobic bacteria and instead of endocytosis endosymbiosis occurred, that is the association became mutually beneficial for both the bacteria and resulted in early aerobic heterotrophic eukaryote. Further, when this eukaryote engulfed photosynthetic bacteria another endosymbiotic relationship evolved and resulted in an autotrophic eukaryote. This theory also explains double membrane of these organelles, one derived from each of the organism involved.

Since the autonomy is not complete and the chloroplasts depend on nuclear genome for majority of their proteins and enzymes, hence, these organelles are known as semiautonomous in nature. For example, in chloroplast the enzyme RuBisCo is made up of multiple units of polypeptides assembled into two types of subunits called the large chain and small chain. The large chain gene is a part of chloroplast DNA but the small chain genes are located on the nuclear genome. The small chain subunit is thus synthesized in the cytosol and is imported into the stroma.

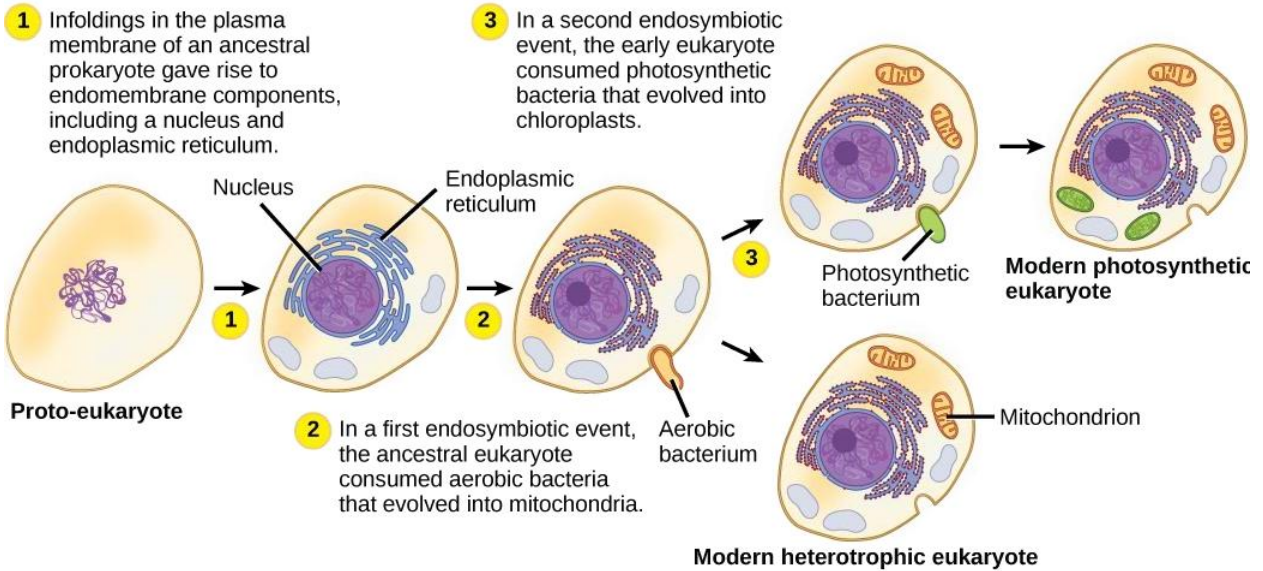
## **Endosymbiotic theory**

Evidences suggest that chloroplasts originated from oxygen evolving photosynthetic bacteria via the process of endosymbiosis about 1 billion years ago. The primitive eukaryotic cells (which were probably anaerobic) engulfed the photosynthetic bacteria and these then later established a symbiotic relationship with their partners. Similarly the mitochondria evolved from bacteria being endocytosed. This probably is the reason why the basic process of ATP synthesis is similar in both the organelles.

Most of the chloroplast proteins are encoded by the nucleus therefore it is hypothesized that an extensive transfer of genes from organelle to the nucleus must have occurred during evolution.

## Chloroplast

### The ENDOSYMBIOTIC THEORY

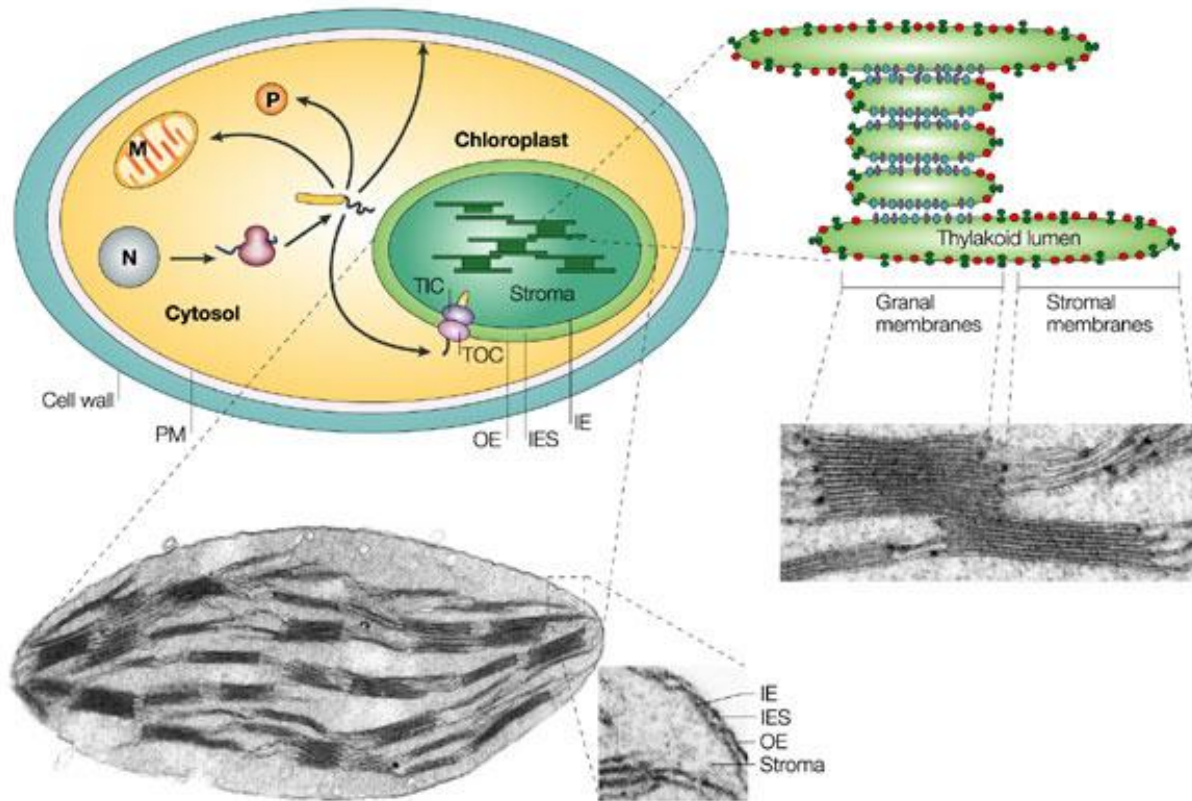


Source: [http://cnx.org/content/m47175/latest/figure\\_23\\_01\\_04.png](http://cnx.org/content/m47175/latest/figure_23_01_04.png)

### Sorting and import of proteins

As discussed earlier 95% of chloroplast proteins are encoded by the nuclear genes and are imported into the chloroplasts after they have been synthesized in the cytoplasm (following the signal hypothesis). The proteins targeted to the chloroplasts are recognized by a stretch of 30-100 amino acids located on the N-terminal called transit peptide. Transit peptides are recognized by the receptors located on the outer membrane called a guiding complex. Guiding complex directs the protein to a translocase (Toc complex- A multiprotein complex) located in the outer chloroplast membrane.

## Chloroplast



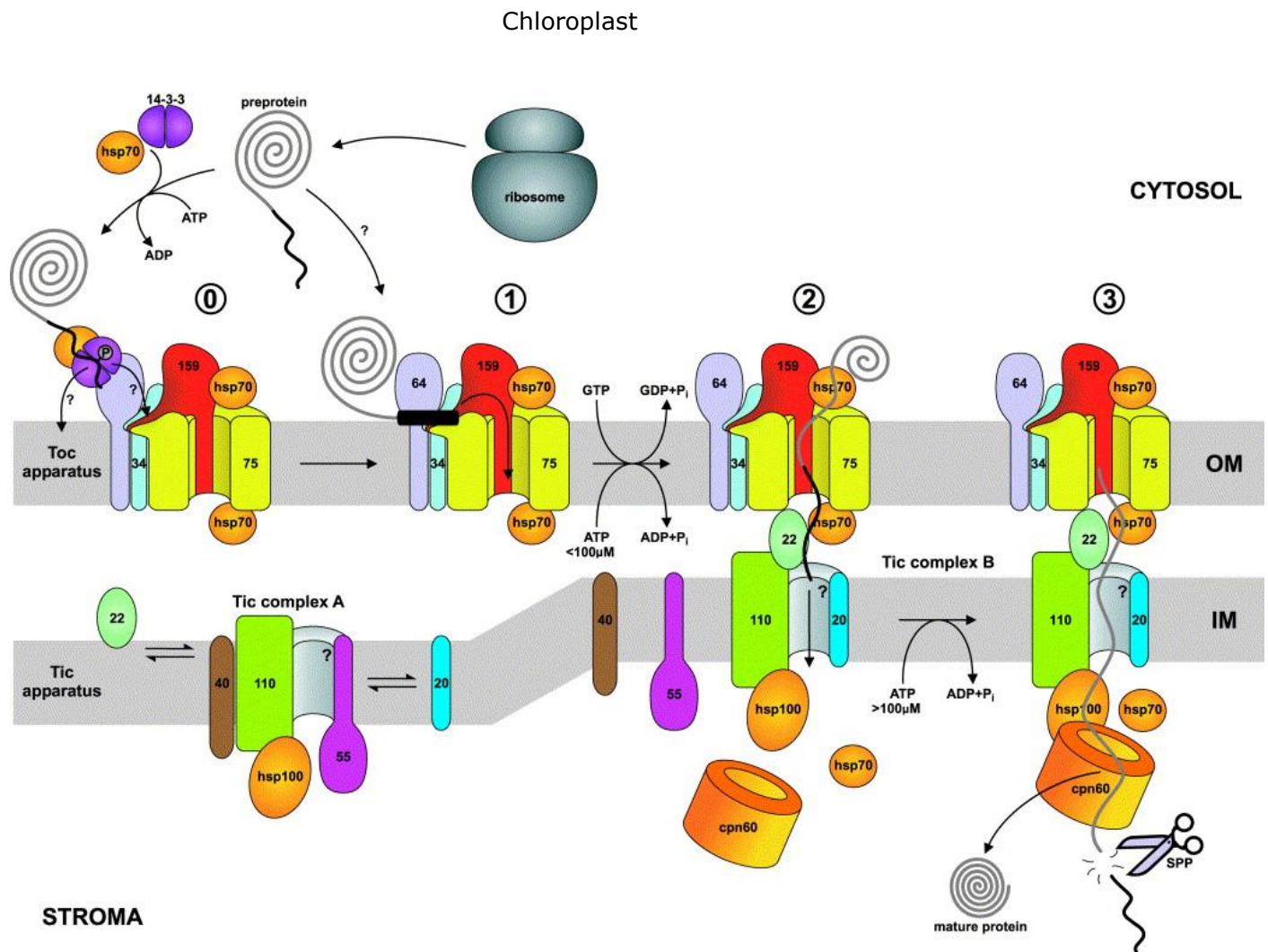
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Source: [http://www.nature.com/nrm/journal/v5/n3/box/nrm1333\\_BX1.html](http://www.nature.com/nrm/journal/v5/n3/box/nrm1333_BX1.html) (Displayed with permission)

The proteins are maintained in their unfolded state by chaperone Hsp70 as they are ferried from the cytoplasm into the stroma. This import is driven by the energy released by ATP hydrolysis catalyzed by Hsp70. On reaching the stroma the transit peptide is cleaved and the proteins either fold into their native state by binding with Hsp60 or remain unfolded by binding to the stromal Hsp70.

The proteins after transport by the Toc complex are transferred to the translocase (Tic a multiprotein complex) located in the inner membrane. A chaperone Hsp100 located on the inner side serves to pull the protein through the inner membrane into the stroma. The signal sequence is cleaved by a stromal enzyme **stromal processing peptidase (SPP)** and the protein now associates with the stromal Hsp70 proteins for folding.





**Figure:** A working model for the chloroplast protein import mechanism as described below.

Source: <http://origin-ars.els-cdn.com/content/image/1-s2.0-S0167488902001763-gr1.jpg>

Stage 0: As chloroplast preproteins emerge from 80S ribosomes, they are bound by a cytosolic guidance complex which docks at Toc64

Stage 1: Certain preproteins may bypass this guidance step and proceed directly to the core Toc complex. Preproteins unload from the guidance complex and pass to a trimeric receptor complex comprising Toc159, Toc34 and Toc75, either directly or via the lipid matrix of the outer envelope membrane. Subsequently, the import mechanism can be divided into three distinct stages, as indicated, based on energetic requirements. At stage 1 (energy-independent binding) the preprotein interacts reversibly with the heterotrimeric Toc receptor complex.

## Chloroplast

Stage 2: Progression to stage 2 (or formation of the early import intermediate) requires ATP at low concentrations in the intermembrane space, and GTP. At this stage, the preprotein is inserted across the outer envelope membrane and is in contact with components of the Tic apparatus.

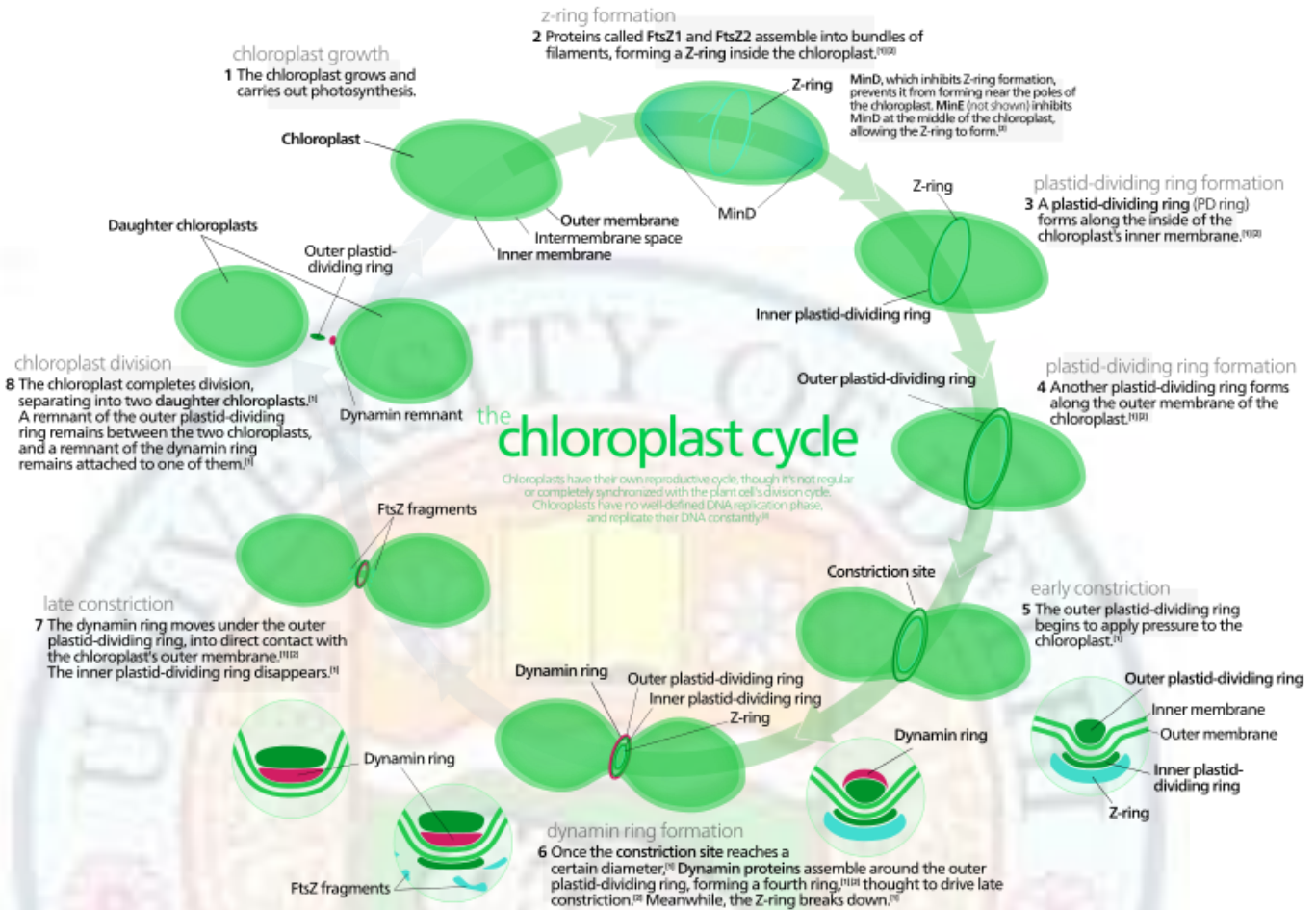
Stage 3: (complete translocation) requires high concentrations of ATP in the stroma. The preprotein is translocated simultaneously across both envelope membranes at a contact site, the transit peptide is cleaved by the stromal processing peptidase (SPP) and the mature protein takes on its final conformation. Data from different laboratories suggests that there may be two distinct Tic complexes (Tic complexes A and B). Tic complex B was arbitrarily chosen for the illustrated import reaction. A very similar diagram could be drawn to show an import reaction involving Tic complex A.

Numbers indicate the predicted molecular mass of each protein, and therefore identify the different Toc/Tic components. OM denotes outer envelope membrane, and IM denotes inner envelope membrane.

### **Biogenesis**

Chloroplasts arise from pre-existing chloroplasts through binary fission. The figure shows the formation of two small chloroplasts from a single large chloroplast. Later, each chloroplast grows in size. We already know, plastids can interchange into one another, for example etioplasts can change into chloroplasts and vice-versa.

## Chloroplast



Source: [http://en.wikipedia.org/wiki/File:Chloroplast\\_division.svg](http://en.wikipedia.org/wiki/File:Chloroplast_division.svg)

## Summary

- Chloroplasts are member of plastid family and are found only in eukaryotes.
- They have three membranes systems: two membranes of the envelope and one of the thylakoids.
- They have three compartments: the inter membrane system between the outer and the inner membrane, stroma enclosed by the inner reaches membrane and thylakoid lumen surrounded by thylakoid membrane.
- Composition of inter membrane space is almost similar to cytosol, stroma has enzymes of Calvin cycle, ct DNA, ribosomes, DNA replicating, transcription and translation enzymes, lipid granules, starch granules and water; thylakoid lumen has protons concentration higher than the stroma.

## Chloroplast

- ATP synthesis takes place in stroma but the phosphorylating complex is present in thylakoid membrane.
- Thylakoid membrane also has components of light reaction of photosynthesis.
- Chloroplasts divide by fission and have evolved from free living photosynthetic bacteria.
- Chloroplasts are semiautonomous in nature.
- Chloroplast DNA ranges from 120-160kb and has around 130 genes. It resembles prokaryotic genome in its functioning.

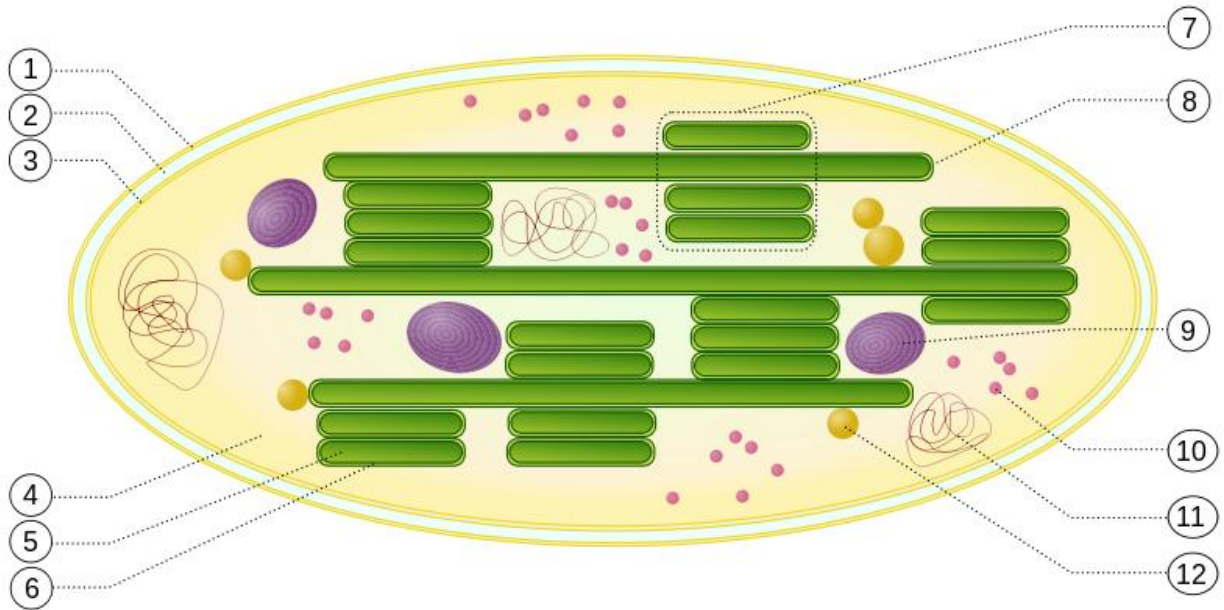
### Exercises

1. Apart from nucleus, name one organelle that has double membrane envelope.
2. Where do you find members of electron transport chain in chloroplast?
3. How many compartments are present in chloroplast?
4. What do you understand by semiautonomous nature of chloroplast?
5. Enlist differences between mitochondrial and nuclear DNA.
6. True or false
  - a) The ribosomes of chloroplast, mitochondria and prokaryotes are similar. (T/F)
  - b) Chloroplasts have originated from free-living photosynthetic bacteria. (T/F)
  - c) Photolysis occurs in the stroma of chloroplast. (T/F)
  - d) Grana are joined to each other by stromal thylakoids. (T/F)
  - e) Chloroplast genome has unique genetic code for protein synthesis. (T/F)
7. Chloroplasts divide by
  - a) amitosis
  - b) fission
  - c) mitosis
  - d) meiosis
8. One of the following statement does not hold true for chloroplast DNA
  - a) It is circular
  - b) It is not associated with histone proteins
  - c) It cannot replicate in bacteria.
  - d) Chloroplast mRNA can be translated in bacteria.
9. All the following events take place in the stroma of chloroplast, except
  - a) Conversion of ADP to ATP
  - b) Fixation of CO<sub>2</sub> and formation of 3-phosphoglyceric acid
  - c) Breakdown of H<sub>2</sub>O into H<sup>+</sup>, e<sup>-</sup> and O<sub>2</sub>.

## Chloroplast

d) Calvin cycle

10. Identify the organelle and label its various parts



Source: <http://en.wikipedia.org/wiki/File:Chloroplast.svg>

## Glossary

**ATPase:** Enzyme that catalyses the hydrolysis of ATP.

**Chemiosmosis( chemiosmotic coupling):** Mechanism in which a gradient of hydrogen ions(pH gradient) across a membrane is used to drive ATP production.

**Electron transport chain:** Series of electron carrier molecules pass electrons down the chain from successively lower energy levels to a final acceptor molecule.

**Endosymbiosis:** A theory which traces the origin of mitochondria and chloroplast from free living prokaryotes.

**Granum/Grana:** thylakoids arranged in stacks in the stroma of chloroplast.

**Marker Enzymes:** Enzymes specific to an organelle.

**Photosystems:** A group of chlorophyll molecules present on the thylakoid membrane that trap energy from sun and pass it to the reaction centre.

**Photophosphorylation:** Formation of ATP by creating a proton gradient across thylakoid membrane using sunlight.

**Semiautonomous organelles:** organelles that have their own DNA and carry out some of their functions without the help of nucleus.

**Signal sequence:** Short continuous sequence of amino acid that eventually determines the location of the protein in the cell.

## Chloroplast

**Stroma:** Matrix of chloroplast, rich in photosynthetic enzymes, ct DNA, ribosomes and has thylakoid membrane system embedded in it.

**Thylakoid:** A disc shaped phospholipid membrane rich in proteins that carry out light reactions of photosynthesis.

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## Chloroplast

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