2. The **stroma or matrix**. It occupies most part of the chloroplast. It represents a kind of gel-fluid phase and surrounds the thylakoids. Some 50 per cent of the chloroplast proteins are found in the stroma. It also comprises ribosomes, DNA, osmiophilic granules etc.

Osmiophilic granules are minute structures which are observed after impregnation with osmium salt and that is why these are called osmiophilic granules. These remain concerned with lipid metabolism such as biosynthesis of chlorophylls and carotenoids.

Ribosomes are found lying free in the matrix (stroma) region and these are 70S type, similar to those found in prokaryotic cells. These participate in protein synthesis within the chloroplast.

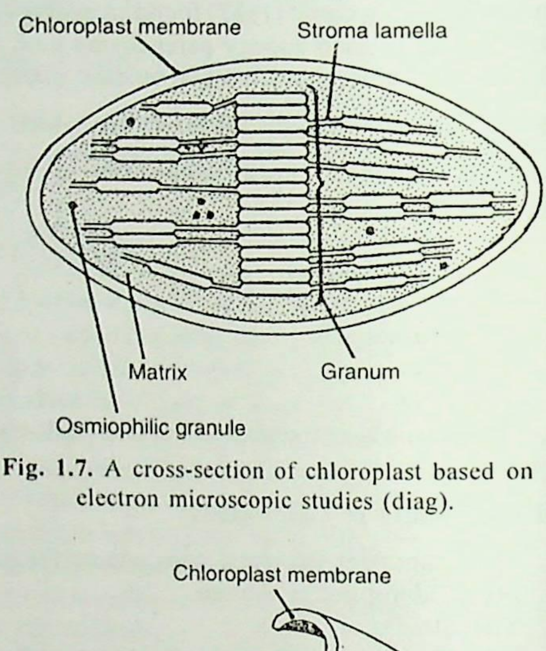

Fig. 1.7. A cross-section of chloroplast based on electron microscopic studies (diag).

DNA content in a chloroplast is about $4–8 \times 10^8$ daltons, and equal to that of bacteria. Likewise, it is circular and non-histone type (similar to prokaryotic DNA). About 15 to 30 molecules of DNA are found in each chloroplast. RNA molecules are also found lying free or with ribosomes.

3. The **thylakoids**. These consist of flattened vesicles arranged as a membranous network. The inner surface of a thylakoid encloses an **intra thylakoid space** and its outer the surface remains in contact with stroma. The thylakoids may be stacked like a pile of coins, forming **grana** or they may be unstacked, called **stroma thylakoids**. The stroma thylakoid comprises a system of anastomosing tubules that are joined to the grana thylakoids. The number of thylakoids in each granum may be from a few to 50 or more. The thylakoids contain about 50 per cent of the protein and all the components essential for photosynthesis.

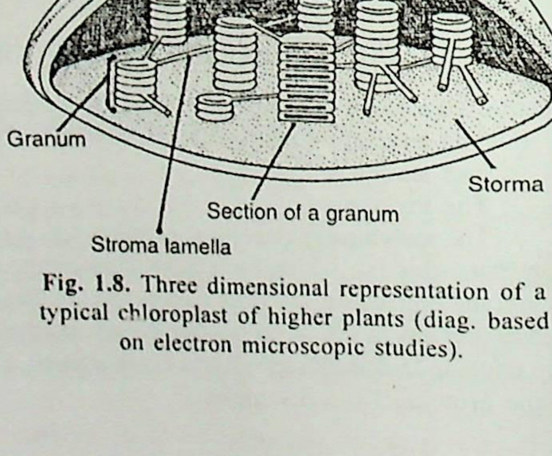

Fig. 1.8. Three dimensional representation of a typical chloroplast of higher plants (diag. based on electron microscopic studies).

Molecular Organisation of Thylakoids

Molecular organisation of thylakoids is largely based on the fluid lipid-protein mosaic model of the membrane.

Lipids represent about 50 per cent of the thylakoid membrane. Some of these are directly involved in photosynthesis, such as **chlorophylls, carotenoids** and **plastoquinones** and rest are structural lipids mainly as **glycolipids, sulfolipids** and a few **phospholipids**. Structural lipids are commonly highly unsaturated to confer the thylakoid membrane a higher degree of fluidity.

The **protein components** of the thylakoid membrane are represented by 30 to 50 polypeptides forming five major supra-molecular complexes as follows :

(1) **Photosystem I (PS I)**. It contains a reactive centre composed of P 700, several polypeptides, a lower chlorophyll **a/b** ratio (as compared to PS II), and β-carotene. PS I is found present in the unstacked membranes and it also acts as a light trap.

(2) **Photosystem II (PS II).** It contains two intrinsic proteins that bind to a reaction centre comprising chlorophyll P 680. It has a high ratio of chlorophyll **a** over **b** and β-carotene. PS IIs are associated with the light harvesting complex and work as a light trap in photosynthesis. This complex is found in the stacked membranes of the grana.

(3) **Cytochrome b/f**. This complex contains one cytochrome *f*, two cytochromes of b-563, one FeS centre and a polypeptide. This system is uniformly distributed in the grana.

PS I, PS II and Cyt b/f components are related to the electron transport and are linked by mobile electron carriers comprising plastoquinone, plastocyanin and ferredoxin. The electron transport through PS II and PS I finally results in the reduction of coenzyme $NADP^+$. Simultaneously, the transfer of protons from the outside to the inside of the thylakoid membrane occurs.

(4) **ATP synthetase**. This complex comprises a CF_0 hydrophobic portion, a proteolipid that makes a proton channel, and a CF_1 (coupling factor) that synthesises ATP from ADP and Pi (inorganic phosphate), using the proton gradient provided by the electron transport. This system resembles that of mitochondria.

(5) **Light harvesting complex**. Its main function is to capture solar energy. It contains two main polypeptides and both chlorophyll **a** and **b**. This complex is mainly present in stacked membranes and lacks photochemical activity. It is associated with PS II and may also be related to PS I.

Fine Structure of the Thylakoid Membrane

Fine structure of thylakoid membrane has been suitably studied by freeze fracturing and electron microscopy. Studies revealed that the thylakoid membrane consists of two leaflets, a protoplasmic (P) and an exoplasmic (E). Each leaflet has a true surface (S) and a fractured surface (F).

The thylakoid membrane appears as a smooth continuum, representing one half of the lipid bilayer, with particles of various sizes. These particles are believed to be protein aggregates that are asymmetrically localised within the lipid matrix. A large number of small particles are found in unstacked and stacked parts of PF. Relatively, a few but larger particles are found in the stacked region of EF and still lesser number of larger particles are found in the unstacked region of EF (*i.e.* unstacked intra-thylakoid space). Thus, smaller particles are nearly similarly distributed in both stacked (grana) and unstacked (stromal) region of PF whereas, larger particles are more concentrated in the stacked region.

It is believed that larger EF particles comprise complete PS II (*i.e.* PS II associated with light harvesting complex). The proportion of PS II in both stacked and unstacked regions of the thylakoid is the same as that of EF particles. Thus, the distribution of particles of photosystems and light harvesting complexes in a chloroplast show a heterogenous organisation.

While large EF particles are identified as PS II systems associated with light harvesting complexes, the nature of small PF particles is difficult to interpret but these may correspond to the other protein complexes. The figure 1.9 shows distribution of the main complexes within the thylakoid membrane both in stacked (granal) and unstacked (stromal) regions. It also reveals that *ATP synthetase* is only present in the stromal surface of the grana and the stromal thylakoids and the stacked regions contain the other three complexes.

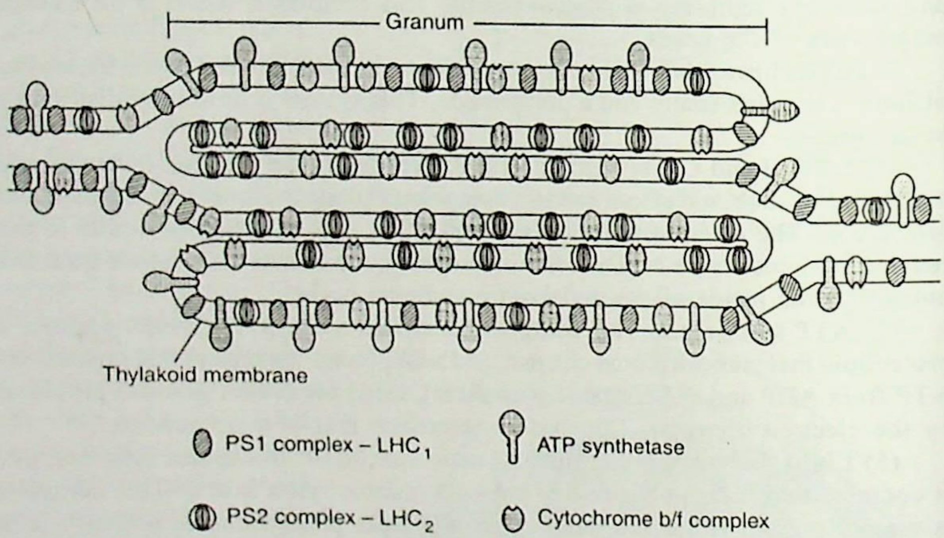

Fig. 1.9. Diagram showing the distribution of the main complex within the granal and stromal regions of the thylakoid membrane.

(C) Functions of Chloroplasts

Some important functions of the chloroplast are as follows:

1. These are the main sites of photosynthesis in which both light and dark reactions are found. Light reaction takes place in the grana region whereas dark reaction takes place in the stroma region.

2. The granum is the site for the production of assimilatory powers using light energy, *i.e.* reduction of NADP and photophosphorylation are found in this region.

3. It participates in photorespiration, and is the seat of glycolic acid production which functions as the substrate of photorespiration.

4. Some amount of protein synthesis takes place in the chloroplast which is used in its structural organisation. The last steps in the biosynthesis of enzyme RUBISCO take place in the chloroplast. The other major protein is associated with the *ATP synthetase* complex present in the thylakoid membrane.

5. It also carries out lipid metabolism to synthesise pigment molecules.

6. In certain cases, chloroplasts are converted into chromoplasts, such as in fruits of tomato.

7. The chloroplast has its own genetic system and is self replicating.

The genetic autonomy of a chloroplast and the occurrence of carbohydrate, lipid, protein and nucleic acid metabolisms within it has led many scientists to believe that the chloroplast is a **prokaryotic cell within an eukaryotic cell**, which during a long course of evolution has developed a symbiotic relationship because of their mutual interdependence. Chloroplasts receive water, CO_2, eyzymes of Calvin cycle and protection through the cytoplasm and in return supply the manufactured food material to the cell.

(D) Origin of Chloroplasts

Many hypotheses have been proposed but two are important.

(*i*) **Origin from proplastids.** The proplastids are fine globular double membrane particles, and during the course of their development, the formation of infoldings from the inner membrane occurs parallel to the surface. They are further differentiated into stroma and lamellae. Some biologists hold a strong opinion about the nuclear origin of plastids, like that of mitochondria. This fact leads us to consider the nuclear origin of plastids.

However, experiments on *Euglena* have contradicted this fact, in which case plastids can arise only from the pre-existing plastids, *i.e.* they are autonomous structures. These are cytoplasmically inherited from generation to generation.

(*ii*) **Autonomous origin.** This concept accommodates the fact that plastids are autonomous structures which have the potentiality for changing from one to other. However, the change may be reversible or irreversible. The sequence may be from initial proplastids to leucoplasts and chloroplasts and then into chromoplasts.

Leucoplasts and chloroplasts are considered as active stages of plastids while chromoplasts as the degenerated ones. Some cytologists consider chloroplast as semiautonomous organelle.

MITOCHONDRIA

Earlier mitochondria, as cytoplasmic particles, were known to Kalliker (1880), W. Flemming (1882) and Altman (1890) but the name mitochondria was given by C. Benda (1897) to these new structures. Mitochondria is a Greek word derived from *mitos* (means filament) and *chondros* (means granules). Since then, many names have been given to this organelle, *e.g.* bioplasts, chondriome, chondriosome, chondriocont etc. Their presence in plants was discovered by F. Meves (1904). They are found in every living cell except prokaryotic ones.

The **shape** of mitochondria is variable. Occasionally their shape is influenced by environment and physiological conditions. However, they may be granular, club-shaped, tennis-racket shaped, vesicular or rod-shaped. Like shape, the **size** also varies. The rod-shaped mitochondria are 0.5 μ to 2 μ but may attain a maximum length of 7μ. The mitocohondria, in general, show uniform distribution and orientation but are characteristic to the species in question. The number of mitochondria per cell also varies, *e.g.* in the cell of rat liver, 500, 1000 and sometimes 2500 mitochondria per cell have been reported. In Sea Urchin, the number varies from 14,000 to 1,50,000 per cell.

Structure of Mitochondria

A typical mitochondria is a sausage-shaped body of about 15,000 Å long and 5000 Å in diameter. The detailed structure of mitochondria has been studied with the help of an electron microscope. It remains externally bounded by two membranes, the outer and the inner membranes. Both membranes have a trilaminar (unit membrane) structure. Both membranes differ in composition and function.

The outer membrane is smooth and contains about 40 per cent lipid content (high lipid content) with lipid/protein ratio of 0.8. Most proteins are intrinisic type which appear to have channels for the passage of solutes. Such proteins are also called **porin** (the protein of pores). Porins are synthesised on free ribosomes and then integrated into the outer membrane.

The **inner membrane** shows a complex infolding projecting into the matrix. These foldings are called mitochondrial crest or cristae and on its inner surface (towards matrix) are found F_1 particles (also called elementary particles or oxysomes) of 8.5 nm. F_1 particles have a hollow stalk, 30 to 35 Å wide and 45 to 50 Å long and a rounded head, 75 to 80 Å dia. These are spaced at roughly 100 Å interval along the membrane and there are between 10^4 and 10^5 F_1 particles per mitochondrion.

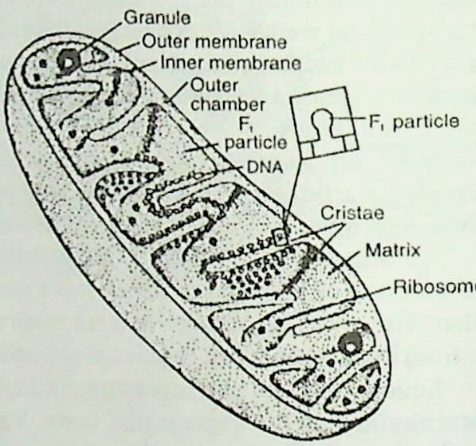

Fig. 1.10. A three-dimensional and cut open portion of mitchondrion.

The inner membrane has 0.3 lipid/protein ratio. It carries all components of the respiratory chain and the oxidative phosphorylation system. It also has several **carriers** or **translocators** for the permeation of phosphate, glutamate, aspartate, ADP and ATP. The respiratory chain represents about 20% and the phosphorylating system about 15% of the total protein content of the membrane.

Each mitochondrion has two compartments, the **inner** one filled with mitochondrial matrix surrounded by the inner membrane and the outer one located between the inner and outer membranes. A fairly large number of enzymes are found in the mitochondria. Some of them are found as the **constituents** of the membranes and others as **soluble** enzymes distributed in the compartments or the matrix.

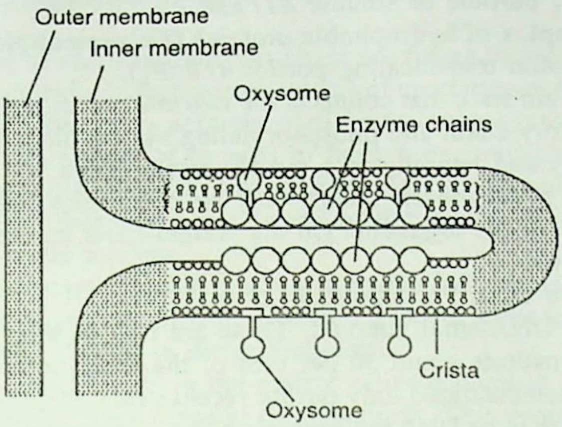

Fig. 1.11. The molecular and internal organisation of a portion of crista.

The **distribution of mitochondrial enzymes** may be summarised as follows:

Outer membrane - Monoamine oxidase, NADH-cyt. c reductase, Kynurenine hydroxylase and fatty acid CoA ligase.

Space between two membranes- Adenylate kinase and nucleoside diphosphokinase.

Inner membrane - Respiratory chain enzymes, ATP synthetase, Succinate dehydrogenase, β-hydroxybutyrate dehydrogenase, Carnitine fatty acid acyl transferase.

Matrix - Malate and isocitrate dehydrogenases, Fumarase and Aconitase, Citrate synthetase, α-keto acid dehydrogenases and β-oxidation enzymes.

The matrix also contains DNA, RNA, ribosomes and enzymes involved in nucleic acid metabolism and protein synthesis.

The **components of repiratory chains** are organised in the form of four complexes:

Complex I (NADH-Q-reductase). It consists of 15 subunits and contains FMN and six iron-sulphur centres. This complex is able to translocate protons across it (*i.e.* from matrix to cytosol side).

Complex II (succinate-Q-reductase). It consists of two polypeptides, FAD and three iron-sulphur centres. It is unable to translocate protons across the membrane.

Complex III (QH_2-cytochrome-c-reductase). It contains a number of subunits, cytochrome *b*, cytochrome c_1 and iron sulphur protein.

Complex IV (Cytochrome-c-oxidase). It consists of several polypeptides, two cytochromes (a and a_3) and two copper atoms. It shows transmembranal orientation helpful in vectorial transport of protons across the membrane.

The **phosphorylating system** is represented by the F_1-*ATPase* which is a multipeptide complex. It has three main parts:

(*i*) The F_1 particle or soluble *ATPase*.

(*ii*) A complex of hydrophobic proteins (*i.e.* proteolipids) which represent the proton translocating portion (*i.e.* F_0).

(*iii*) A protein stalk that connects the two and contains the coupling factors.

The respiratory chain and phosphorylating system have a fine topology that runs transversely and laterally in a mosaic arrangement.

Chemically, mitochondria mainly consists of proteins and lipids constituting about 90 to 99% of the organelle. On dry weight basis its chemical composition may be as follows:

Protein-65 to 70%, Phospholipids-25 to 30%

RNA-0.5%; DNA-small amount. These are rich in Mn.

Enzymes constitute about 30 per cent of the total protein. The presence of DNA has been demonstrated only during recent years. Depending upon the size, there may be one or more DNA molecules in a single mitochondrion. In *Neurospora*, yeast, *Phaseolus vulgaris*, mitochondria contain DNA molecules of larger size. The mitochondrial DNA differs from nuclear DNA in basic composition, conformations (circular and non-histone type) and genetic complexity and also in the larger number of cytosine-guanine base pairs. It has been found that mit. DNA is sufficient to code for about 25 polypeptide chains, but for structural proteins only. However, the protein present in cytochromes and other enzymes is synthesised under the control of the nucleus. A few biologists believe that mit. DNA represents a second genetic system in a cell. In its stroma or matrix region 70 S type of ribosomes are found lying free. In mammalian cells, 55 S type of ribosomes with subunits 25 S and 35 S are found in the mitochondria.

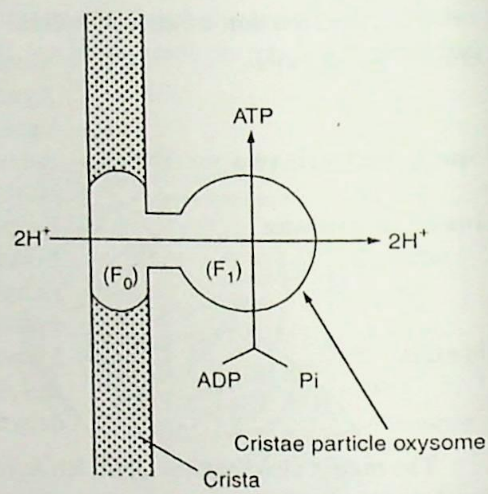

Fig. 1.12. ATP synthesis by inner membrane particle of mitochondrion.

Functions of Mitochondria

Following are important functions of mitochondria:

1. Christian Duve called mitochondria a power house of the cell as these are sources of readily available forms of energy to be used in various activities. The energy is in the form of ATP.

2. In mitochondria, the ATP molecules are formed during aerobic respiration (Krebs cycle). In it, the F_0-F_1 combination functions as *ATP synthetase* that catalyses ATP formation (Fig. 1.9).

3. The chemical reactions of Krebs cycle take place in the matrix of mitochondria, where in, 2 GTP molecules, per glucose molecule oxidation, are also formed in GTP mills. GTP are then converted into ATP.

4. These are the organelles of CO_2 release during respiration, thus increasing pollution.

5. The enzymes of electron transport chain are found in the inner membrane or cristae of mitochondrion. In fact, some proteins of inner membrane act as electron transporting enzymes and these actively participate in the synthesis of ATP and release of electrons in favour of oxygen to form water molecules.

6. Biogenesis of mitochondrial ribosomes (70 S type) takes place in the matrix of the mitochondria. Ribosomal proteins are synthesised in the cytosol (on ER) under the control of the nucleus.

7. Mitochondria also participate in **photorespiration** in C_3 plants. Photorespiration is the process in which carbon dioxide is released in the presence of light. In the process, NH_3 is also released in the mitochondria.

8. Glyoxylate cycle occurs in the matrix of mitochondria.

9. Protein synthesis takes place in mitochondria. This protein in used in its structural organisation. About 90% of the mitochondrial proteins are coded by nuclear genes.

10. Nucleic acid metabolism, *i.e.* replication (formation of new DNA) and transcription (formation of RNAs), are found in the mitochondria.

11. Each mitochondrion is capable of replicating itself either by budding or by constriction.

12. Mitochondria are also capable of changing their shape and sometimes may show movement within the cytoplasm.

Origin of Mitochondria

Some important views are as follows:

(*i*) **Autonomous Replication.** This concept believes that the mitochondria are autonomous organelles with independent hereditary action and living in the cell symbiotically. This would then represent a second genetic system in the cell. Of course, they pass on from one generation to another and in many cases their multiplication simultaneously with cell division has been observed. The concept may appeal, but its universal application is doubted. However, the budding of mitochondria is known in several cases. These may also arise by the formation of constriction of septum.

(ii) **Promitochondrial Origin.** Mitochondria arise from smaller particles called *promitochondria* which are present in a considerable number in the meristematic cells. With the growth of the cell, the promitochondrial particles increase in volume and their inner membrane starts forming folds at right angles to the surface. Later these folds become differentiated into cristae. Some biologists believe that promitochondria may arise from the nuclear envelope. Therefore, it would be more proper to say that mitochondria are of nuclear origin. Two views have been put forward to explain the mechanism of a nuclear origin. Firstly, the promitochondria may arise by means of evagination from nuclear membrane forming vesicles. These vesicles grow to give rise to promitochondria. Secondly, the promitochondria may arise from the organised folding of endoplasmic reticulum. The nuclear origin of mitochondria is further supported by Armentront *et al.* (1968). However, the problem is still disputed.