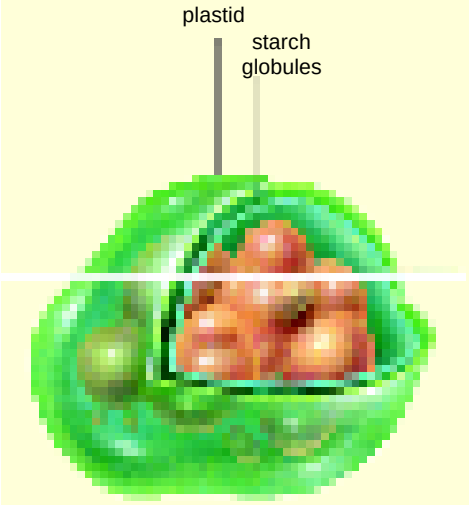
CHAPTER

11

Plastids

(Chloroplasts, Photosynthesis and Vacuoles)



Starch filling plastids in potato cells.

Plant cells are readily distinguished from animal cells by the presence of two types of membrane-bounded com partments– **vacuoles** and **plastids**. Both organelles are related to the immobile life-style of plant cells.

Plastids are present in all living plant cells and in *Euglena* (a protozoan). They are small bodies found free in the cyto-plasm. Plastids are often more or less spherical or disc-shaped (1 µm to 1 mm in diameter), but may be elongated or lobed or show amoeboid characteristics. Their other identifying features are their double bounding membranes, the possession of **plastoglobuli** (spherical lipid droplets; store of lipids surplus to current requirements) and an internal membrane fretwork of many discrete internal vesicles. All plastids in a particular plant species contain multiple copies of same relatively small genome (DNA) and 70S-type ribosomes. They are self-replicating or-ganelles containing a protein-synthesizing capacity comparable to that of mitochondria. They perform most important biological activities as the synthesis of food and storage of carbohydrates, lipids and proteins. Plastids are absent in the cells of fungi, bacteria, animals and male sperm cells of certain higher plants.

HISTORICAL

Chloroplasts were described as early as seventeenth cen-tury by **Nehemiah Grew** and **Antonie van Leeuwenhoek**. The term plastid was used by **Schimper** in 1885 ; he also classified the plastids of plants. **A. Meyer** in 1883, **F. Schmitz** in 1884 and **A.F.W. Schimper** in 1885 made detailed cytological studies of these cell organelles and showed that chloroplasts always arise

from pre-existing chloroplasts. In 1918, **Wilstatter** and **Stoll** isolated and characterized the green pigments–chlorophylls *a* and *b*. **K. Porter** and **S. Granick** (1947) described the ultrastructure of grana of chloroplasts. The studies of **Julius Sachs** in the mid-nineteenth century show that chlorophyll was confined to the chloroplasts and was not distributed throughout the plant cell. He also showed that sunlight caused chloroplasts to absorb carbon dioxide and that chlorophyll is formed in chloroplasts only in the presence of light.

Dutrochet (1837) recognized that chlorophyll was essential to oxygen evolution by plants. **Liebig**, in 1845 indicated that carbon dioxide was the source of all organic compounds synthesized by green plants. In 1845, **von Mayer** recognized that green plants convert the solar energy into the chemical energy of organic matter :

$$CO_2 + H_2O \xrightarrow[\text{green plants}]{\text{sunlight}} \text{Organic matter} + O_2$$

In 1862, **Sachs** proved that starch was synthesized by plants in a light-dependent reaction (photosynthesis).

In 1931, an American biochemist, **Cornelius van Niel** observed that a certain type of photosynthetic bacteria fixed carbon dioxide in the presence of hydrogen sulphide. In this process no oxygen was evolved. Instead globules of sulphur were formed as a waste product. He concluded that during bacterial photosynthesis carbon dioxide was not split, rather hydrogen sulphide was broken down, the resultant hydrogen reduced carbon dioxide and sulphur was left behind :

$$6\,CO_2 + 12\,H_2S \longrightarrow C_6H_{12}O_6 + 6H_2O + 12S$$

This led van Niel to hypothesize that (1) oxygen produced during photosynthesis of higher plants comes from water and not from carbon dioxide. (2) Water is the hydrogen donor. (3) CO_2 molecules are incorporated intact into carbohydrates. He proposed the following reaction for all photosynthetic organisms :

$$\underset{\substack{\text{Hydrogen}\\\text{acceptor}}}{CO_2} + \underset{\substack{\text{Hydrogen}\\\text{donor}\\(H_2O \text{ or } H_2S)}}{2H_2A} \xrightarrow[\text{chlorophyll}]{\text{light}} \underset{\substack{\text{Reduced}\\\text{acceptor}}}{(CH_2O)} + \underset{\substack{\text{Dehydrogenated}\\\text{donor}\\(1/2\ O_2 \text{ or } S)}}{2A} + H_2O$$

In 1932, **Emerson** and **Arnold** carried out the flashing light experiment and showed the existence of light and dark reactions. They introduced the concept of **photosynthetic unit** (or **PS I**) which is thought to be activated when light impinges on a photosynthetic unit.

An English biochemist, **Robert Hill** (1937) demonstrated **photolysis** of water by isolated chloroplasts in the presence of suitable electron acceptor (*e.g.*, ferricyanide)

$$2H_2O + \underset{\text{Ferricyanide}}{4Fe^{3+}} \xrightarrow[\text{chloroplast}]{\text{illuminated}} O_2 + 4H^+ + \underset{\text{Ferrocyanide}}{4Fe^{2+}}$$

In 1941, **Ruben** and **Kamen** used O^{18} to show that in photosynthesis oxygen comes from water. **Calvin** and **Benson** (1948) showed that phosphoglycerate was an early product of CO_2 fixation. In 1954, **Arnon**, **Allen** and **Whatley** used $^{14}CO_2$ to show fixation of CO_2 by isolated chloroplasts. **Melvin Calvin** (1945–1954) made experiments with unicellular green alga *Chlorella* and used radioactive form of CO_2 ($^{14}CO_2$) to work out those anabolic reactions by which CO_2 is fixed into hexoses and other carbohydrates. These reactions are found to be independent of light and are called **dark reactions**,

biochemical reactions, **Calvin cycle** or **C_3 cycle** (since it involves the formation of a 3-carbon product). Calvin was awarded the Nobel prize in 1960.

Hill and **Bendall** (1960), proposed Z-sheme for electron transport from water to NADPH during photosynthesis. It linked the two photosystems—PS I and PS II.

In 1966, two Australian workers, **M.D. Hatch** and **G.R. Slack** suggested an alternative pathway for carbon fixation in corn and some other hot-weather plants. It is called **C_4 cycle**, since it involves a four carbon compound.

TYPES OF THE PLASTIDS

The term 'plastid' is derived from the Greek word "*plastikas*" (= formed or moulded) and was used by **A.F.W. Schimper** in 1885. **Schimper** classified the plastids into following types according to their structure, pigments and the functions :

1. Leucoplasts

The leucoplasts (Gr., *leuco* = white ; *plast* = living) are the colourless plastids which are found in embryonic and germ cells. They are also found in meristematic cells and in those regions of the plant which are not receiving light. Plastids located in the cotyledons and the primordium of the stem are colourless (leucoplastes) but eventually become filled with chlorophyll and transform into chloroplasts. **True leucoplasts** occur in fully differentiated cells such as epidermal and internal plant tissues. They never become green and photosynthetic. True leucoplasts do not contain thylakoids and even ribosomes (**Carde**, 1984). They store the food materials as carbohydrates, lipids and proteins and accordingly are of following types :

(i) Amyloplasts. The amyloplasts (L., *amyl*=starch ; Gr., *plast*=living) are those leucoplasts which synthesize and store the starch. The amyloplasts occur in those cells which store the starch. The outer membrane of the amyloplst encloses the stroma and contains one to eight starch granules. In some plant tissues such as potato tuber, amyloplasts can grow to be as large as an average animal cell. In them starch granules may become so large that they rupture the encasing membrane. Starch granules of amyloplasts are typically composed of concentric layers of starch.

(ii) Elaioplasts. The elaioplasts store the lipids (oils) and occur in seeds of monocotyledons and dicotyledons. They also include sterol-rich **sterinochloroplast**.

(iii) Proteinoplasts. The proteinoplasts are the protein storing plastids which mostly occur in seeds and contain few thylakoids (**Heinrich**, 1966).

2. Chromoplasts

The chromoplasts (Gr., *chroma*=colour; *plast*=living) are the coloured plastids containing **carotenoids** and other pigments. They impart colour (*e.g.*, yellow, orange and red) to certain portions of plants such as flower petals (*e.g.*, daffodils, rose), fruits (*e.g.*, tomatoes) and some roots (*e.g.*, carrots). Chromoplast structure is quite diverse ; they may be round, ellipsoidal, or even needle-shaped, and the carotenoids that they contain may be localized in droplets or in crystalline structures. The function of chromoplasts is not clear but in many cases (*e.g.*, flowers and fruits) the colour they produce probably plays a role in attracting insects and other animals for pollination or seed dispersal.

In general, chromoplasts have a reduced chlorophyll content and are, thus, less active photosynthetically. The red colour of ripe tomatoes is the result of chromoplasts that contain the red pigment **lycopene** which is a member of carotenoid family. Chromoplasts of blue-green algae or cyanobacteria contain various pigments such as **phycoerythrin**, **phycocyanin**, **chlorophyll a** and **carotenoids**. Chromoplasts are of following two types :

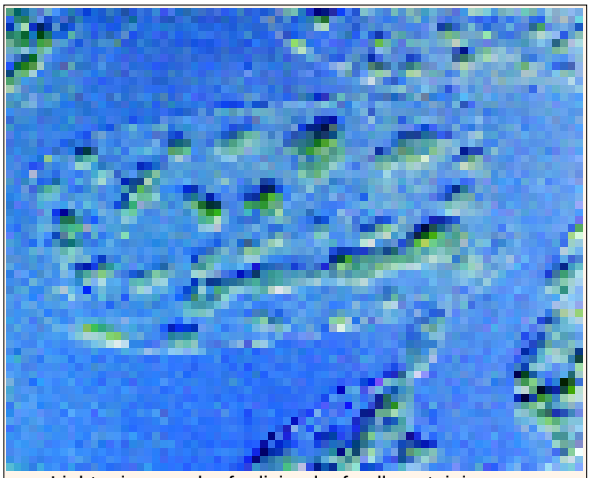
(i) Phaeoplast. The phaeoplast (Gr., *phaeo*=dark or brown; *plast*=living) contains the pigment **fucoxanthin** which absorbs the light. The phaeoplasts occur in the diatoms, dinoflagellates and brown algae.

(ii) Rhodoplast. The rhodoplast (Gr., *rhode*= red; *plast*=living) contains the pigment **phaeoerythrin** which absorbs the light. The rhodoplasts occur in the red algae.

3. Chloroplasts

The chloroplast (Gr., *chlor*=green; *plast*=living) is most widely occurring chromoplast of the plants. It occurs mostly in the green algae and higher plants. The chloroplast contains the pigment chlorophyll a and chlorophyll b and DNA and RNA.

According to **Schimper**, different kinds of plastids can transform into one another, as shown in following figure :



Light micrograph of a living leaf cell containing a number of chloroplasts.

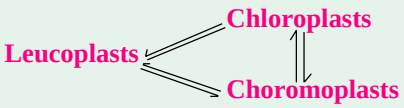


Fig. 11.1. Interconversion of three kinds of plastids.

Development of Plastids

All plastids, including chloroplasts, develop from **proplastids**, which are relatively small organelles present in meristematic cells. Proplastids develop according to the need of each differentiated cell. If a leaf is grown in darkness, its proplastids enlarge and develop into **etioplasts**. These have a semi-crystalline array of internal membranes that contain **protochlorophyll** (a yellow chlorophyll precursor) instead of chlorophyll. When exposed to light, the etioplasts rapidly develop into chloropalsts by converting protochlorophyll to chlorophyll and by synthesizing new membrane, pigments, photosynthetic enzymes and components of electron transport chain (**Thomson** and **Watley**, 1980 ; **Mullet**, 1988).

Light regulates many processes in plants. In ways that are still unclear, photoreceptors including phytochromes control the transcription of many light activated genes involved in chloroplast development, not only in the chloroplast itself but also in the nucleus. Approximately one-fifth of the 120 chloroplast genes are regulated in a light-dependent manner.

Phytochromes. Although there are several types of photoreceptor molecules in plants, the best characterized is the large (1,24,000 dalton) protein, called **phytochrome**. This protein contains a pigment that is responsive to light and can exist in two interconvertible forms : an **inactive form** produced by exposure to far red light (between visible red and infrared), and an **active form** produced by red light. Phytochrome is known to mediate many light-activated responses, including chloroplast rotation, plastid differentiation, seed germination, stem elongation, leaf initiation and flowering. For example, *Mougeotia* is an alga in which each cylindrical cell contains a single plate-like chloroplast. In response to light, the chloroplast rotates until it is perpendicular to the incident light. The photoreceptor molecules that initiate the response are phytochrome and a blue light receptor located on or very near the plasma membrane. Illumination of these receptors with a microbeam causes an influx of Ca^{2+} which binds to calmodulin. Calmodulin activates a network of actin filaments, attached both to the outer membrane of the chloroplast envelope and to the plasma membrane which mediates the rotation (**Wagner** and **Klein**, 1981).

CHLOROPLASTS

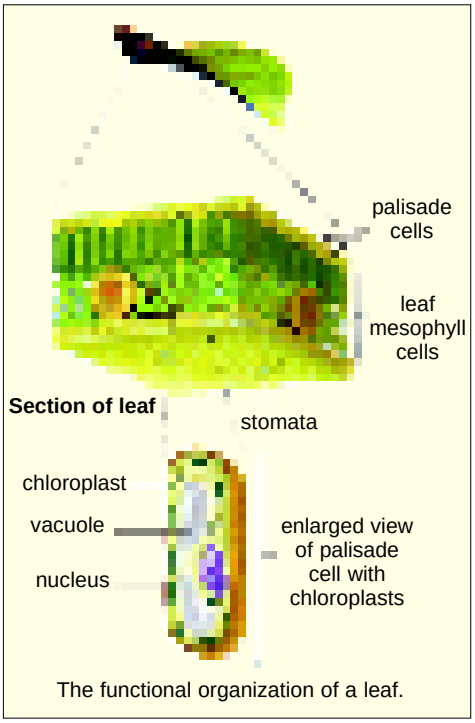
We will describe here only the chloroplasts in detail because of two reasons. Firstly they are most common plastids of many plant cells and secondly they perform the photosynthetic activity of greatest biological importance. By the process of photosynthesis the chloroplast synthesizes the carbohydrates which contain energy in the form of chemical energy. The chemical energy is utilized by all living beings to perform various life activities.

DISTRIBUTION

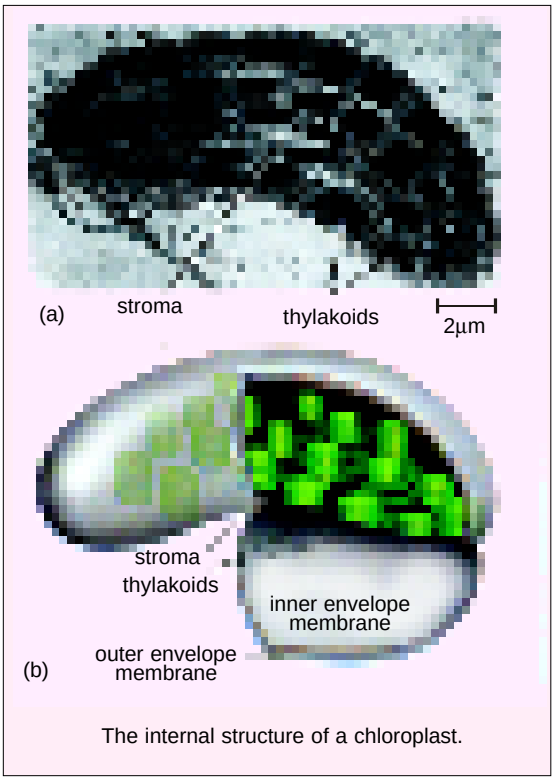
The chloroplasts remain distributed homogeneously in the cytoplasm of plant cells. But in certain cells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. The chloroplasts have a definite orientation in the cell cytoplasm. Since chloroplasts are motile organelles, they show passive and active movements.

MORPHOLOGY

Shape. Higher plant chloroplasts are generally biconvex or plano-convex. However, in different plant cells, chloroplasts may have various shapes, *viz*., filamentous, saucer-shaped, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.



The functional organization of a leaf.



The internal structure of a chloroplast.

Size. The size of the chloroplasts varies from species to species. The chloroplasts generally measure 2–3µm in hickness and 5–10µm in diameter (*e.g.*, *Chlamydomonas*). The chloroplasts of polyploid plant cells are comparatively larger than the chloroplasts of the diploid plant cells. Generally, chloroplasts of plants grown in the shade are larger and contain more chlorophyll than those of plants grown in sunlight.

Number. The number of the chloroplasts varies from cell to cell and from species to species and is related with the physiological state of the cell, but it usually remains constant for a particular plant cell. The algae usually have a single huge chloroplast. The cells of the higher plants have 20 to 40 chloroplasts. According to a calculation, the leaf of *Ricinus communis* contains about 400,000 chloroplasts per square millimeter of surface area. When the number of chloroplasts is inadequate, it is increased by division; when excessive, it is reduced by degeneration.

Comparison of Chloroplasts and Mitochondria

Chloroplasts carry out their energy inter-conversions by chemiosmotic mechanisms in much the same way that mitochondria do and they are organized on the same principles. Thus, each chloroplast contains three distinct membranes which define three separate internal compartments—the intermembrane space, the stroma and the thylakoid space. The thylakoid membrane contains all the energy generating systems of chloroplasts.

Like the mitochondria, chloroplasts have a highly permeable outer membrane ; a much less permeable inner membrane, in which special carrier or transport proteins are embedded ; and a narrow intermembrane space in between. The inner membrane surrounds a large space called the **stroma**, which is analogous to the mitochondrial matrix and contains various enzymes, ribosomes, RNAs and DNA.

However, there is an important difference between the two : the inner membrane of the chloroplast is not folded into cristae and does not contain an electorn-transport chain. Instead, the photosynthetic light-absorbing system, the electorn-transport chain and an ATP synthetase are all contained in a third distinct membrane that forms a set of flattened disc-like sacs, the **thylakoids** (Fig. 11.2).

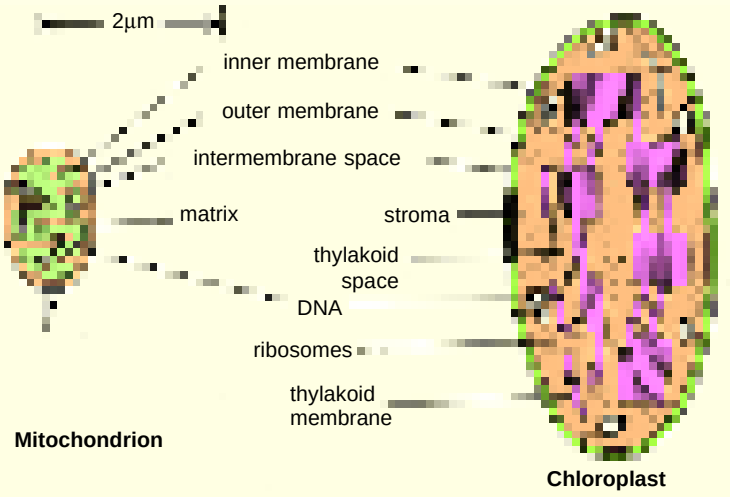


Fig. 11.2. Comparison of a mitochondrion and a chloroplast (after Alberts *et al.*, 1989).

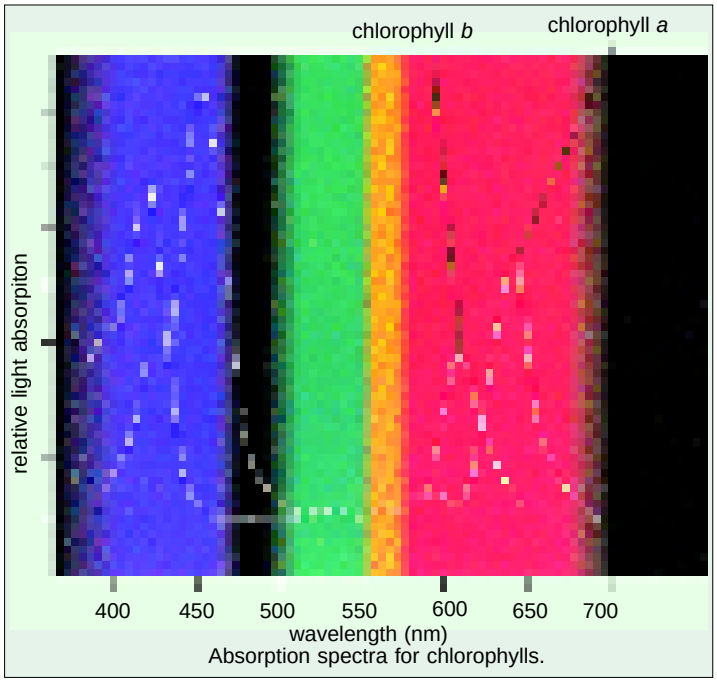
In a general way, one might view the chloroplast as a greatly enlarged mitochondria in which the cristae are converted into a series of interconnected submitochondrial particles in the matrix space. The knobbed ends of the chloroplast ATP synthetases (F_0 - F_1 coupling factors), where ATP is made, protrude from the thylakoid membrane into the stroma, just as they protrude into the matrix from the membrane of each mitochondrial crista.

ISOLATION AND CHEMICAL COMPOSITION

Chloroplasts are routinely isolated from plant tissues by differential centrifugation following the disruption of the cells. Leaves are homogenized in an ice-cold buffered isotonic saline solution (*e.g.*, 0.35 M NaCl) at pH 8.0. The disruption is generally carried out with bursts of Waring blender. After filtration through a nylon gauze (20 µm pore size) to remove the larger particles of debris (*e.g.*, cell nuclei, tissue fragments and unbroken cells), the chloroplasts are separated by unbroken cells), the chloroplasts are separated by centrifugation of 200g for 1 minute. The chloroplast rich pellet is then resuspended and centrifuged again at 2000 g for 45 seconds to re-sediment the chloroplasts. Chloroplast preparations obtained by this method are generally mixtures of intact and broken organelles. Because the chemical composition, rate of photosynthetic activity and other properties of intact chloroplasts differ significantly from those of damaged organelles, it is often desirable to separate the two populations. This may be achieved by isopycnic density gradient centrifugation of the chloroplast preparation.

Details of ultrastructure of grana lamellae have been worked out by electron microscopy (fixation by gluteraldehyde and staining by osmium) and freeze-fracture technique.

The isolated chloroplasts of higher plants are found to contain the chemical composition shown in Table 11-1. The chloroplasts are composed of the carbohydrates, lipids, proteins, chlorophyll, carotenoids (carotene and xanthophylls), DNA, RNA and certain enzymes and coenzymes. The chloroplasts also contain some metallic atoms as Fe, Cu, Mn and Zn.



Absorption spectra for chlorophylls.

The carbohydrates occur in very low percentage in the chloroplasts. The most common carbohydrates of the chloroplasts are the starch and sugar phosphates.

The chloroplasts contain 20–30 per cent lipids of its dry weight. The lipids are composed of 50 per cent fats, 20 per cent sterols, 16 per cent waxes and 7 to 20 per cent phospholipids. The most common alcohols of the lipids are the choline, inositol, glycerol, ethanolamine.

The proteins constitute 35 to 55 per cent of the chloroplast. About 80 per cent proteins are insoluble and forming the unit membranes of the chloroplasts along with the lipids. The 20 per cent proteins are soluble and occur in the form of the enzymes.

The chlorophyll is a green pigment of the chloroplasts. The chlorophyll contains an asymetrical molecule which has hydrophilic head of four rings of the pyrols and hydrophobic tail of the phytol chain. Chemically the chlorophyll is a porphyrin like the animal pigment haemoglobin and cytochromes except besides the iron (Fe), it contains Mg atom in between the rings of the pyrols which remain connected with each other by the methyl groups. The chlorophyll consists of 75 per cent **chlorophyll** *a* and 25 per cent **chlorophyll** *b*.

The carotenoids are carotenes and xanthophylls, both of which are related to vitamin A. The carotenes have hydrophobic chains of unsaturated hydrocarbons in their molecules. The xanthophylls contain many hydroxy groups in their molecules.

DNA of chloroplast of *Chlamydomonas* represents non-chromosomal genetic system and has been found to be related with cytoplasmic heredity. **Ruth Sager**, who is pioneer on nonchromosomal genes, was able to prepare genetic map of *Chlamydomonas* chloroplast. She has shown that the genome of the chloroplast of *Chlamydomonas* is circular like that of bacteria.

Table 11-1. **Chemical composition of chloroplasts of higher plants.**

Chemical constituents	Per cent dry weight	Components (per cent)
1. Proteins	35–55	Insoluble 80%
2. Lipids	20–30	Fats 50%
		Sterols 20%
		Wax 16%
		Phosphatides 2–7%

Chemical constituents	Per cent dry weight	Components (per cent)
3. Carbohydrates	Variable	Starch, sugar, phosphates 3–7%
4. Chorophyll	9.0	Chlorophyll *a* 75% Chlorophyll *b* 25%
5. Carotenoids	4.5	Xanthophyll 75% Carotene 25%
6. Nucleic acids		
RNA	3–4	
DNA	< 0.02–.01	

ULTRASTRUCTURE

A chloroplast comprises the following three main components (Fig.11.3) :

1. Envelope

The entire chloroplast is bounded by an **envelope** which is made of a double unit membranes. Across this double membrane envelope occurs exchange of molecules between chloroplast and cytosol (cytoplasmic matrix). Isolated membranes of envelope of chloroplast lack chlorophyll pigment and cytochromes but have a yellow colour due to the presence of small amounts of carotenoids. They contain only 1 to 2 per cent of the total protein of the chloroplast.

2. Stroma

The matrix or stroma fills most of the volume of the chloroplasts and is a kind of gel-fluid phase that surrounds the thylakoids (grana). It contains about 50 per cent of the proteins of the chloroplast, most of which are soluble type. The stroma also contains ribosomes and DNA molecules (*i.e.*, 80 DNA molecules per chloroplast per cell of *Chlamydomonas*; 20 to 40 DNA molecules per chloroplast per cell of leaf of maize), both of which are involved in the synthesis of some of the structural proteins of the chloroplast. The stroma is the place where CO_2 fixation occurs and where the synthesis of sugars, starch, fatty acids and some proteins takes place.

3. Thylakoids

The thylakoids (thylakoid = sac-like) consists of flattened and closed vesicles arranged as a membranous network. The outer surface of the thylakoid is in contact with the stroma, and its inner surface encloses an **intrathylakoid space** (the third compartment). Thylakoids may be stacked like a neat pile of coins, forming **grana** or they may be unstacked, **intergranal**, or **stromal thylakoids**, forming a system of anastomosing tubules that are joined to the **grana thylakoids**. There may be 40 to 80 grana in the matrix of a chloroplast. The number of thylakoids per granum may vary from 1 to 50 or more. For example, there may be single thylakoid (*e.g.*, red alga), paired thylakoids (*e.g.*, Chrysophyta), triple thylakoids and multiple thylakoids (*e.g.*, green algae and higher plants) (Fig. 11.4).

Molecular Organization of Thylakoids

Molecular organization of the membrane of thylakoids is based on the fluid-mosaic model of the membrane which represents following main characteristics: **fluidity**, **asymmetry** and **economy** (*i.e.*, lack of movement in the third dimension). Lipids represent about 50 per cent of the thylakoid membrane; these include those directly involved in photosynthesis (called **functional lipids**) such as **chlorophylls**, **carotenoids** and **plastoquinones**. **Structural lipids** of thylakoids include glycolipids, sulpholipids and a few phospholipids. Most of these structural lipids are highly unsaturated which confer to the membrane of thylakoids a high degree of fluidity.

The protein components of thylakoid membrane are represented by 30 to 50 polypeptides which are disposed in the following five major supramolecular complexes (Fig. 11.5), which can be isolated with mild detergent :

1. Photosystem I (PS I). This complex contains a reactive centre composed of P700 (Type of pigment which is bleached at the wavelength of 700 nm), several polypeptides, a lower chlorophyll *a/b* ratio and β-carotene. It acts as a light trap and is present in unstacked thylakoid membranes. In it light induced reduction of $NADP^+$ takes place.

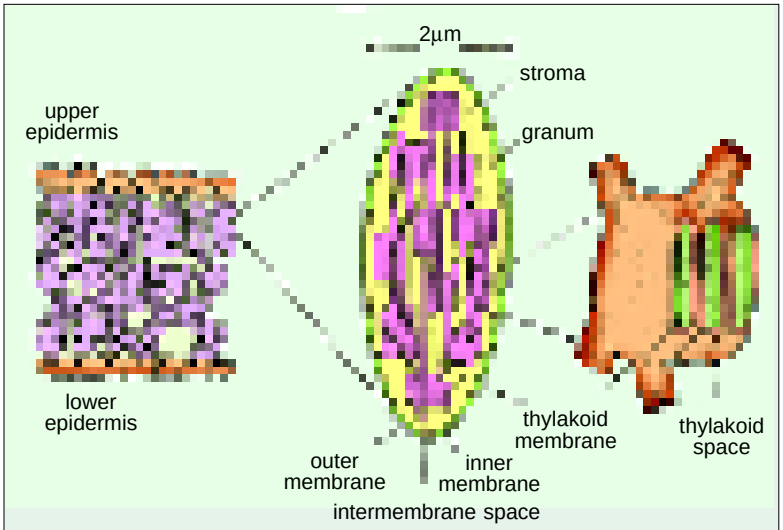


Fig. 11.3. A—Distribution of chloroplasts in mesophyll cells of a leaf; B—Ultrastructure of a chloroplast; C—Details of a granum (after Alberts *et al.*, 1989).

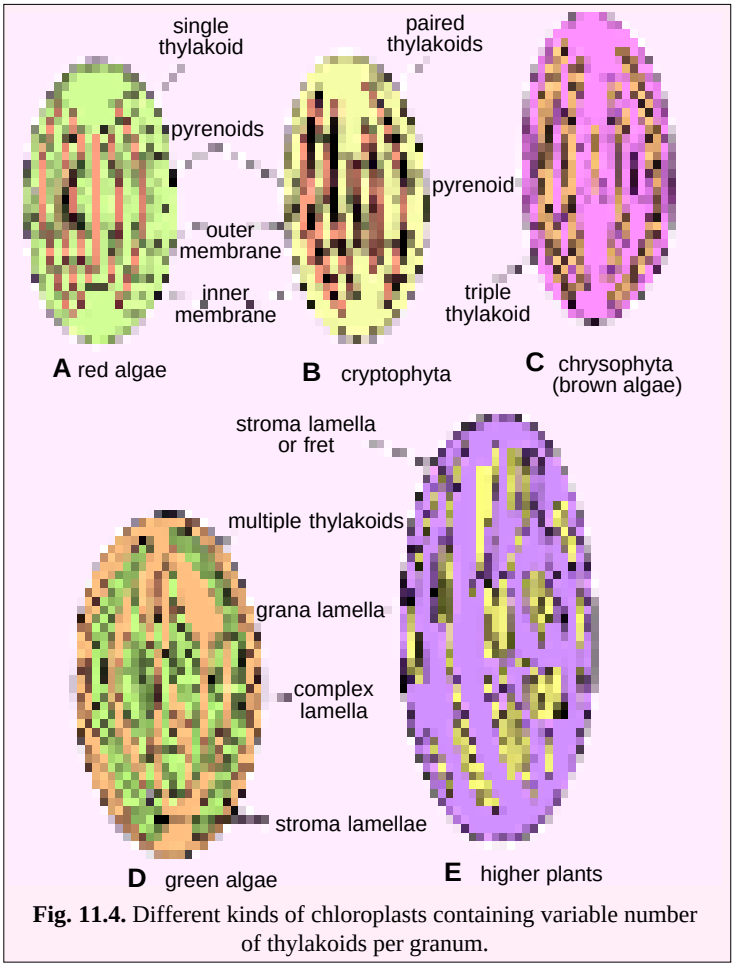


Fig. 11.4. Different kinds of chloroplasts containing variable number of thylakoids per granum.

2. Photosystem II (PS II). This complex comprises two intrinsic proteins that bind to the reaction centre of chlorophyll P680 (The pigment that bleaches when absorbing light at 680 nm). It contains a high ratio of chlorophyll *a/b* and β-carotene. Frequently, the PS IIs are associated with the light-harvesting complex and are involved in light induced release of O_2 from H_2O (*i.e.*, photolysis of water). PS II works as a light trap in photosynthesis and is mainly present in the stacked thylakoid membranes of grana.

3. Cytochrome b/f. This complex contains one cytochrome F, two cytochromes of b 563, one FeS centre and a polypeptide. It is uniformly distributed in the grana and acts as the electron carrier.

These three complexes are related to the electron transport and are linked by **mobile electron carriers** (*i.e.*, plastoquinone, plastocyanin and ferredoxin). Electron transport through PS II and PS I finally results in the reduction of the coenzyme $NADP^+$. Simultaneously, the transfer of protons from the outside to the inside of the thylakoid membrane occurs.

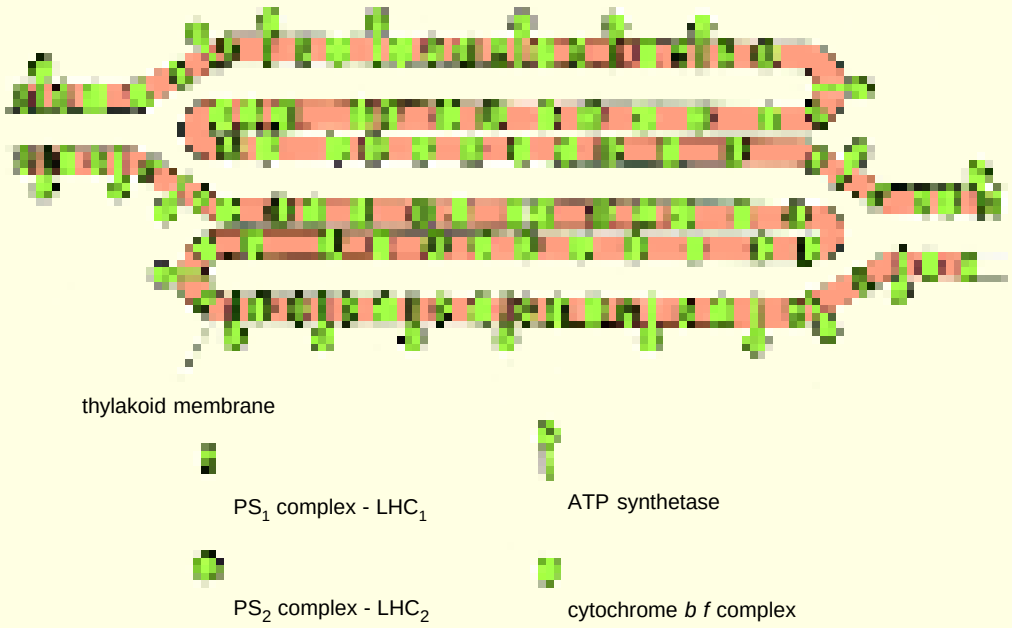


Fig. 11.5. Diagram showing the distribution of the main complexes within the thylakoid membranes both in the granal or stacked and stromal or unstacked regions (after De Robertis and De Robertis, Jr., 1987).

4. ATP synthetase. As in mitochondria, this complex consists of a **CF_0** hydrophobic portion, a proteolipid that makes a proton channel, and a **CF_1** (or coupling factor one) that synthesizes ATP from ADP and Pi, using the proton gradient provided by the electorn transport. ATP synthetase complexes are located in stacked membrane (grana).

5. Light harvesting complex (LHC). The main function of LH complex is to capture solar energy. It contains two main polypeptides and both cholorophyll *a* and *b*. LH complex is mainly associated with PS II, but may also be associated with PS I (**Anderson**, 1975). LHC is localized in stacked membranes and lacks photochemical activity.

Mutation and chloroplast structure. The organization of chloroplasts and other plastids is often modifed due to mutation. **D. Von Wettstein** (1956) reported that the plastids of normal barley plant have a well organized system of grana and stroma. But the plastids of an albino mutant of barley, fail to develop beyond a particular stage and there occurs no differentiation of grana and stroma. Further, the plastids of a yellow-green mutant of barley develop somewhat further than plastids of an albino plant.

FUNCTIONS OF THE CHLOROPLAST : PHOTOSYNTHESIS

It is well evident now that the process of photosynthesis consists of the following two steps:

1. **Light reaction**. It is also called **Hill reaction**, **photosynthetic electron transfer reaction** or **photochemical reactions.** In light reaction solar energy is trapped in the form of chemical energy of ATP and as reducing power in NADPH. During it, oxygen is evolved by photolysis or splitting of water molecule. Light reaction occurs in thylakoid membranes. 2. **Dark reaction**. It is also called **Calvin reaction**, **photosynthetic carbon reduction cycle** (**PCR cycle**), **carbon-fixation reaction** or

thermo-chemical reaction. In dark reaction, the reducing capacity of NADPH and the energy of ATP are utilized in the conversion of carbon dioxide to carbohydrate. Such a process of "**carbon fixation**" or "**CO_2-fixation**" occurs in the stroma of chloroplast (Fig. 11.6).

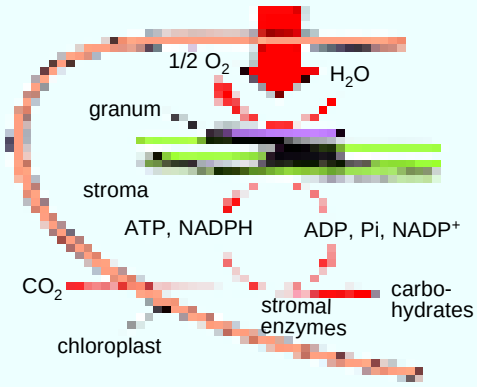


Fig. 11.6. Localization of the light and dark reaction of photosynthesis. The light reaction is catalyzed by chloroplast lamellae, especially in the grana. The dark reaction is catalyzed by enzymes of the stroma (after Dyson, 1978).

1. Light Reaction

The most important step of light reaction is harvesting of the maximum amount of solar energy for conversion into chemical energy. The photosynthetic light reaction is completed by passing through the following processes:

(i) Light absorption by photosynthetic pigment. Einstein suggested in 1905 that light and other electromagnetic radiations travel in discrete packets called **quanta** or **photons** and that when light interacts with matter it does so by annihilating complete photons, never a part of one. Further, according to Einstein's **photoelectric theory**, it takes one photon to eject one electron. Increasing the intensity of light, or flux of photons, only increases the number of electrons ejected, not their velocities. On the other hand, changing the wavelenght of light does change the velocity of ejected electrons, implying that the energy of a photon must be related to its wavelength.

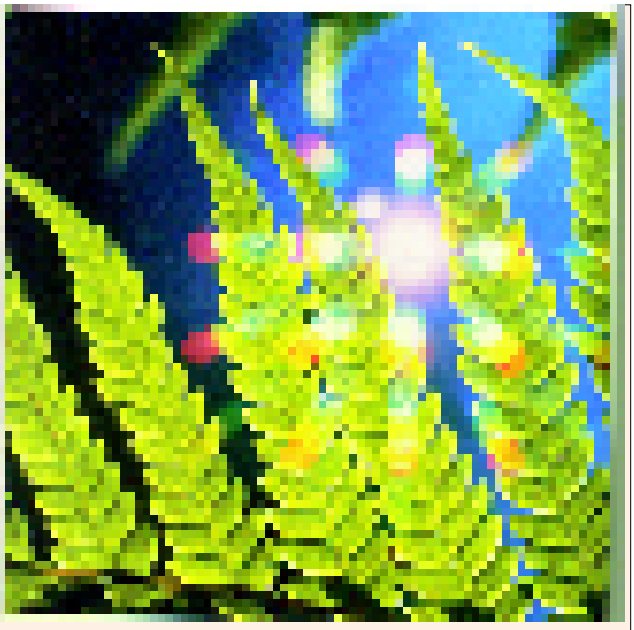
When a molecule absorbs a photon of light, it is absorbing a **quantum** of energy. Several things can happen to this energy : (i) It can be dissipated in molecular motion, manifest as heat. (ii) It can be reemitted as a new photon of light at a longer wavelength, with the shift representing losses to other processes—if remission occurs very quickly, it is called **fluorescence** ; if there is a long lag (milliseconds to seconds) between absorption and reemission, the process is called **phosphorescence**. (iii) The energy of light can cause a chemical change in the compound that absorbs it. It is this latter possiblility that can happen when a molecule of chlorophyll absorbs a photon. In fact, chloroplast acts as an energy transducer, *i.e.*, it can convert light energy to chemical energy, much as a solar battery uses light to run a transistor radio.

Within a fraction of a second after light is absorbed by a photosynthetic pigment, the molecule is altered ; some electrons associated with the pigment are raised to new energetic heights, changing their spin or modifying their position. If enough energy is absorbed, an electron may even be ejected and, thus, oxidation occurs. When these events happen, we say that the molecule is in an **excited state**.

Photosynthetic units. The basic photosynthetic units seem to be groups of roughly 300 pigment molecules located in the chloroplast membranes (thylakoid disc). Although all the pigment molecules in the unit (carotenoids, chlorophyll *b*, etc.) are capable of capturing light energy, they must transfer it to a single key chlorophyll *a* molecule called the **reaction centre**. The latter then loses an electron to a series of electorn carrier molecules. Thus, the other 299 accessory pigment molecules are referred to as **antenna molecules** or **antenna pigments**, to designate their role in the capture of light energy.

Dual pigment systems. Higher plants are found to have two types of photosynthetic units, associated with two different pigment systems, which absorb light of different wavelenghts. **Pigment system I** or **photosystem I (PS I)** units occur in the thylakoid membrane in the form of small and densely packed particles. Each PS I unit consists of about 200 molecules of chlorophyll *a* and 50 carotene molecules. The reaction centre (chlorophyll *a* molecule) is called **P 700** because it has a

maximum light absorption at 700 nanometers. Energy funneling into P 700 is responsible for the ejection of an electorn from the chlorophyll. **Pigment system II** or **photosystem II (PS II)** units occur in the thylakoid membrane in the form of larger, more widely spaced particles or ES particles (or quantosomes). Each PS II unit consists of approximately 200 molecules of chlorophyll *a*, 200 molecules of carotenols, chlorophyll *b*, and chlorophyll *c* or *d*, depending upon the species. Its reaction-centre chlorophyll *a* is designated **P 690** or **shorter-wavelength trap**. In the thylakoid membrane PS I and PS II are probably arranged near one another forming the so-called **Z-scheme** (**Hill** and **Bendall**, 1960) because they are functionally related ; excitation energy originating in one can be shunted to the other system. However, two photosystems are coupled chemically rather than through direct energy transfer.



A sunburst through the leaves of a fern depicts the harvesting of light energy by photosynthesis, the ultimate source of energy for nearly all life on earth.

(ii) Electron transport systems and oxidation of water. When the P680 of photosystem II acquires a sufficient quantum of energy, it emits a pair of electrons. These electrons with high potential

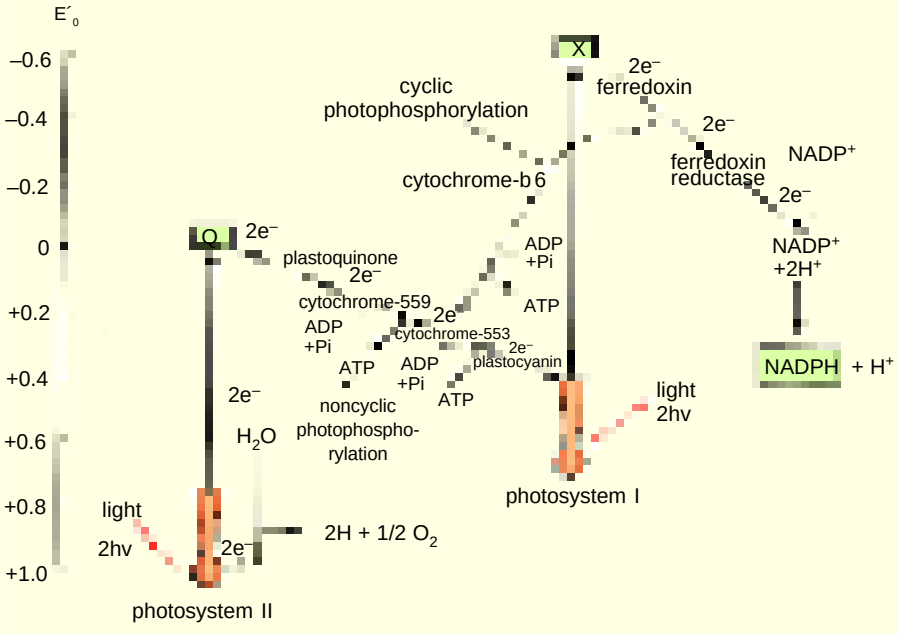


Fig. 11.7. Photosynthetic light reaction showing major events occurring in photosystem I and photosystem II of chloroplasts.

energy move down an electron transport chain (of thylakoid membrane) and during this process ATP molecules are formed (Fig. 11.7). Two electrons are passed through an electron acceptor **Q** (which is a quinone) to an electron transport chain involving four electron carriers (**plastoquinone**, **cytochrome-559**, **cytochrome-553** or **cytochrome f** and **plastocyanin**), before being passed on to PS I. The electrons are passed through four carriers successively at lower energy levels, so that at each step energy is released, which is harvested in the production of two ATP molecules (from ADP + Pi). The electron lost by P 680 is ultimately accepted by P700 of photosystem I. P700 (PS I) also captures light, and for absorbing each photon, it ejects an electorn. This electron is replaced by an electron from PS II and this flow of electron continues as long as the light is available. The electrons liberated from P700 are passed through acceptor x, to an electron transport chain (**ferredoxin**, **ferredoxin NADP reductase**) at successive lower energy levels. Finally these electorns reach NADP coenzyme, each molecule of which receives an electron, enabling it to pick up a H^+ ion (proton), thus, producing two molecules of NADPH from one molecule of H_2O used in PS II :

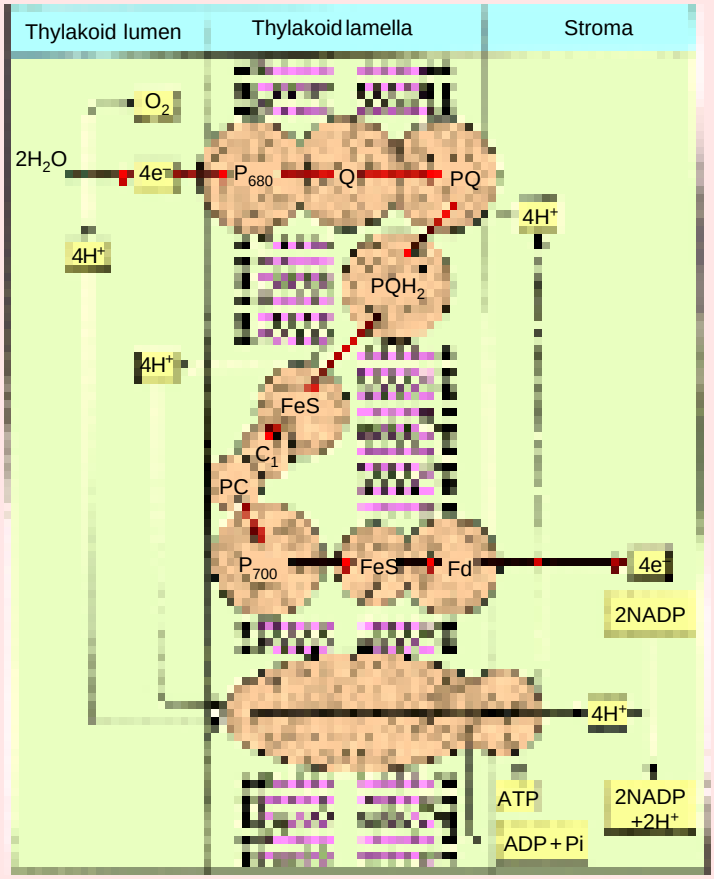


Fig. 11.8. Model for chemiosmotic coupling. Locations of electron transfer intermediates in the thylakoid lamella (membrane) and enzymes involved in ATP synthesis and proton (H^+) transport have been tentatively depicted (after Sheeler and Bianchi, 1987).

$$2 e^- + 2H^+ + 2NaDP^+ \xrightarrow{2hv} 2NADPH$$

The oxidized P680 regains its electrons by the photolysis of water into $2H^+$, $2e^-$ and oxygen:

$$H_2O \xrightarrow{2quanta\ or\ 2hv} \frac{1}{2} O_2 + 2H^+ + 2e^-$$

Oxygen is given out by photosynthesizing plants. The protons (H^+) accumulate inside the thylakoid membrane resulting in a **proton gradient**. The energy released by the protons when they diffuse across the thylakoid membrane into the stroma (along H^+ concentration gradient) is used to produce ATP molecules, by **CF0 - CF1 ATP synthetase** in the membrane. As synthesis of ATP occurs in light and the process is not cyclic (*i.e.*, it needs a constant supply of water molecules to be oxidized and NADP to be reduced), the process is called **non-cyclic photophosphorylation**.

ATP production by **cyclic photophosphorylation** also occurs during the light reaction. This process involves another electorn transfer mechanism involving **cytochrome b6** and starting with P700; the ultimate acceptor of the de-energized electron is also P700 of photosystem I.

All the molecules of ATP and NADP generated in the light reaction of photosynthesis are used by soluble enzymes of stroma of chloroplast during the dark reaction. Total light reaction can be summarized as follows :

$$H_2O + 2NADP + 2\ ADP + 2Pi \xrightarrow{4hv} \frac{1}{2} O_2 + 2\ NADPH + 2ATP$$

2. Dark Reaction

The dark reaction is completed by passing through following three main phases:

(i) Phase 1 : Carboxylation. During this phase of dark reaction, three molecules of carbon dioxide (3C) are attached to three molecules of **ribulose 1,5, biphosphate** (**RuBP** ; this pentose sugar was previously termed RuDP or ribulose diphosphate ; 15C) to produce short- lived six-carbon intermediates. This process is called **carboxylation** and is catalyzed by the enzyme **RuBP carboxylase**, **carboxydismutase** or **"Rubisco"** (which is widely acclaimed as one of the most abundant proteins present on the planet Earth; see **Alberts** *et al.*, 1989). Rubisco is a large protein molecule (500,000 dalton MW) comprising 16 per cent of chloroplast protein. The six carbon intermediates are immediately broken down into six molecules of **PGA** or **3- phosphoglyceric acid** (*i.e.* a C-3 or three carbon compound, 6C×3C = 18C).

Calvin with a student. The sequence of steps in the light independent stage was investigated by a team led by Melvin Calvin.

(ii) Phase 2 : Glycolytic reversal. By utilizing six ATP molecules, these six molecules of PGA are transformed into six molecules of **1,3, diphosphoglyceric acid** (*i.e.*, 6C × 3C = 18C ; 3C from $3CO_2$ and 15C from 3 RuBP). These in turn get converted into six molecules of **glyceraldehyde-3-phosphate**, **3- phosphoglyceraldehyde** (**PGAL**) or **3-phosphoglyceric acid** (triose) by utilizing six NADPH molecules.

(iii) Phase 3 : Regeneration of RuBP. For the continuous running of Calvin cycle, there must

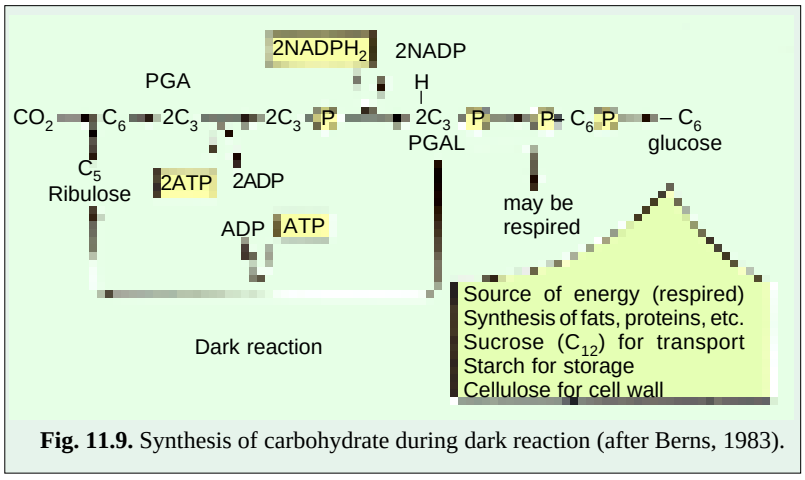


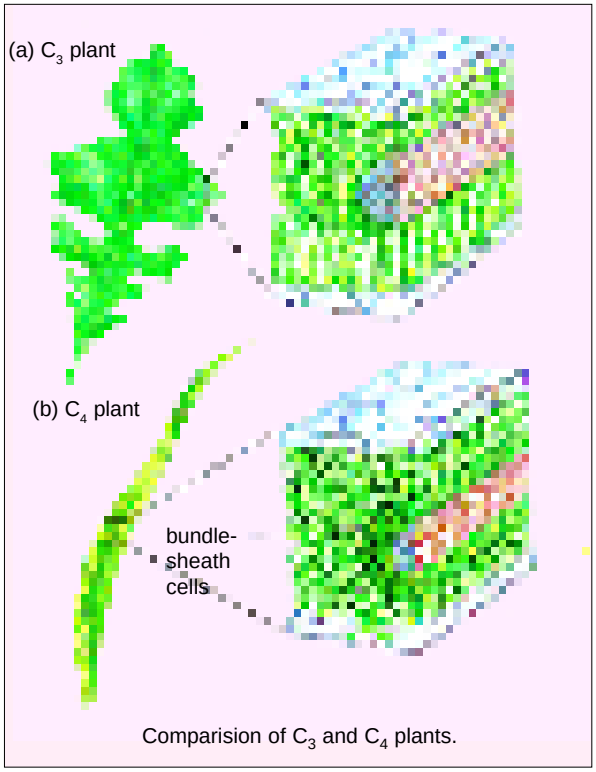
Fig. 11.9. Synthesis of carbohydrate during dark reaction (after Berns, 1983).

be a regular supply of ATP and NADPH and also sufficient amount of RuBP. Three molecules of RuBP (3C × 5C = 15C) are regenerated by a complex series of reactions which utilize three ATP molecules and five molecules of 3-phosphoglyceric acid (5C × 3C = 15C). This leaves one molecule of PGAL as the net gain of one Calvin cycle. Two turns of Calvin cycle result in the production of one molecule of **glucose**. This glucose is used by the plant to form a large variety of organic compounds required for its structure and function (*e.g.*, starch, cellulose, lipids, amino acids and proteins, etc.). The dark reaction may be summed up as follows :

$$6\,RuBP + 6CO_2 + 18\,ATP + 12\,NADPH \longrightarrow 6RuBP + C_6H_{12}O_6 + 18ADP + 18Pi + 12\,NADP$$

Hatch and Slack Pathway or C_4 Pathway of CO_2 - fixation in Angiosperms

In many angiosperms (*e.g.*, maize, sugarcane, sorghum) having Krantz anatomy (*i.e.*,bundle sheath with chloroplasts), an alternative pathway of CO_2 fixation occurs called **C_4 pathway of carbon dioxide fixation**. In the mesophyll cells of leaf in the presence of **PEP carboxylase enzyme**, the CO_2 is assimilated by carboxylation of **PEP** (a 3-carbon acceptor, called **phosphoenol pyruvate**) to produce a 4-carbon compound, called **oxaloacetic acid** or **OAA** (OAA is an intermediate in the Krebs cycle of respiration). OAA is converted into another intermediate of Krebs cycles, the **malic acid** (4C) or **aspartic acid** in some cases. This acid is then transferred (probably by diffusion) to the cells surrounding the vascular bundle, the **bundle sheath cells** having enzymes of Calvin cycle. Here, malic acid undrgoes decarboxylation to produce pyruvic acid (3C) and CO_2. CO_2 enters the Calvin cycle (or dark reaction) to produce a molecule of 3-phosphoglyceric acid. Sugars formed in Calvin cycle are transported into the phloem.



Comparision of C_3 and C_4 plants.

The pyruvic acid generated in the bundle sheath cells is transferred back to the mesophyll. It is converted to PEP by the

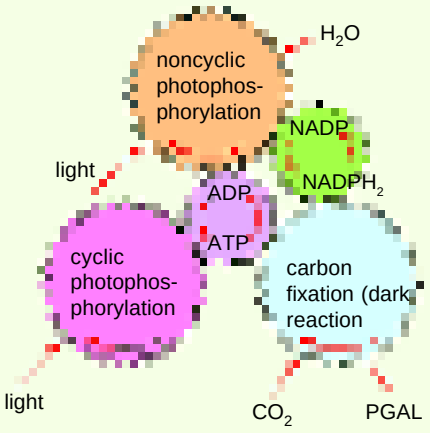


Fig. 11.10. Diagram showing relationship betwen photophosphorylation and carbon fixation. The entire photosynthetic process can be visualized as a series of interlocking gears, with the energy from light turning the two photophosphorylation gears. The turning of the cyclic-phosphorylation gear causes the gear of ATP synthesis to turn, and the turning of noncyclic-photophosphorylation gear causes both the ATP synthesis and NADPH- synthesis gears to turn. These two gears cause the carbon fixation gear to turn, with resultant production of carbohydrate (PGAL; triose) from CO_2 (after Berns, 1983).

expenditure of an ATP molecule. But because the conversion results in the formation of AMP (and not ADP), there remains a requirement of 2ATP for the regeneration of ATP from AMP.

The C-4 pathway is more energy-expensive than the C-3 pathway. While C-3 pathway requires 18 ATP for the synthesis of one molecule of glucose (*i.e.*, 9 ATP molecules per Calvin cycle: $9 \times 2 = 18$ ATP molecules), the C-4 pathway requires 30 ATP molecules (12 ATP more than the C-3 cycle). But realizing that many tropical plants would otherwise lose more than half of the photosynthetic carbon in photorespiration,the C-4 pathway is of adaptive advantage. Further, production of OAA in C-4 plants is a favourable step, since it permits closure of stomata and allows conservation of water.

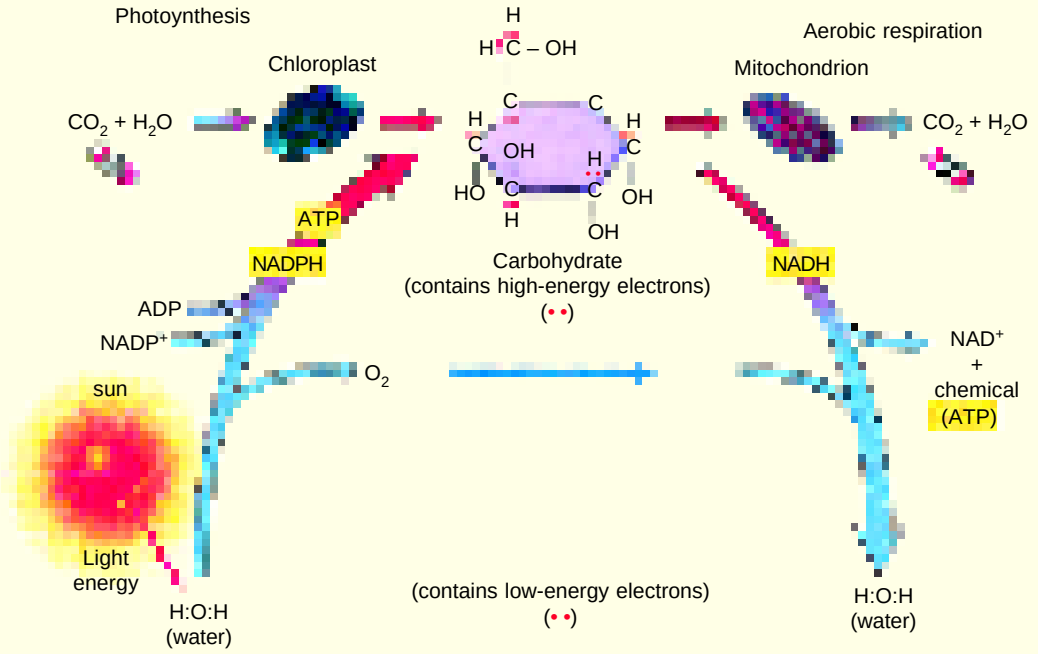
CHLOROPLAST AS SEMIAUTONOMOUS ORGANELLE

Like the mitochondria, the chloroplasts have their own DNA, RNAs and protein synthetic machinery and are semiautonomous in nature.

1. DNA of chloroplast. Recently the chloroplasts of the algae and higher plants are found to contain DNA molecules. First of all **Ris** and **Plant** (1962) have reported DNA molecule in the chloroplast of the *Chlamydomonas*. Later on DNA molecule has been reported from the chloroplasts of other algae and higher plants. In general, chloroplasts have a double helical DNA circle with an average length of 45 µm (about 135,000 base pairs). The replication of chloroplast DNA has been followed with ^{3}H-thymidine. Maps of the location of genes (genetic maps) have been made in several chloroplast DNAs by the help of restriction enzymes. The gene for the large subunit of carboxydismutase enzyme has been fully sequenced and is found to contain 1425 nucleotides.

2. Ribosomes of chloroplasts. The chloroplasts contain the ribosomes which are smaller than the cytoplsmic ribosomes. The ribosomes of the chloroplast are of 70S type and resemble with the bacterial ribosomes. The ribosomes of the chloroplasts consist of two ribosomal RNAs, 23S rRNA and 16S rRNA (**Stutz** and **Noll**, 1967, **Bager** and **Hamilton**, 1967). **Lyttleton** (1962) has also separated polyribosomes or polysomes from the chloroplast. The chloroplasts also contain aminoacyl-tRNAs, aminoacyl-tRNA synthetases, methionyl-tRNA.

3. Protein synthesis. According to most recent studies (see **Hall**, *et al.*, 1974). the DNA of chloroplast codes for chloroplast mRNA, rRNA, tRNA, and ribsomal proteins. It also codes for certain structural proteins of thylakoid membranes. The synthesis of other chloroplast components as chlorophyll, carotenoids, lipids and photosynthetic and starch synthesizing enzymes, is controlled by nuclear genes. The 70S ribosomes of *Euglena* chloroplast are found to require Mg^{++} for their stability and also have a requirement for N-formy1 methionyl-tRNA in chain initiation protein synthesis like the bacteria. The protein synthetic mechanism of chloroplasts is inhibited by chloramphenicol like that of mitochondria and bacteria (**Ellis**, 1969).



An overview of the energetics of photosynthesis and aerobic respiration.

The mode of synthesis of proteins of chloroplasts indicates towards their **semiautonomous** or **symbiotic nature**. For example, of the 30 known thylakoid polypeptides that function in photosynthesis, so far 9 have been demonstrated to be synthesized on chloroplastic ribosomes and 9 are coded by nuclear genes and synthesized on cytoplasmic ribosomes (**von Wettstein**, 1981). Synthesis of carboxydismutase (C Dase) presents a good case of cooperative action of two genetic systems (*i.e.*, chloroplastic and nuclear genetic systems). C Dase comprises 16 subunits : 8 subunits of high molecular weight (55,000 daltons) and 8 subunits of much smaller molecular weight (14,000 daltons). The large subunit is coded by genes present in chloroplastic DNA, while the small subunit is produced by nuclear genes. The small subunit (called P20) is synthesized as a precursor weighing 20,000 daltons on free ribosomes ; it then enters **post-translationally** into the stroma to be cleaved to attain its final size. It is postulated that the chloroplastic envelope has receptor sites that recognize the proteins that are to be incorporated into the organelle. The extra sequence (acting as the **signal**) that is present in P20 is composed of acidic amino acids, in contrast to the hydrophobic ones in the signal sequence of secretory proteins. After entering the chloroplast the signal sequences are removed by a protease enzyme, which is present in the envelope of chloroplast, and the small subunit of C Dase is released into the stroma (**Ellis**, 1981). Thus, chloroplast proteins may be synthesized by three avenues : (1) by an exclusive chloroplastic mechanism, (2) by a mechanism involving nuclear genes and chloroplastic ribosomes, and (3) by nuclear genes and cytoplasmic ribosomes.

Protein transport into chloroplasts resembles transport into mitochondria in many respects : both occur post-translationally, both require energy, and both utilize hydrophilic amino-terminal signal peptides that are removed after use. However, there is at least one important difference that while mitochondria exploit the electrochemical gradient across their inner membrane to help drive the transport, chloroplasts (which have an electrochemical gradient across their thylakoid but not their inner membrane) appear to employ only ATP hydrolysis to import across their double-membrane outer envelope.

Translocation of proteins into the thylakoid space of chloroplasts requires two signal peptides and two translocation events. The precursor polypeptide contains an amino-terminal chloroplast signal peptide followed immediately by a thylakoid signal peptide. The **chloroplast signal peptide** initiates translocation into the stroma through a membrane contact site by a mechanism similar to that used for translocation into mitochondrial matrix. The signal peptide is then cleaved off, unmasking the thylakoid signal peptide, which initiates translocation across the thylakoid membrane (Fig. 11.12).

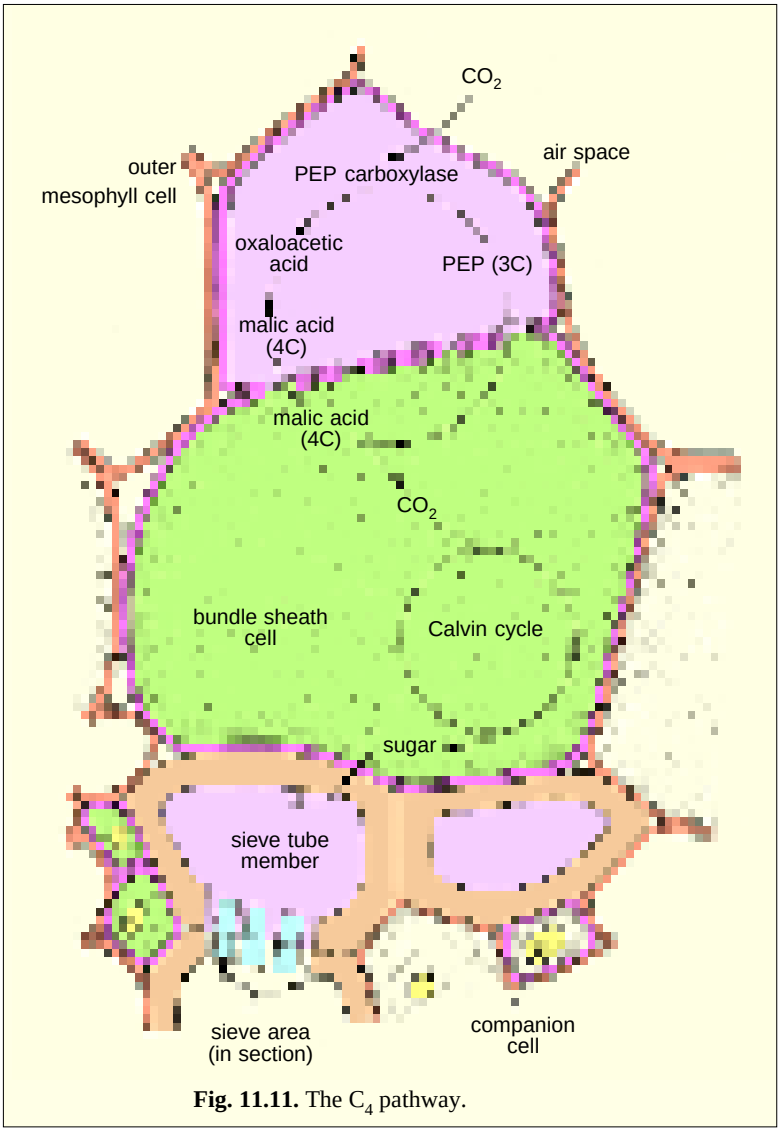


Fig. 11.11. The C_4 pathway.

BIOGENESIS OF CHLOROPLAST

The chloroplasts never originated *de novo*. Since the classic work of **Schimper** and **Meyer** (1883) it has been accepted that chloroplasts multiply by fission, a process that implies growth of the daughter organelles. This is easily observed in the alga *Nitella*, which contain a single huge chloroplast. In *Nitella* a division cycle of 18 hours has been cinematographically recorded for the chloroplast.

During the development of the chloroplast, the first structure to appear is the so-called **proplastid**, which has a double membrane. Development of proplastid into chloroplast takes place in the following steps :

1. In the presence of light, the inner membrane grows and gives off vesicles into the matrix that are transformed into discs (Fig. 11.13). These intrachloroplastic membranes are the thylakoids which, in certain regions, pile closely to form the grana. In the mature chloroplast the thylakoids are no longer connected to the inner membrane, but the grana remain united by intergranal thylakoids.

2. In the absence of light, a reverse sequence of changes takes place. This is the process of **etiolation**, in which the leaves lose their green pigment and the chloroplast membranes become disorganized. The chloroplasts are transformed into **etioplasts**, in which there is a paracrystalline

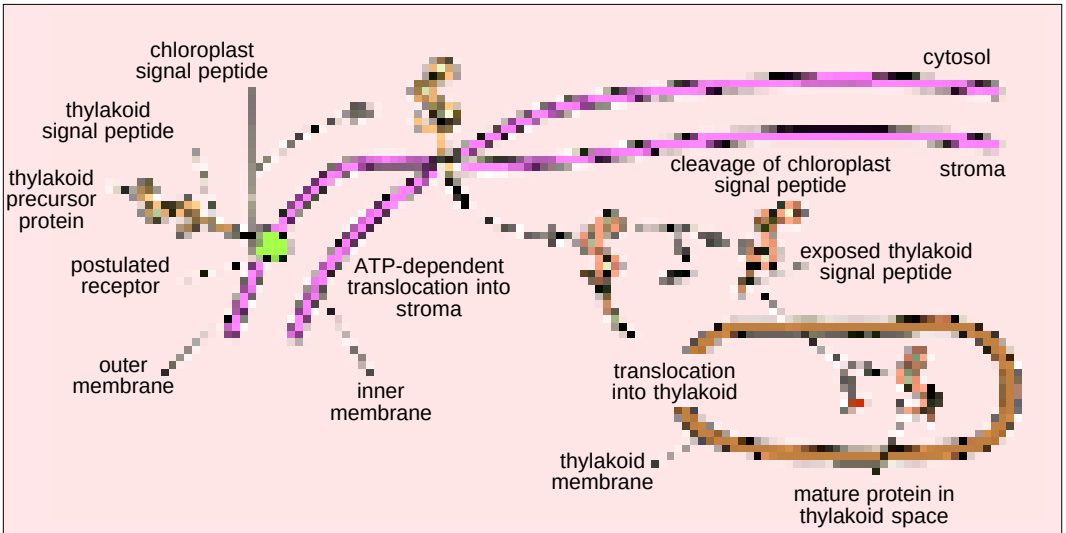


Fig. 11.12. Mode of translocation of proteins into stroma and thylakoid of chloroplast by the help of signal peptides (after Albert *et al*., 1989).

arrangement of tubules forming the so-called **prolamellar body**. Attached to these bodies are young thylakoid membranes that lack photosynthetic activity.

The regular crystal lattice of two prolamellar bodies surrounded by young thylakoid membranes is observed by **Osumi** *et al.*, (1984). If etiolated plants are re-exposed to light, thylakoids are reformed and the prolamellar material is used for assembly.

The symbiotic origin of the chloroplast. In certain characteristics, the chloroplasts are comparable with that of a semi-autonomous or symbiotic organism living within the plant cells. They divide, grow and differentiate; they contain circular DNA, ribosomal RNA, messenger RNA and are able to conduct protein synthesis. By visualizing these similarities between chloroplast and micro-organism, it has been suggested that chloroplast might have resulted from a symbiotic relationship between

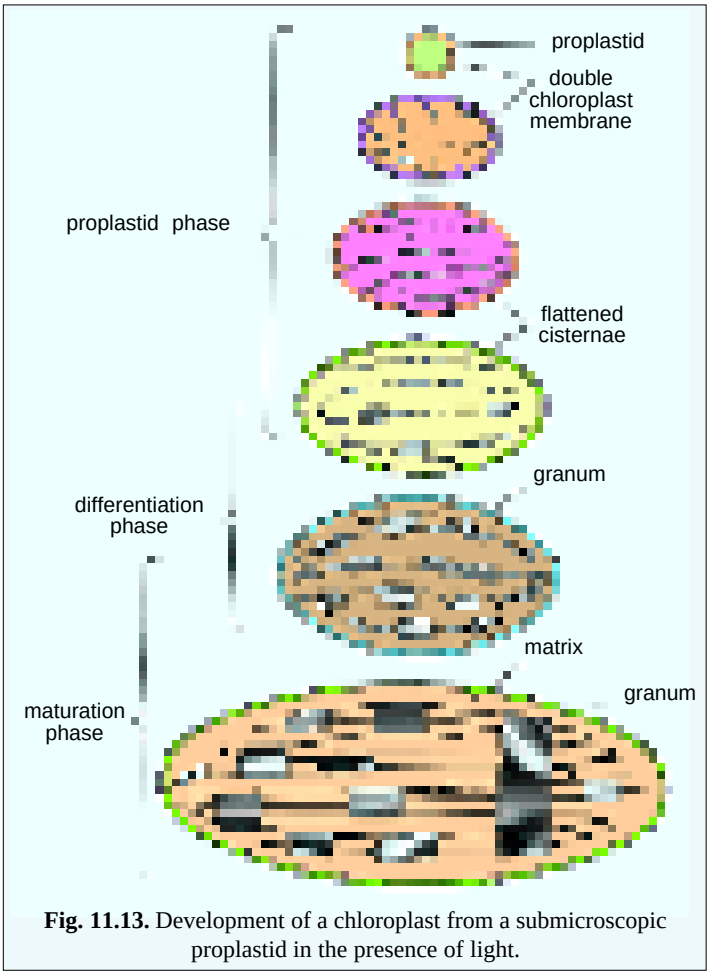


Fig. 11.13. Development of a chloroplast from a submicroscopic proplastid in the presence of light.

an autotrophic micro-organism, one which is able to transform radiant energy from sunlight and heterotrophic host cell. The symbiotic origin of the chloroplast appears very justified but **Kirk** (1966) has shown that certain important enzymes which are necessary for the development of the chlorophyll and for the photosynthetic mechanism are synthesized according to the codes of the nuclear DNA. There still exists certain doubt about the symbiotic origin of the chloroplast.

AMYLOPLASTS

Amyloplasts or starch granules are leucoplasts which lack any visible pigment and are involved in the synthesis and storage of various kinds of carbohydrates.

Structure and Function

The amyloplasts resemble proplastids and differ from them only in size. The contain less number of lamellae, but, can build up thylakoid structure found in chloroplast, in the presence of light. In the dark they synthesize and store starch. The conversion of glucose into starch is a chief characteristic of amyloplasts. However, chloroplasts can also synthesize starch and store it in stroma region. But, this starch in chloroplasts disappears quickly and is, therefore, known as **transitory starch**. The starch of amyloplasts on the other hand can be stored for longer periods and is, therefore, called **reserve starch**.

Origin of Amyloplasts

The starch development begins with the formation of a particle in the stroma. This particle consists of several tubuli and thylakoids and is surrounded by additional rings of starch. It grows till the amyloplast is filled with starch. Ultimately, the tubuli are pressed against the wall and gradually, starch granule exceeds the size of amyloplast. Finally, the membraneous wall of amyloplasts ruptures and withers away, so that only the starch granules can be seen.

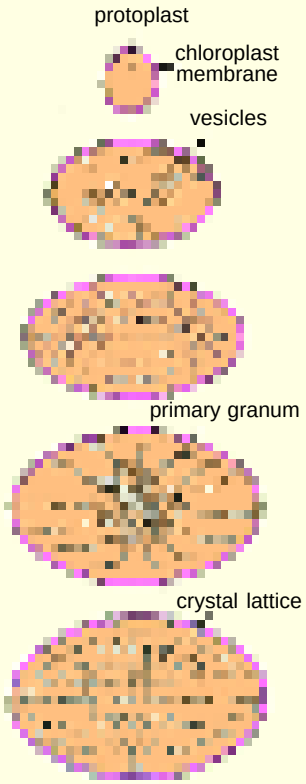


Fig. 11.14. Development of a chloroplast from submicroscopic proplastid in dark.

CHROMOPLASTS

The plastids which contain pigments other than chlorophyll are the chromoplasts. They can be originated from chloroplasts and also from leucoplasts. The common example of their derivation from chloroplasts can be observed in the petals which are green initially but become coloured subsequently. Similarly in carrot roots, chromoplasts are derived from leucoplasts as is clear from the fact that the roots have no colour in the beginning but become coloured at a later stage.

Chromoplasts impart colour to roots (Carrot), fruit (Tomato) and petals (Rose).

Structure and Function

Chromoplasts appear to be products of degeneration or disintegration of chloroplasts or leucoplasts. Their specific function in plant cell is still little understood. However, their presence in floweres definitely serves in attracting insects for pollination and propagation.

Origin of Chromoplasts

The chromoplasts can originate either from chloroplasts or from leucoplasts :

(i) When a chloroplast transforms into a chromoplast, it has been observed that some yellow coloured droplets called **globulins** appear in the former. In the course of development chlorophyll and starch of chlorplasts gradually decrease, large globuli are formed, the lamellar structure breaks down and stroma is disorganized. Ultimately, globuli are arranged along plastid membrane and the centre of the plastid appears empty due to disintegration of stroma.

(ii) During the transformation of leucoplasts into chromoplasts, certain fibrils appear which give rise to crystals filling up the whole plastids. These crystals are normally found in the form of sheet-like structures containing large quantities of carotenoids.

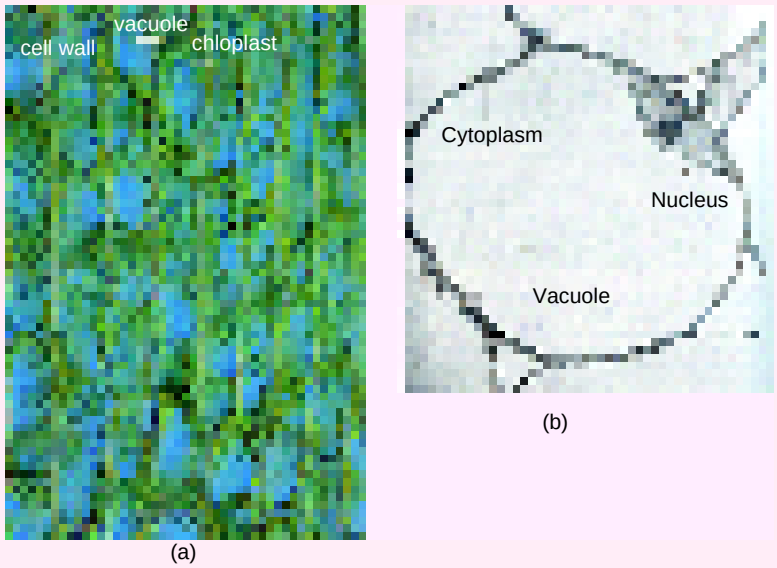
VACUOLES

The most conspicuous compartment in most plant cells is a very large, fluid-filled vesicle called a **vacuole**. There may be several vacuoles in a single cell, each separated from the cytoplasm by a single unit membrane, called the **tonoplast**. Generally vacuoles occupy more than 30 per cent of the cell volume; but this may vary from 5 per cent to 90 per cent, depending on the cell type. Conventionally, plant cell biologists do not consider the vacuole to be part of the cytoplasm ; they tend to consider only three parts in a plant cell : nucleus, vacuole and cytoplasm—the latter containing all the other membrane-bounded organelles, including the plastids.

In immature and actively dividing plant cells the vacuoles are quite small. These vacuoles arise initially in young dividing cells, probably by the progressive fusion of vesicles derived from the Golgi apparatus. They are structurally and functionally related to lysosomes in animal cells and may contain a wide range of hydrolytic enzymes. In addition, they usually contain sugars, salts, acids and nitrogenous compounds such as alkaloids and anthocyanin pigments. The pH of plant vacuoles may be as high as 9 to 10 due to large quantities of alkaline substances or as low as 3 due to the accumulation of quantities of acids (*e.g.*, citric, oxalic and tartaric acids).

Functions of Vacuoles

A plant vacuole has a variety of functions. It can act as a storage organelle for both nutrients and waste products ; as a lysosomal compartment (**Boller** and **Kende**, 1979), as a economical way of increasing the size of the cells, and as a con-



(a) (b)

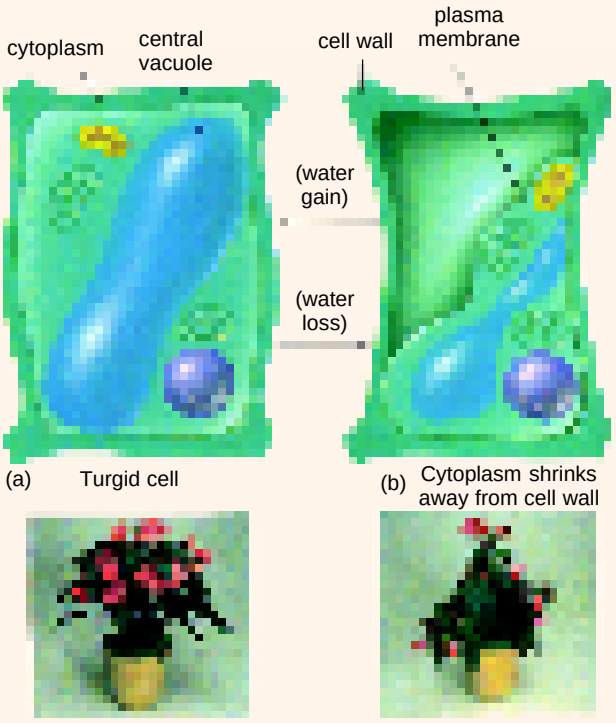
Plant cell vacuoles (a) Vacuoles seen in *Elodea* leaf (b) TEM of a cell showing vacuole.

troller of turgor pressure (To recall, turgor provides support for the individual plant cell and contributes to the rigidity of the leaves and younger parts of the plant). Different vacuoles with distinct functions (*e.g.*, lysosomal and storage) are often present in the same cell, we have already described the role of lysosomal vacuole, now, let us examine the storage function of a vacuole.

Storage functions of plant vacuoles. Plant vacuoles can store many type of molecules. In particular, they can sequester substances that are potentially harmful for the plant cell, if they are present in bulk in the cytoplasm. For example, the vacuoles of certain specialized cells contain such interesting products as rubber (in *Hevea brasiliensis*) or opium (in *Papaver somniferum*). Even Na+ ions are stored in these organelles, where their osmotic activity contributes to turgor pressure. Analysis of the giant cells of the alga *Nitella* indicates that **Na+ pumps** located in the tonoplast maintain low concentration of Na+ in the cytosol and four to five fold higher concentrations in the vacuole ; and since the vacuole occupies a much greater volume than the cytoplasm in *Nitella*, the greater bulk of cellular Na+ is in the vacuole.

The vacuole has an important **homeostatic function** in plant cells that are subjected to wide variations in their environment. For example, when the pH in the environment drops, the flux of H^+ into the cytoplasm is buffered by increased transport of H^+ into the vacuole. Similarly, many plant cells maintain turgor pressure at remarkable constant levels in the face of large changes in the tonicity of the fluids in their immediate environment. They do so by changing the somatic pressure of the cytoplasm and vacuole—in part by controlled breakdown and resynthesis of polymers such as **polyphosphate** in the vacuole, and in part by altering transport rate across the plasma membrane and the tonoplast. The permeability of these two membranes is partly regulated by turgor pressure and is determined by the distinct set of membrane transport proteins that transfer specific sugars, amino acids, and other metabolites across each lipid bilayer. The substances in the vacuole differ qualitatively and quantitatively from those in the cytoplasm. The tonoplast has little mechanical strength, however, the hydrostatic pressure must remain roughly equal in cytoplasm and vacuole, and two compartments must act together is osmotic balance to maintain turgor.

Some of the products stored by vacuoles have a **metabolic function**. For example, succulent plants open their stomata and take up carbon dioxide at night (when transpiration losses are less than in the day) and convert it to **malic acid**. This acid is stored in vacuoles until the following day, when light energy can be used to convert it to sugar while the stomata are kept shut. As a second example, proteins can be stored for years as



The central vacuole and turgor pressure in plant cells. (a) (top) when water is plentiful, it fills the central vacuole and help maintain the cell's shape (bottom). The pressure of water supports leaves (b)(top) when water is scarce, the central vacuole shrinks and cell becomes soft and shrunken (bottom) wilted plant.

reserves for future growth in the vacuoles of the storage cells of many seeds, such as those of peas and beans. When the seeds germinate, the proteins are hydrolyzed and the amino acids are mobilized to form a food supply for the developing embryo.

Other molecules stored in vacuoles are involved in the interactions of the plant with animals or with other plants. The anthocyanin pigments, for example, colour the petals of some flowers so that they attract pollinating insects. Other molecules defend the plant against predators. Noxious metabolites released from vacuoles, when the cells are eaten or otherwise damaged, range from poisonous alkaloids to unpalatable inhibitors of digestion. The **trypsin inhibitors** commonly found in seeds and the **wound induced protease inhibitors** of leaf cells (to inhibit both insect and microbial proteases), both accumulate in the vacuole and are presumably designed to interfere with the digestive processes of herbivores.

REVISION QUESTIONS

1. What is the chloroplst? Describe the ultrastructure of the chloroplast. What are some of the more obvious similarities and differences between chloroplasts and mitochondria?
2. Define the following : granum, thylakoid, chromoplast, leucoplast, proplastid.
3. What part of chloroplast is associated with the light reaction ? Where dark reaction takes place?
4. Define the following : photon; a quantum of energy ; fluorescence; photoelectric effect. What are the possible fates of an absorbed photon ?
5. What is the Calvin cycle, and what is its purpose? Describe it.
6. Write an essay on “chloroplast and photosynthesis.”
7. Write short notes on the following :

   (*i*) Chromatophores; (*ii*) Quantosome concept; (*iii*) Pyrenoid; (*iv*) Photosynthetic pigments of chloroplasts; (*v*) Dark reaction; and (*vi*) Origin of chloroplasts.
8. Describe the molecular organization of the thylakoids.
9. Write an account of structure and function of plant vacuoles.