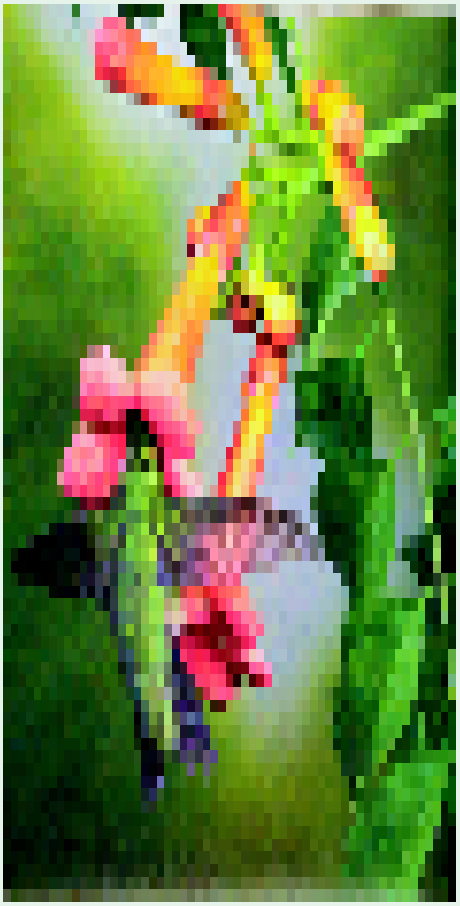
CHAPTER

10

Mitochondria



With wings beating 60 times per second, the ruby-throated hummingbird has a metabolic rate 50 times that of a human. The muscles of its wings are packed with mitochondria, which supply the ATP needed to meet the bird's energy demands.

The mitochondria (Gr., *mito*=thread, *chondrion* =granule) are filamentous or granular cytoplasmic or ganelles of all aerobic cells of higher animals and plants and also of certain micro-organisms including Algae, Protozoa and Fungi. These are absent in bacterial cells. The mitochondria have lipoprotein framework which contains many enzymes and coenzymes required for energy metabolism. They also contain a specific DNA for the cytoplasmic inheritance and ribosomes for the protein synthesis.

HISTORICAL

The mitochondria were first observed by **Kolliker** in 1850 as granular structures in the striated muscles. In 1888, he isolated them from insect muscles (which contain many slab-like mitochondria ; Fig. 10.1) and showed that they swelled in water and contain a membrane around them. In 1882, **Flemming** named them as **fila**. **Richard Altmann** (1890) developed a specific stain that had useful specificity for the mitochondria. He named this organelle, the **bioblast**. Altmann correctly speculated that bioblasts were autonomous elementary living particles that made a genetic and metabolic impact on the cells. The present name mitochondria was assigned by **Benda** (1897–98) to them. He stained mitochondria with alizarin and crystal violet. **Michaelis** (1900) used the supravital stain Janus green to demonstrate that mitochondria were oxidation-reduction sites in the cell. In 1912, **Kingsbury** suggested that the oxidation reactions mediated by mitochondria were normal cellular processes. **Otto Warburg** (1883–1970), who is considered as 'the father of respirometry', in 1910 isolated mitochondria ("large granules") by low-speed centrifugation of tissues disrupted by grinding.

He showed that these granules contained enzymes catalyzing oxidative cellular reactions.

Various steps of **glycolysis** were discovered by two German biochemists **Embden** and **Meyerhof** [**Gustav G. Embden** (1874–1933); **Otto F. Meyerhof** (1884–1951)]. Meyerhof got Nobel Prize in 1922 along with English biophysicist **A.V. Hill**, for the discovery of oxygen and metabolism of lactic acid in muscle (*i.e.*, production of heat in muscle). **Lohmann** (1931) discovered **ATP** in muscle. **Lipmann** (German biochemist in U.S. ; born 1899) discovered **coenzyme A** and showed its significance in intermediary metabolism. In 1941, he introduced the concept of "high energy phosphates" and "high energy phosphate bonds" (*i.e.*, ATP) in bioenergetics. **Warburg** linked the phenomenon of ATP formation to the oxidation of glyceraldehyde phosphate. **Meyerhof** showed the formation of ATP from phosphopyruvate and **Kalckar** related oxidative phosphoryalation to respiration. **Sir Hans Adolph Krebs** (German biochemist in England ; born 1900), in 1937, worked out various reactions of the **citric acid cycle** (or tricarboxylic acid or TCA cycle). His contribution was remarkable, because, up to that time radioactively labelled compounds were not available for biological studies and cellular sites of the reactions were not known with certainty. Krebs received the Nobel Prize in 1953 along with Lipmann for his discovery of the citric acid cycle.

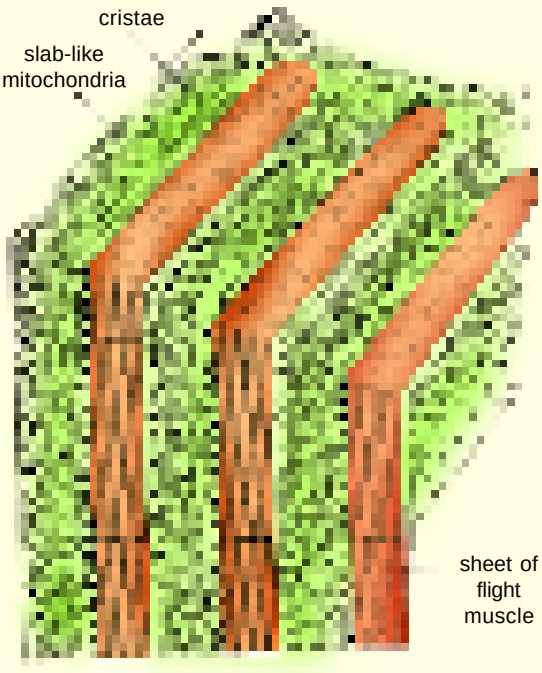


Fig. 10.1. Mitochondria of the flight muscle of a dragon fly, showing profuse cristae.

Kennedy and **Lehninger** (1948–1950) showed that the citric acid cycle (Krebs cycle), oxidative phosphorylation and fatty acid oxidation took place in the mitochondria. In 1951, **Lehninger** proved that oxidative phosphorylation requires electron transport. Among these early investigators of ETS the Nobel Prize recipients were **Warburg**, **Szent-Gyorgyi** and **Kuhn**. In 1961, **Mitchell** proposed the highly acclaimed "**chemiosmotic-coupling hypothesis**" for the ATP-production in mitochondria. He got the Nobel Prize in 1978 for the development of this model.

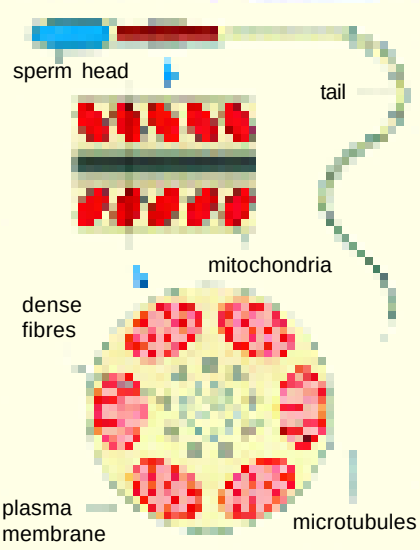
Palade (1954) described the ultra structure of cristale. In 1963, **Nass** and **Nass** demonstrated the presence of **DNA fibres** in the matrix of mitochondria of embryonic cells. **Attardi**, **Attardi** and **Aloni** (1971) reported the 70S type ribosomes inside the mitochondria.

Previously the mitochondria have been known by various names such as **fuchsinophilic granules**, **parabasal bodies**, **plasmosomes**, **plastosomes**, **fila**, **vermicules**, **bioblasts** and **chondriosomes**.

DISTRIBUTION OR LOCALIZATION

The mitochondria move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells their distribution is very restricted. The distribution and number of mitochondria (and also of mitochondrial cristae) are often correlated with type of function the cell performs. Typically mitochondria with many cristae are associated with mechanical and osmotic work situations, where there are sustained demands for ATP and where space is at a premium, *e.g.*,

between muscle fibres, in the basal infolding of kidney tubule cells, and in a portion of inner segment of rod and cone cells of retina. Myocardial muscle cells have numerous large mitochondria called **sarcosomes**, that reflect the great amount of work done by these cells. Since the work of hepatic cells is mainly biosynthetic and degraditive, and work locations are spread throughout the cell, in these cells, it may be more efficient to have a large number of "low key" sources of ATP production distributed throughout the cell. Often mitochondria occur in greater concentrations at work sites, for example, in the oocyte of *Thyone briaeus*, rows of mitochondria are closely associated with RER membranes, where ATP is required for protein biosynthesis. Mitochondria are particularly numerous in regions where ATP-driven osmotic work occurs, *e.g.*, brush border of kidney proximal tubules, the infolding of the plasma membrane of dogfish salt glands and Malpighian tubules of insects, the contractile vacuoles of some protozoans (*Paramecium*). Non-myelinated axons contain many mitochondria that are poor ATP factories, since each has only a single crista. In this case, there is a great requirement for **monoamine oxidase**, an enzyme present in outer mitochondrial membrane that oxidatively deaminates monoamines including neurotransmitters (acetylcholine).



A cross-section of the tail of a human sperm showing the '9 + 2' arrangement of microtubule and mitochondria.

ORIENTATION

The mitochondria have definite orientation. For example, in cylindrical cells the mitochondria usually remain orientated in basal apical direction and lie parallel to the main axis. In leucocytes, the mitochondria remain arranged radially with respect to the centrioles. As they move about in the mitochondria form long moving filaments or chains, while in others they remain fixed in one position where they provide ATP directly to a site of high ATP utilization, *e.g.*, they are packed between adjacent myofibrils in a cardiac muscle cell or wrapped tightly around the flagellum of sperm (Fig.10.2).

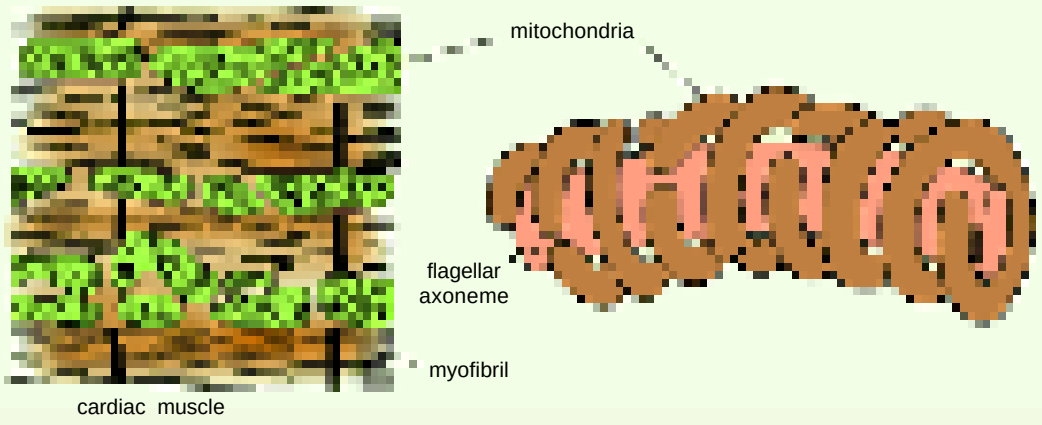


Fig. 10.2. Location of mitochondria near high ATP utilization : A— In cardiac muscles; B— In sperm tail (after Alberts *et al.*, 1989).

MORPHOLOGY

Number. The number of mitochondria in a cell depends on the type and functional state of the cell. It varies from cell to cell and from species to species. Certain cells contain exceptionally large number

of the mitochondria, *e.g.*, the *Amoeba*, *Chaos chaos* contain 50,000; eggs of sea urchin contain 140,000 to 150,000 and oocytes of amphibians contain 300,000 mitochondria. Certain cells, *viz.*, liver cells of rat contain only 500 to 1600 mitochondria. The cells of green plants contain less number of mitochondria in comparison to animal cells because in plant cells the function of mitochondria is taken over by the chloroplasts. Some algal cells may contain only one mitochondrion.

Shape. The mitochondria may be filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape. Mitochondria are granular in primary spermatocyte or rat, or club-shaped in liver cells (Fig.10.3).

Time-lapse microcinematography of living cells shows that mitochondria are remarkably mobile and plastic organelles, constantly changing their shape. They sometimes fuse with one another and then separate again. For example, in certain euglenoid cells, the mitochondria fuse into a reticulate structure during the day and dissociate during darkness. Similar changes have been reported in yeast species, apparently in response to culture conditions (see **Reid** and **Leech** 1980).

Size. Normally mitochondria vary in size from 0.5 µm to 2.0 µm and, therefore, are not distinctly visible under the light microscope. Sometimes their length may reach up to 7 µm.

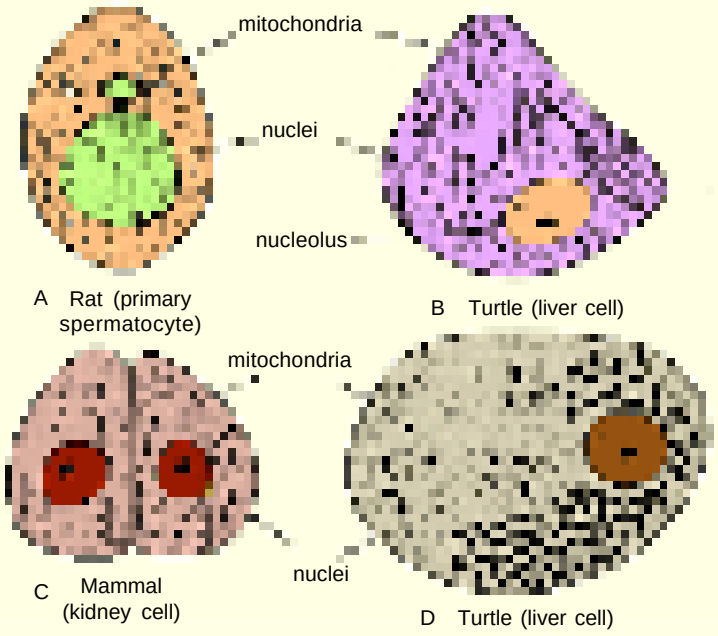


Fig.10.3. Mitochondria of different type of animal cells.

Structure. Each mitochondrion is bound by two highly specialized membranes that play a crucial part in its activities. Each of the mitochondrial membrane is 6 nm in thickness and fluid-mosaic in ultrastructure. The **outer membrane** is quite smooth and has many copies of a transport protein called **porin** which forms large aqueous channels through the lipid bilayer. This membrane, thus, resembles a sieve that is permeable to all molecules of 10,000 daltons or less, including small proteins. Inside and separated from the outer membrane by a 6–8 nm wide space is present the **inner membrane** (Fig.10.4). The inner membrane is not smooth but is impermeable and highly convoluted, forming a series of infoldings, known as **cristae**, in the matrix space.

Thus, mitochondria are double membrane envelopes in which the inner membrane divides the mitochondrial space into two distinct chambers : 1. The **outer compartment, peri-mitochondrial space** or the **inter-membrane space** between outer membrane and inner membrane. This space is continuous into the core of the crests or cristae. 2. The **inner compartment**, **inner chamber** or **matrix space**, which is filled with a dense, homogeneous, gel-like proteinaceous material, called **mitochondrial matrix**. The mitochondrial matrix contains lipids, proteins, circular DNA molecules, 55S ribosomes and certain **granules** which are related to the ability of mitochondria to accumulate ions. Granules are prominent in the mitochondria of cells concerned with the transport of ions and water, including kidney tubule cells,

epithelial cells of the small intestine, and the osteoblasts of bone-forming cells. Further, the inner membrane has an outer **cytosol** or **C face** toward the perimitochondrial space and an inner **matrix** or **M face** toward matrix.

In general, the cristae of plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like (**Hall**, **Flowers** and **Roberts**, 1974), but, in many Protozoa and in steroid synthesizing tissues including the adrenal cortex and corpus luteum, they occur as regularly packed tubules (**Tyler**, 1973). The cristae greatly increase the area of inner membrane, so that in liver cell mitochondria, the cristae membrane is 3–4 times greater than the outer membranre area. Some mitochondria, particularly those from heart, kidney and skeletal muscles have more extensive cristae arrangements than liver mitochondria. In comparison to these, other mitochondria (*e.g.*, from fibroblasts, nerve axons and most plant tissues) have relatively few cristae. For example, mitochondria in epithelial cells of carotid bodies (or **glomus carotica** which are chemoreceptors, sensitive to changes in blood chemistry and lie near the bifurcations of carotid arteries) have only four to five cristae and mitochondria from non-myelinated axons of rabbit brain have only a single crista.

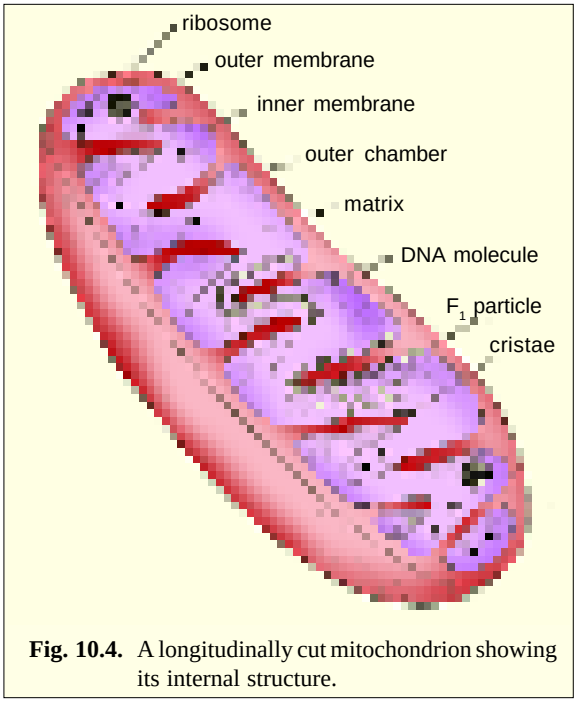


Fig. 10.4. A longitudinally cut mitochondrion showing its internal structure.

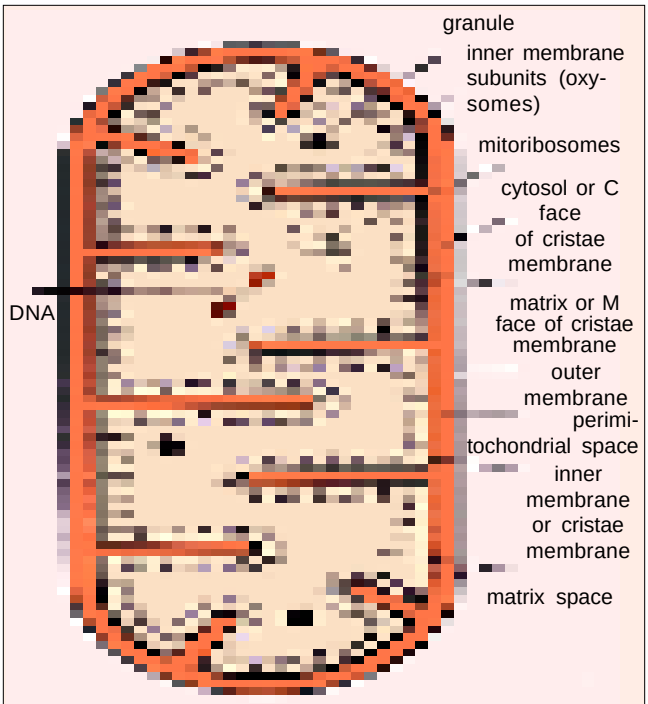


Fig. 10.5. A mitochondrion in sectional view to show its numerous oxysomes.

Attached to M face of inner mitochondrial membrane are repeated units of stalked particles, called **elementary particles**, **inner membrane subunits** or **oxysomes** (Fig.10.5). They are also identified as **F_1 particles** or **F_0-F_1 particles** and are meant for ATP synthesis (phosphorylation) and also for ATP oxidation (*i.e.*, acting as ATP synthetase and ATPase) (**Racker**, 1967). F_0-F_1 particles are regularly spaced at intervals of 10 nm on the inner surface of inner mitochondrial membrane. According to some estimates, there are 10^4 to 10^5 elementary particles per mitochondrion. When the mitochondrial cristae are disrupted by sonic vibrations or by detergent action, they produce **submitochondrial vesicles** of inverted orientation. In these vesicles, F_0-F_1 particles are seen attached on their outer surface (Fig.10.6). These submitochondrial vesicles are able to per-

form respiratory chain phosphorylation. However, in the absence of F_0-F_1 particles, these vesicles lose their capacity of phosphorylation as shown by **resolution** (*i.e.*, removal by urea or trypsin treatment) and **reconstitution** of these particles (Fig.10.6).

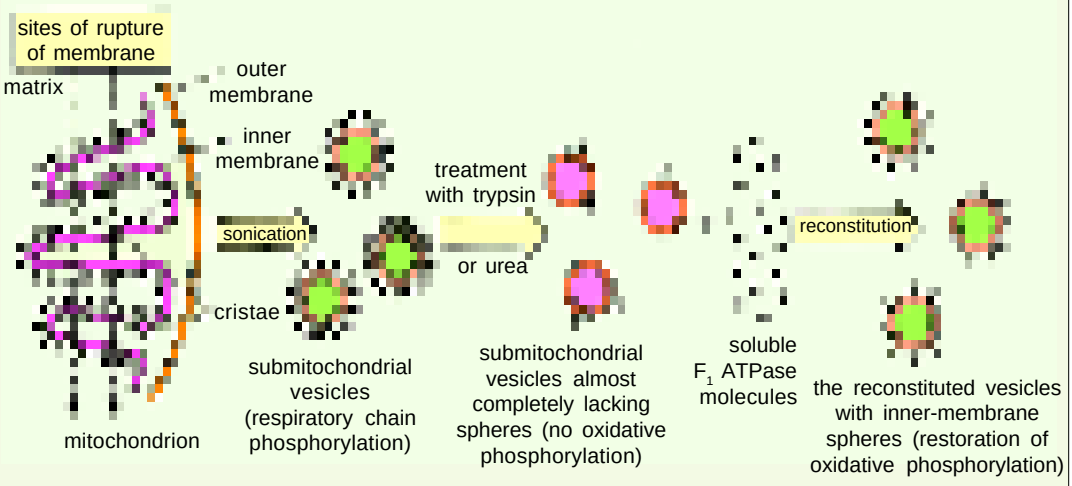


Fig. 10.6. Method of formation of small mitochondrial vesicles having oxysomes on their surface. Experimental results of resolution and reconstitution of oxysomes also have been shown (after Sheeler and Bianchi, 1987).

ISOLATION

Mitochondria have been studied by following three types of methods :

1. Direct Observation of Mitochondria

The examination of mitochondria in living cells is somewhat difficult because of their low refractive index. However, they can be observed easily in cells cultured *in vitro*, particularly under darkfield illumination and phase contrast microscope. Such an examination has been greatly facilitated by colouration with vital stain **Janus green** which stains living mitochondria greenish blue due to its action with cytochrome oxidase system present in the mitochondria. This system maintains the vital dye in its oxidized (coloured) state. In the surrounding cytoplasm the stain is reduced to a colourless leukobase.

Fluorescent dyes (*e.g.*, rhodamine 123), which are more sensitive, have been used in isolated mitochondria and intact cultured cells. Such stains are more suitable for *in situ* metabolic studies of mitochondria.

2. Cytochemical Marking of Mitochondrial Enzymes

Different parts of mitochondria have distinct marker enzymes for histochemical markings, such as **cytochrome oxidase** for inner membrane, **monoamine oxidase** for outer membrane, **malate dehydrogenase** for matrix and **adenylate kinase** for outer chamber.

3. Isolation

Mitochondria can be easily isolated by cell fractionation brought about by differential centrifugation. Homogeneous fractions of mitochondria have been obtained from liver, skeletal muscle, heart, and some other tissues. In differential centrifugation mitochondria sediment at 5000 to 24000 g, while in living cells at the ultracentrifugation (20,000 to 400,000 g) mitochondria are deposited intact at the centrifugal pole.

The two mitochondrial membranes have been separated by **density gradient centrifugation**. The outer membrane is separated by causing a swelling which can be brought about by breakage followed by contraction of inner membrane and matrix. Certain detergents such as digitonin and lubrol are often used for this purpose. Since outer membrane is lighter and much stronger, centrifugal force is needed to separate it. When outer membrane is removed with digitonin, the so-called **mitoplast** is formed. Mitoplast includes inner membrane with unfolded cristae and matrix. Mitoplast is found to carry out oxidative phosphorylation. The isolated outer membrane is revealed by negative staining and shows a "folded-bag" appearance (Fig.10.7). Such isolation of two membranes and compartments has enabled localization of various enzyme systems of mitochondria.

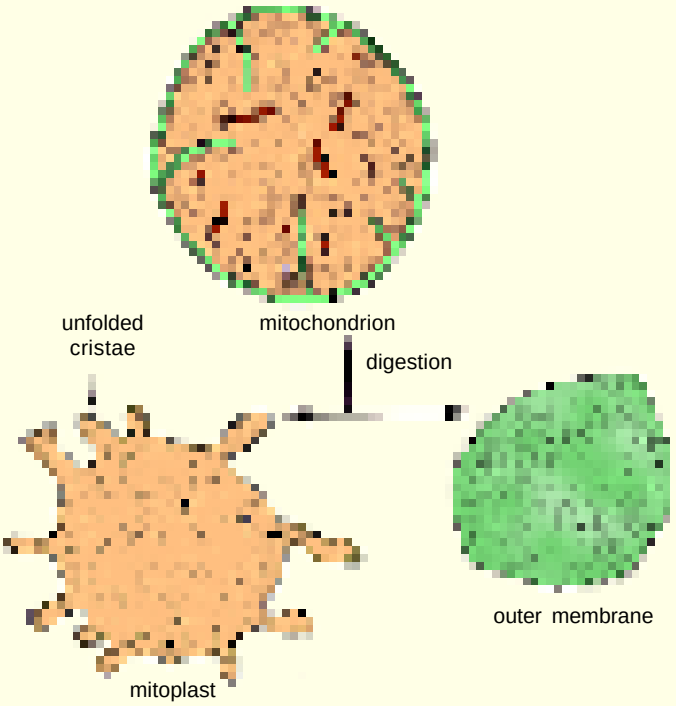


Fig. 10.7. Action of digitonin on liver mitochondrion to produce mitoplast and "folded-bag" of outer membrane (after De Robertis and De Robertis, Jr., 1987).

CHEMICAL COMPOSITION

The gross chemical composition of the mitochondria varies in different animal and plant cells. However the mitochondria are found to contain 65 to 70 per cent proteins, 25 to 30 per cent lipids, 0.5 per cent RNA and small amount of the DNA. The lipid contents of the mitochondria are composed of 90 per cent phospholipids (lecithin and cephalin), 5 per cent or less cholesterol and 5 per cent free fatty acids and triglycerides. The inner membrane is rich in one type of phospholipid, called **cardiolipin** which makes this membrane impermeable to a variety of ions and small molecules (*e.g.*, Na^+, K^+, Cl^-, NAD^+, AMP, GTP, CoA and so on).

The outer mitochondrial membrane has typical ratio of 50 per cent proteins and 50 per cent phospholipids of 'unit membrane'. However, it contains more unsaturated fatty acids and less cholesterol. It has been estimated that in the mitochondria of liver 67 per cent of the total mitochondrial protein is located in the matrix, 21 per cent is located in the inner membrane, 6 per cent is situated in the outer membrane and 6 per cent is found in the outer chamber. Each of these four mitochondrial regions contains a special set of proteins that mediate distinct functions :

1. Enzymes of outer membrane. Besides porin, other proteins of this membrane include enzymes involved in mitochondrial lipid synthesis and those enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix. Certain important enzymes of this membrane are monoamine oxidase, rotenone-insensitive NADH-cytochrome-C-reductase, kynurenine hydroxyalase, and fatty acid CoA ligase.

2. Enzymes of intermembrane space. This space contains several enzymes that use the ATP molecules passing out of the matrix to phosphorylate other nucleotides. The main enzymes of this part are adenylate kinase and nucleoside diphosphokinase.

3. Enzymes of inner membrane. This membrane contains proteins with three types of functions: 1. those that carry out the oxidation reactions of the respiratory chain; 2. an enzyme complex, called **ATP synthetase** that makes ATP in matrix ; and 3. specific transport proteins (see Table 10-1) that regulate the passage of metabolites into and out of the matrix. Since an electrochemical gradient, that drives ATP synthetase, is established across this membrane by the respiratory chain, it is important that the membrane be impermeable to small ions. The significant enzymes of inner membrane are enzymes of electron transport pathways, *viz.*, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), diphosphopyridine nucleotide (DPN) dehydrogenase, four cytochromes (Cyt. b, Cyt. c, Cyt.c_1, Cyt. a and Cyt. a_3), ubiquinone or coenzyme Q_{10}, non-heme copper and iron, ATP synthetase, succinate dehydrogenase; β-hydroxybutyrate dehydrogenase; carnitive fatty acid acyl transferase (Fig. 10.8).

4. Enzymes of mitochondrial matrix. The mitochondrial matrix contains a highly concentrated mixture of hundreds of enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle or Krebs cycle. The matrix also contains several identical copies of the mitochondrial DNA, special 55S mitochondrial ribosomes, tRNAs and various enzymes required for the expression of mitochondrial genes. Thus, the mitochondrial matrix contains the following enzymes : malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthetase, α-keto acid dehydrogenase, β-oxidation enzymes. Moreover, the mitochondrial matrix contains different nucleotides, nucleotide coenzymes and inorganic electrolytes—K^+, HPO_4^-, Mg^{++}, Cl^- and SO_4^-.

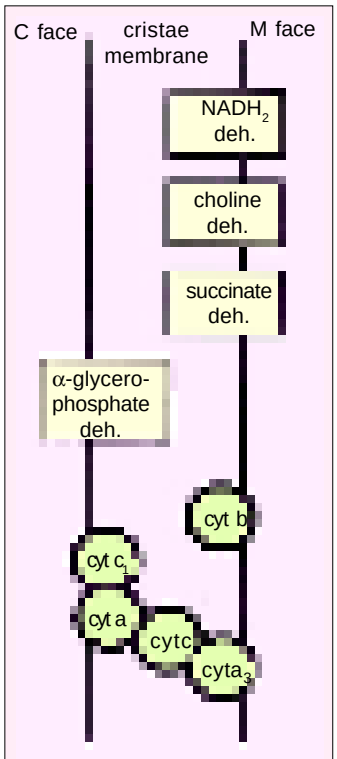


Fig. 10.8. A part of inner mitochondrial membrane (cristae) showing the distribution of different dehydrogenases and cytochromes on M face and C face.

MITOCHONDRIA AND CHLOROPLASTS AS TRANSDUCING SYSTEMS

In cells, energy transformation takes place through the agency of two main transducing systems (*i.e.*, systems that produce energy transformation) represented by mitochondria and chloroplasts. These two organelles of eukaryotic cells in some respects operate in opposite directions. For example, chloroplasts are present only in plant cells and especially adapted to capture light energy and to transduce it into chemical energy, which is stored in covalent bonds between atoms in the different nutrients or **fuel molecules**. In contrast, the mitochondria are the "power plants" or "power houses" that by oxidation, release the energy contained in the fuel molecules and make other forms of chemical energy (Fig.10.9). The main function of chloroplasts is **photosynthesis**, while that of mitochondria is **oxidative phosphorylation**. Finally, photosynthesis is an **endergonic** reaction, which means that it captures energy; oxidative phosphorylation is an **exergonic** reaction, meaning that it releases energy. Table 10-2 has enlisted some of the basic differences between these two transducing systems.

Table 8-1. **Transport systems of inner mitochondrial membrane (Source: Sheeler and Bianchi, 1987).**

System	Exchange
1. Decarboxylate carrier	Exchange for mole-for-mole basis of malate, succinate, fumarate and phosphate between matrix and cytosol.
2. Tricarboxylate carrier	Exchange on mole-for-mole basis citrate and isocitrate between matrix and cytosol.
3. Aspartate-glutamate carrier	Exchange aspartate for glutamate across membrane.
4. α-Ketoglutarate-malate carrier	Specifically exchange α-ketoglutarate for malate across membrane.
5. ADP-ATP carrier	Exchange of ADP for ATP.

FUNCTIONS

The mitochondria perform most important functions such as oxidation, dehydrogenation, oxidative phosphorylation and respiratory chain of the cell. Their structure and enzymatic system are fully adapted for their different functions. They are the actual respiratory organs of the cells where the foodstuffs, *i.e.*, carbohydrates and fats are completely oxidised into CO_2 and H_2O. During the biological oxidation of the carbohydrates and fats large amount of energy is released which is utilized by the mitochondria for synthesis of the energy rich compound known as **adenosine triphosphate** or **ATP**. Because mitochondria synthesize energy rich compound ATP, they are also known as "power houses" of the cell. In animal cells mitochondria produce 95 per cent of ATP molecules, remaining 5 per cent is being produced during anaerobic respiration outside the mitochondria. In plant cells, ATP is also produced by the chloroplasts.

Adenosine triphosphate or ATP

The ATP consists of a purine base **adenine**, a pentose sugar **ribose** and three molecules of the **phosphoric acids** (Fig. 4.30). The adenine and ribose sugar collectively constitute the nucleoside **adenosine** which by having one, two or three phosphate groups forms the adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) respectively. In ATP the last phosphate group is linked with ADP by a special bond known as "**energy rich bond**" because when the last phosphate group of the ATP is released the large amount of energy is released as shown by the following reaction :

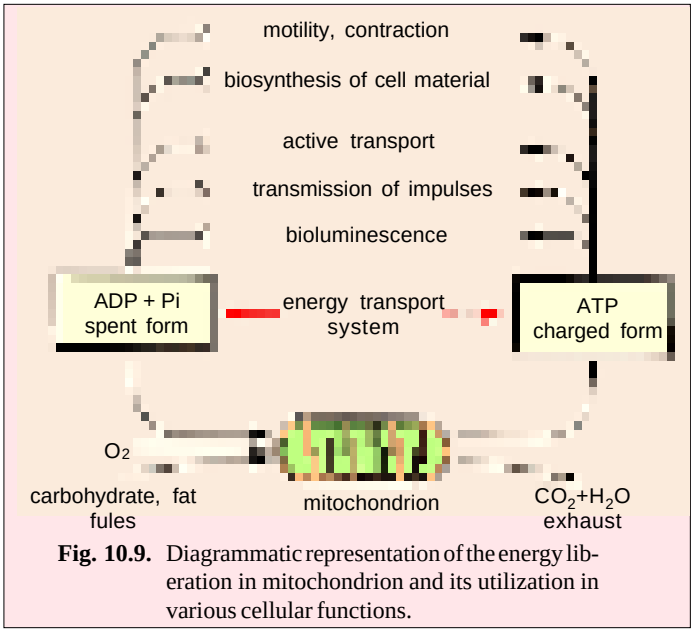


Fig. 10.9. Diagrammatic representation of the energy liberation in mitochondrion and its utilization in various cellular functions.

$$A—P\sim P\sim P \;=\; A–P\sim P \;+\; Pi \;+ 7300 \text{ calories}$$

ATP ADP Phosphate group

In the above reaction, we have seen that by the breaking of the energy rich bond about 7300 calories of energy are released, while the common chemical bond releases only 300 calories of energy. The chemical reactions which synthesize the energy rich bond or~P bond require great amount of energy which is supplied by the oxidation of the foodstuffs in the mitochondria. The utility of energy rich phosphate bond (~P) of the ATP is that great amount of energy is kept stored in the ready state in a very limited space of the cell. The stored chemical energy is disposed of very quickly at the time of the need in various cellular functions such as respiratory cycle, protein and nucleic acid synthesis, nervous transmission, cell division, transportation and bioluminescence, etc.

Table 9-2. **Differences between photosynthesis and oxidative phosphorylation (Source: De Robertis and De Robertis, Jr., 1987).**

Photosynthesis	Oxidative phosphorylation
1. Occurs in the presence of light; thus, periodic	1. Independent of light; thus continuous
2. Uses H_2O and CO_2	2. Uses molecular O_2
3. Liberates O_2	3. Liberates CO_2
4. Hydrolyzes water	4. Forms water
5. Endergonic reaction	5. Exergonic reaction
6. $CO_2 + H_2O$ + energy $\rightarrow$ foodstuff	6. Foodstuff + $O_2 \rightarrow$ $CO_2 + H_2O$ + energy
7. Takes place in chloroplast	7. Takes place in mitochondria

Because the terminal phosphate linkage in ATP is easily cleaved with release of free energy, ATP acts as an efficient phosphate donor in a large number of different phosphorylation reactions. In this way, ATP acts as a carrier molecule like the acetyl CoA and as coenzyme like the CoA or NAD.

Recently, besides ATP, certain other energy rich chemical compounds have been found to be active in the cellular metabolism. These are **cytosine triphosphate (CTP)**, **uridine triphosphate (UTP)** and **guanosine triphosphate (GTP)**. These compounds, however, derive the energy from the ATP by nucleoside diphosphokinases (Fig. 10.9 and Fig.10.10). The energy for the production of ATP or other energy rich molecules is produced during the breakdown of food molecules including carbohydrates, fats and proteins (**catabolic** and **exergonic activities**).

OXIDATION OF CARBOHYDRATES

The carbohydrates enter in the cell in the form of monosaccharides such as glucose or glycogen. These hexose sugars are first broken down into 3-carbon compound (pyruvic acid) by a series of chemical reactions known by many enzymes. The pyruvic acid enters in the mitochondria for its complete oxidation into CO_2 and water. The reactions which involve in the oxidation of glucose into CO_2 and water are known to form the **metabolic pathways** and they can be grouped under the following heads :

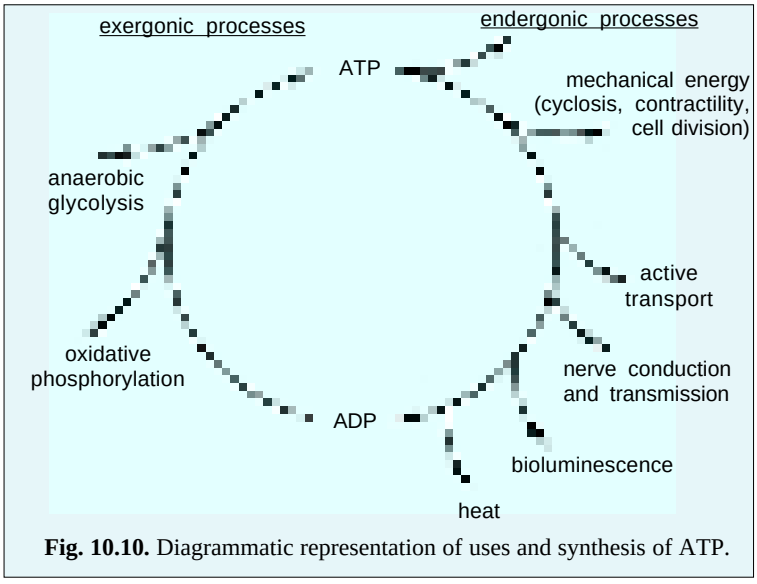


Fig. 10.10. Diagrammatic representation of uses and synthesis of ATP.

(i) Glycolysis or Embden-Meyerhof pathways (EMP) or Embden-Meyerhof-Parnas pathways (EMPP);
(ii) Oxidative decarboxylation;
(iii) Krebs cycle; citric acid cycle or tricarboxylic acid cycle;
(iv) Respiratory chain and oxidative phosphorylation.

1. Glycolysis

Under anaerobic conditions (*i.e.*, in the absence of oxygen) glucose is degraded into lactic acid or lactate by a process called **glycolysis** (*i.e.*, lysis or splitting of glucose), *e.g.*, it commonly occurs in vertebrate muscles when the energy demand in heavy exercise exceeds the available oxygen. [**Note**: According to Circular No. 200 of Committee of Editors of Biochemical Journals Recommendations (1975) the ending *ate* in lact*ate*, pyru*vate*, oxaloac*etate*, citr*ate*, etc., denotes any mixture of free acid and the ionized form(s) (according to pH) in which cations are not specified (see **Martin Jr**., *et al.*, 1983). Most modern textbooks though have adopted this convention for all of the carboxylic acids, but we prefer to stick to the old pattern). If glycolysis is carried out under aerobic conditions the final products are pyruvic acid and coenzyme NADH. Glycolysis is achieved by a series of 10 enzymes all of which are located in the cytosol (cytoplasmic matrix). As shown in Figure 10.11, in this chain of reactions, the product of one enzyme serves as a substrate for the next reaction. To facilitate its analysis, the sequence of glycolysis can be subdivided into following three main steps : (i) Activation (stage I); (ii) Cleavage (stage II); and (iii) Oxidation (stage III).

(i) Activation. In reactions 1 to 3 the glucose molecule is converted into **fructose-1-6- diphosphate**. This step uses two molecules of ATP and involves the following enzymes: hexokinase, phosphoglucose isomerase (or phosphohexoisomerase) and phosphofructokinase.

(ii) Cleavage. In reactions 4 and 5, fructose -1-6-diphosphate splits into two (3-carbon) end products, **glyceraldehyde-3-phosphate** molecules. During the step of cleavage only two enzymes are used: aldolase (fructoaldolase) and triose isomerase (triosephosphate isomerase).

(iii) Oxidation. In reactions 6 to 10, two molecules of glyceraldehyde-3-phosphate are oxidized and ultimately converted into two molecules of **pyruvic acid**. This step produces four molecules of ATP by **substrate-level-phosphorylation** and involves the following enzymes: phosphoglyceric dehydrogenase (glyceraldehyde phosphate dehydrogenase), phosphoglyceric kinase, phosphoglyceromutase, enolase and pyruvic kinase.

The net energy yield of chain reactions of glycolysis is the production of two ATP molecules from one molecule of glucose. Under aerobic conditions, the end products of glycolysis are pyruvic acid and reduced coenzyme NAD (*i.e.*, NADH). NADH carries two electrons, taken from glyceraldehyde-3-phosphate and contains little energy. However, under anaerobic conditions, pyruvic acid remains in the cytosol (cytoplasmic matrix) and is used as a hydrogen acceptor and converted into lactic acid :

$$\text{Pyruvic acid} \xrightarrow[+\text{NADH}]{\text{Lactic dehydrogenase}} \text{Lactic acid} + \text{NAD} + \text{Energy}$$

In above case, the following equation represents the overall reaction of glycolysis :

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + Pi + 2ADP \longrightarrow \underset{\text{Lactic acid}}{2C_3H_6O_3} + 2\,ATP + H_2O$$

Further, pyruvic acid is converted into ethyl alcohol via acetaldehyde by yeast, *Saccharomyces cerevisae* in the absence or deficiency of oxygen. This process is called **alcoholic fermentation :**

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{Glycolysis}} \underset{\text{Pyruvic acid}}{CH_3CO.COOH} \xrightarrow{-CO_2} \underset{\text{Acetaldehyde}}{CH_3CHO} \xrightarrow{+2H} \underset{\text{Ethyl alcohol}}{CH_3CH_2.OH}$$

However, in the cells of higher plants under anaerobic conditions, pyruvic acid is converted into either ethyl alcohol or any organic acid such as malic acid, citric acid, oxalic acid and tartaric acid. The

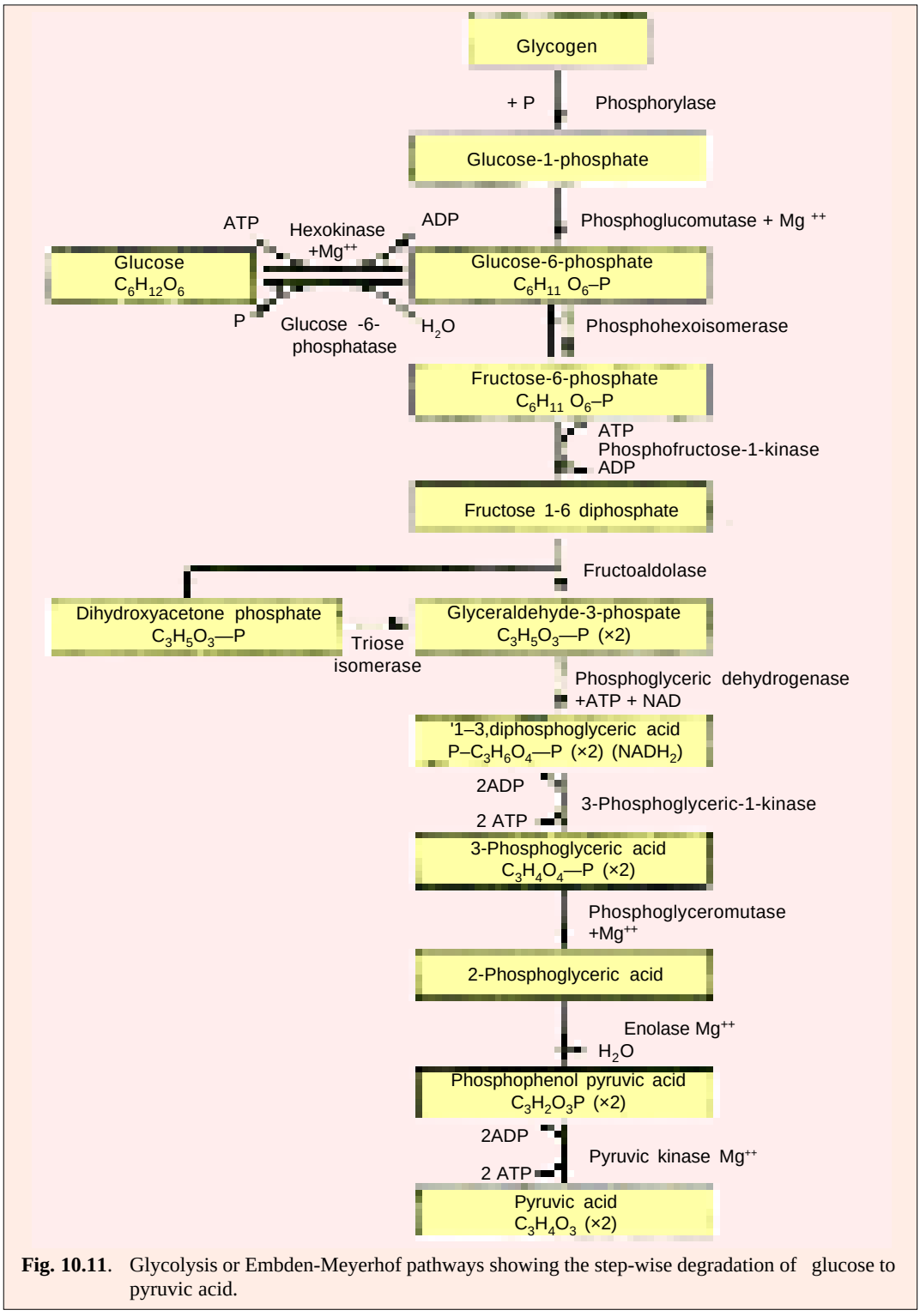


Fig. 10.11. Glycolysis or Embden-Meyerhof pathways showing the step-wise degradation of glucose to pyruvic acid.

anaerobic respiration of higher plants and fermentation of bacteria and yeast have the following differences :

(i) Fermentation is an extracellular process, *i.e.*, the respiratory substrate (*i.e.*, sugar, etc.) is present outside the cell and that too in the liquid medium, while the process of anaerobic respiration is intracellular, *i.e.*, the respiratory substrate is present inside the cell.

(ii) The enzyme (zymase) needed for fermentation is supplied from the micro-organism like yeast, while the enzymes (zymase-complex) required for the process of anaerobic respiration are present in the same cell of the higher plant in which the process is occurring.

However, both processes produce similar end-products as follows :

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow[\text{or Fermentaion}]{\text{Anaerobic Respiration}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2} + \text{Energy}$$

2. Oxidative Decarboxylation

In aerobic organisms, since pyruvic acid still contains a large amount of energy, it must undergo further degradation, but this time inside the mitochondria. This is done in three consecutive steps : **oxidative decarboxylation** (removal of carboxyl or —COOH group), **Krebs cycle** and **oxidative phosphorylation**. Pyruvic acid directly enters the mitochondrial matrix and is converted into **acetyl-CoA** by the help of a huge enzyme, called **pyruvic acid dehydrogenase** (Fig.10.12). The two NADH molecules (which are generated during glycolysis) cannot penetrate directly into the mitochondria, so their electrons are transferred to **dihydroxyacetone phosphate**, which shuttles them into the mitochondria. This process utilizes one ATP molecule for each NADH ; in all two ATP molecules are consumed for two NADH molecules. When both of these NADH pass through ETS, they tend to generate 6 ATP molecules.

Pyruvic acid dehydrogenase and its action. Sometimes two enzymes that catalyze sequential reactions form an **enzyme complex** and the product of the first enzyme does not have to diffuse through the cytoplasm to encounter the second enzyme. The second reaction begins as soon as the first is over. Some large enzyme (multienzyme) aggregates carry out whole series of reactions without losing contact with the substrate. For example, the conversion of pyruvic acid to acetyl CoA proceeds in *three* chemical steps, all of which take place on the same large multienzyme complex (*i.e.*, pyruvate dehydrogenase). Pyruvic acid dehydrogenase occurs in the mitochondrial matrix and is larger than a ribosome in size. It contains multiple copies of **three** enzymes namely **pyruvivc acid dehydrogenase**, **dihydrolipoyl transacetylase** and **dihydrolipoyl dehydrogenase**. It also contains **five** coenzymes (*e.g.*, NAD, coenzyme A, etc.) and **two regulatory proteins** (*e.g.*, protein kinase and protein phosphatase ; both regulating the activity of pyruvic acid dehydrogenase, turning it off whenever ATP levels are high).

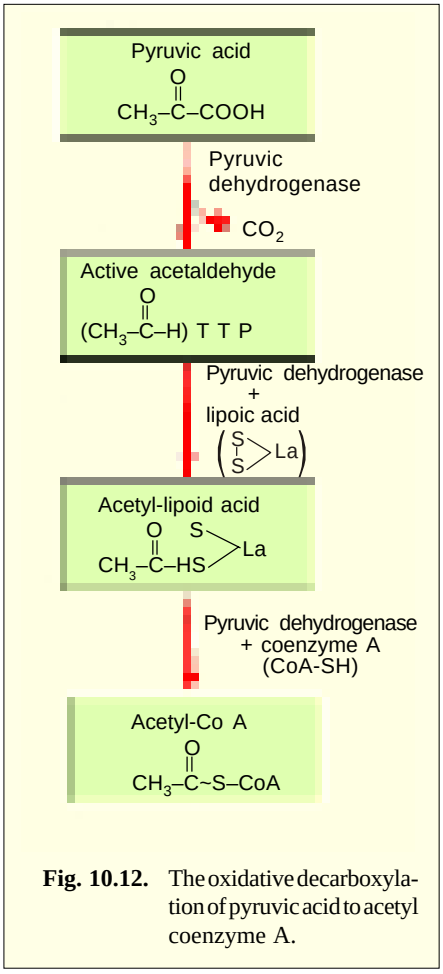


Fig. 10.12. The oxidative decarboxylation of pyruvic acid to acetyl coenzyme A.

Thus, during oxidative decarboxylation of one molecule of pyruvic acid, one mole of CO_2 is produced and one NAD is reduced to NADH. The end product of this reaction is a 2- carbon compound, the **acetyl group** which is attached to coenzyme A to produce the carrier molecule, called **acetyl CoA**.

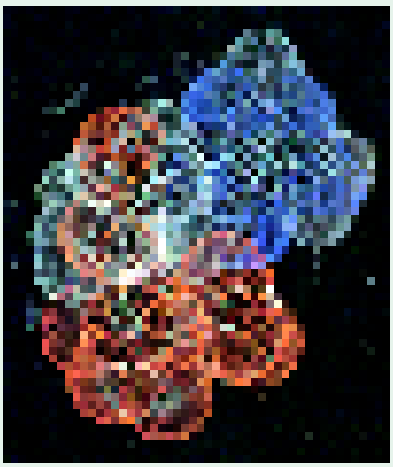
Role of coenzymes in mitochondria. Some coenzymes have a central role in mitochondrial function. **Coenzyme A** (CoA) is part of a group (Table 10-3) that is derived from a nucleoside (adenine-D-ribose) and contains pantothenic acid (a vitamin of B complex) linked to the ribose by pyrophosphoric acid. CoA can be easily transformed into an ester at the thiol end(—SH) by acetyl group making acetyl-CoA. Acetyl-CoA is a carrier molecule in which acetyl group is linked by reactive bonds so that they can be transferred efficiently to other molecules. The same carrier molecule will often participate in many different biosynthetic reactions in which its group (*i.e.*, acetyl group) is needed, *e.g.*, growing fatty acid.

Other mitochondrial coenzymes are **nicotinamide adenine dinucleotide** (**NAD^+**) which contains the vitamin nicotinic acid of B complex and **flavin mononucleotide** (**FMN**) and **flavin adenine dinucleotide** (**FAD**), both of which contain riboflavin or vitamin B_2. NAD^+, FMN and FAD are important coenzymes not only in mitochondria but also in chloroplasts.

Table 10-3. **Some important coenzymes which act as carrier molecules in transfer of a group (Source: Alberts *et al.*, 1989).**

Coenzyme	Group transferred
1. ATP	phosphate
2. NADH, NADPH	hydrogen and electron (hydrogen ions)
3. Coenzyme A	acetyl
4. Biotin	carboxyl
5. S - Adenosyl-methionine	methyl

3. Krebs cycle



In this computer graphics image of ATP, adenosine is blue, pentose is white and the phosphate groups are red.

Two acetyl CoA molecules, produced above by oxidation of one molecule of glucose pass through a series of reactions of Krebs cycle to produce CO_2, H_2O and electrons. Enzymes and coenzymes of Krebs cycle are located in the mitochondrial matrix (some of them such as succinic dehydrogenase, are attached to M face of inner mitochondrial membrane). As illustrated in Figure 10.13, the Krebs cycle involves the condensation of the acetyl group with oxaloacetic acid to make **citric acid** (6-carbon compound). This step is directed by the enzyme **citrate synthase**. From citric acid, H_2O is released twice by **aconitase** enzyme to produce **isocitric acid**. This is followed by a decarboxylation (loss of CO_2) by **isocitric dehydrogenase**, producing 5 carbon **α- ketoglutaric acid**. CO_2 is released by **α- ketoglutarate dehydrogenase** in the presence of CoA to produce **succinyl - CoA** which changes by **succinyl kinase** enzyme (also called succinyl CoA synthetase) into a 4-carbon compound, the **succinic acid** (at this stage one GTP is generated by substrate level phosphorylation). The next enzyme of Krebs cycle, the **succinic dehydrogenase** converts succinic acid into **fumaric acid** and then **fumarase** enzyme produces **malic acid**. The mediation of **malate dehydrogenase** enzyme produces **oxaloacetic acid**, and thereby closes the Krebs cycle.

At each turn of the Krebs cycle, four pairs of hydrogen atoms are removed from the substrate intermediates by enzymatic dehydrogenation and two CO_2 molecules are released. These hydrogen

atoms (or equivalent pairs of electrons) enter the respiratory chain, being accepted by either NAD^+ or FAD. Three pairs of hydrogen molecules are accepted by NAD^+, reducing it into NADH, and one pair by FAD, reducing it into $FADH_2$ (this pair of electrons comes directly from the succinic dehydrogenase reaction). Since it takes two turns of the cycle to metabolize the two acetyl groups that are produced by glycolysis from one molecule of glucose, a total of six molecules of NADH and two of $FADH_2$ are formed. During Krebs cycle are also produced two ATP molecules (*i.e.*, via GTP molecules).

Let us consider the specific function of the Krebs cycle. When a log is to be burned in a fire place, the log must first be chopped up into smaller chunks of fuel. Glucose, the fuel for metabolism, must also be broken into smaller pieces. In glycolysis, glucose is first split into two pyruvic acid molecules. Then, each pyruvic acid molecule is broken into three carbon dioxide fragments: One CO_2 molecule is given off during oxidative decarboxylation (*i.e.*, conversion of pyruvic acid into acetyl-CoA) and two CO_2 molecules are given off in the Krebs cycle. Thus, two turns of Krebs cycle will completely break up one glucose molecule. In fact, chopping off a log into kindling wood does not oxidize the wood. Nor does chopping glucose into smaller carbon fragment oxidize the glucose. The Krebs cycle itself releases no energy, but as the glucose breaks up, it also frees the hydrogen atoms attached to the carbons. Each hydrogen atom contains one proton (H^+) and one electron (e^-). The electrons which are released during complete oxidation of glucose (*i.e.*, glycolysis, oxidative decarboxylation and Krebs cycle) carry most of the energy of the glucose. In the final stage of cell respiration, the electron transport system, these electrons will at last release their energy to the cell.

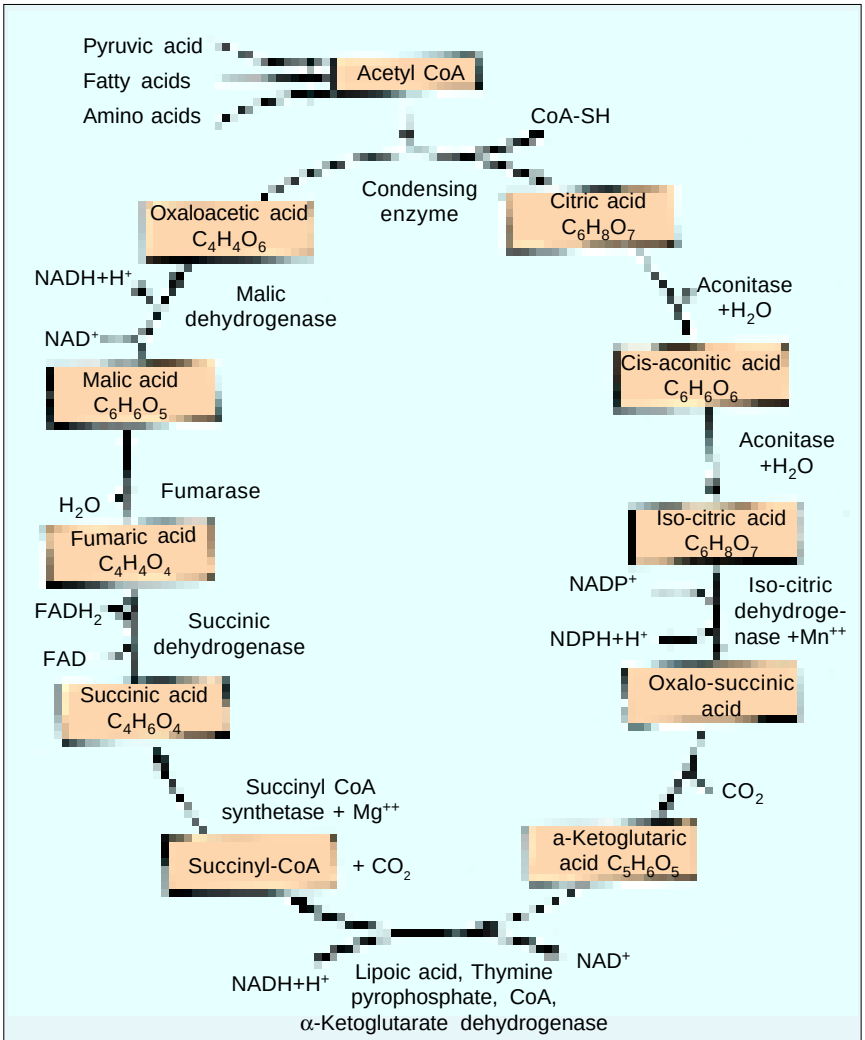
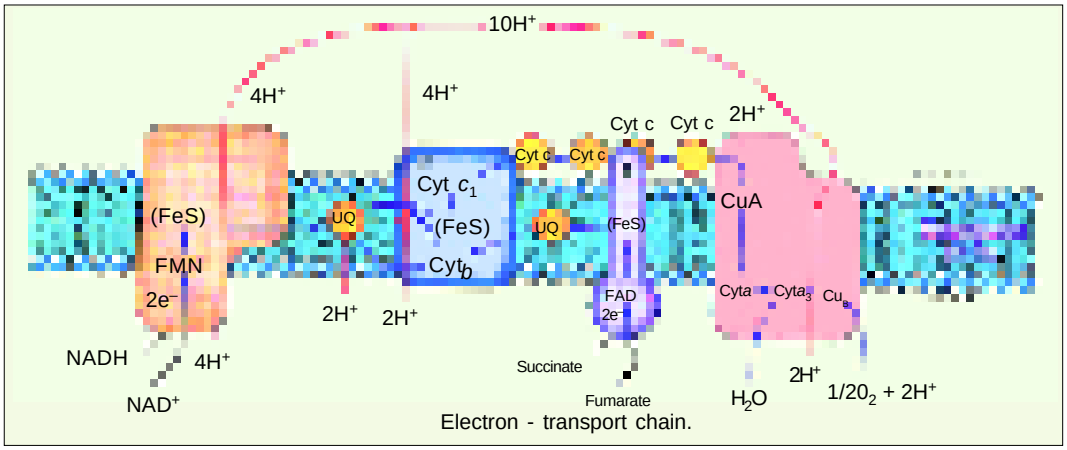


Fig. 10.13. Krebs cycle or tricarboxylic acid cycle.

4. Respiratory Chain and Oxidative Phosphorylation

Two molecules of $FADH_2$ and six molecules of NADH produced in Krebs cycle (from two molecules of acetyl-CoA) are oxidized by molecular O_2 in a **respiratory chain** or **electron transport system** or **ETS** involving a series of enzymes and coenzymes.

In the electron transport system, the successive electron acceptors are at lower and lower energy levels. With each transfer to a lower energy level, the electrons release some of their potential energy. That is why this series is called an **electron cascade,** like a cascade of falling water. At each stage, the released energy is used to form ATP. Since electron transport involves oxidation as well as phosphorylation (*i.e.*, ADP + P = ATP) this process by which cell system traps chemical energy is called **oxidative phosphorylation** (Fig 10.14). The passage of electrons from NAD to oxygen generates 3 ATP molecules, whereas the passage of electrons from FAD to oxygen generates only 2 ATP molecules.



Electron - transport chain.

(A) Compounds that occur in ETS. Following five types of compounds are associated with electron transport system of inner mitochondrial membrane :

(i) Pyridine-linked dehydrogenases require as their coenzyme either NAD^+ or $NADP^+$ both of which can accept two electrons at a time. There are about 200 dehydrogenases for which NAD^+- linked compounds such as pyruvic acid dehydrogenase, are involved in ETS.

(ii) Flavin-linked dehydrogenases (often called **flavoproteins** or **FPs**) require either FAD or FMN. Both are prosthetic groups whose isoalloxazine ring can accept two hydrogen atoms. Flavin- linked enzymes are commonly involved in a number of enzyme systems such as fatty acid oxidation, amino acid oxidation and Krebs cycle activity (*e.g.*, succinic dehydrogenase or SDH).

(iii) Ubiquinones were so named because of their occurrence in so many different organisms and their chemical resemblance to

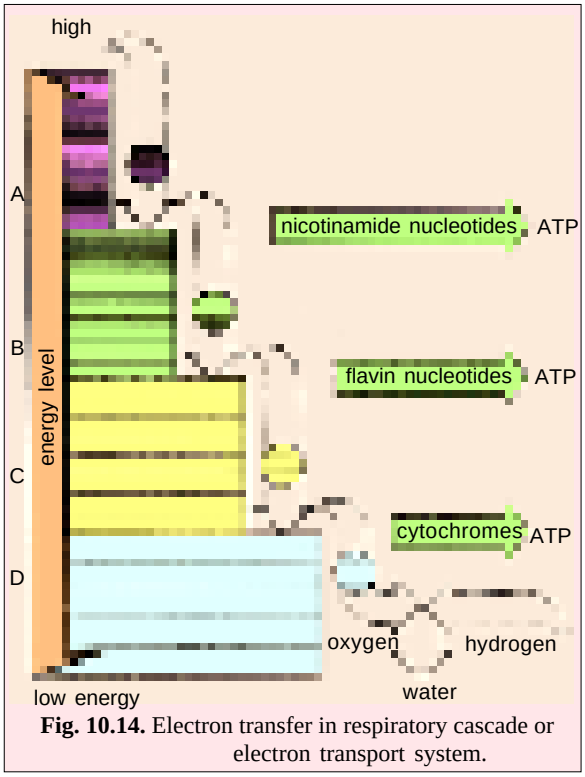


Fig. 10.14. Electron transfer in respiratory cascade or electron transport system.

quinone. They are found in several different forms including the **plastoquinones** of chloroplasts. The form of ubiquinones present in mitochondria is often called **coenzyme Q_{10}** (CoQ_{10} or Q). CoQ_{10} is a lipid soluble and accepts two hydrogen atoms (or two protons and two electrons) at a time.

(iv) Cytochromes are proteins containing iron-porphyrin (haem) groups. There are a large number of cytochromes in cells; most are found in mitochondria, although some also function in the ER and in chloroplasts. Mitochondria have five types of cytochromes which are arranged in the following order in an inner membrane: cyt. b, cyt. c_1., cyt. c, cyt. a and cyt. a_3. All of them transfer electrons by reversible valence changes of the iron atom (trivalent ferric or $Fe^{3+} \rightleftharpoons$ bivalent ferrous or Fe^{2+}).

(v) Iron-sulphur proteins (Fe_2S_2 and Fe_4S_4) are electron carriers of mitochondria containing iron and sulphur in equal amounts. The iron is reversibly oxidized during the electron transfer. Iron- sulphur proteins transfer one electron at a time.

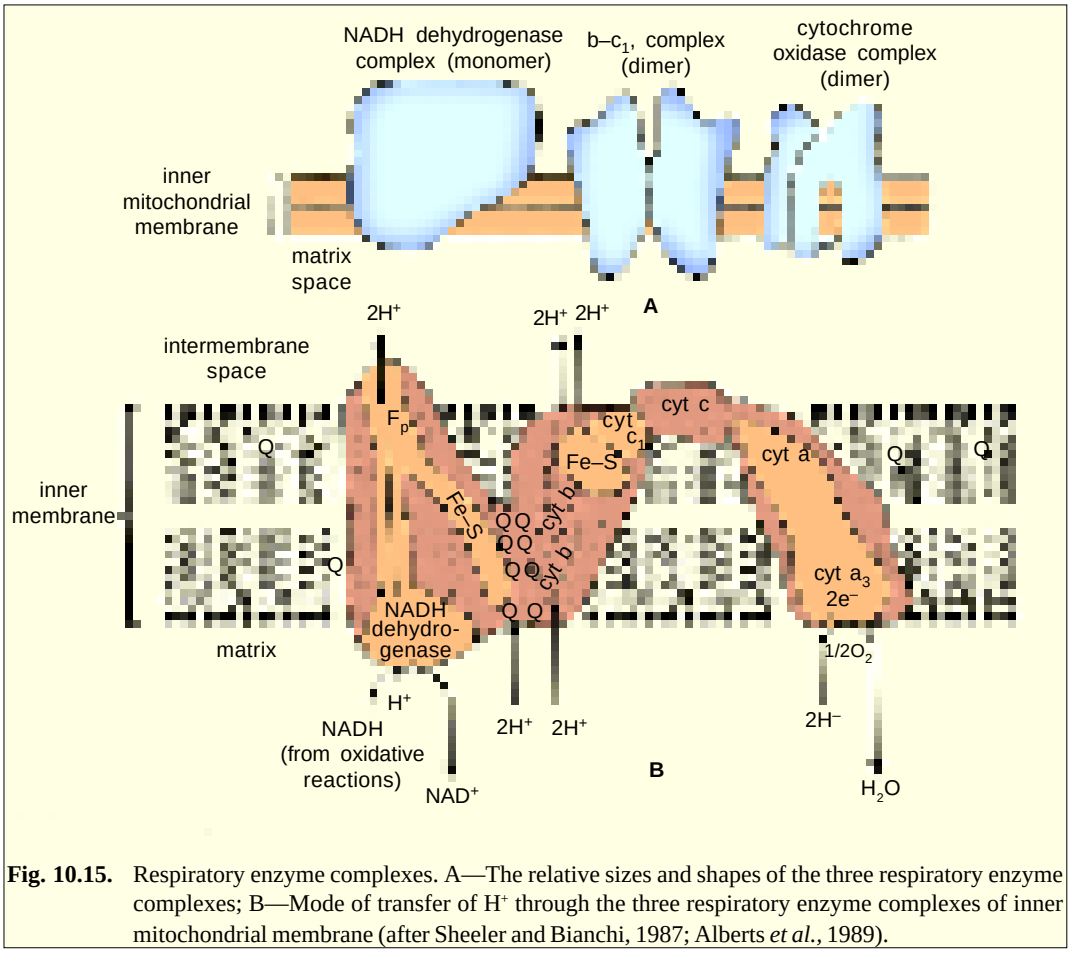


Fig. 10.15. Respiratory enzyme complexes. A—The relative sizes and shapes of the three respiratory enzyme complexes; B—Mode of transfer of H^+ through the three respiratory enzyme complexes of inner mitochondrial membrane (after Sheeler and Bianchi, 1987; Alberts *et al.*, 1989).

All these components of ETS are arranged in the inner mitochondrial membrane in the following sequence: NAD-linked succinic dehydrogenase (SDH), flavoprotein (FAD), non-haem iron protein or iron-sulphur protein, flavoprotein (FAD), cytochrome b, ubiquinone or coenzyme Q_{10}, cytochrome c_1, cytochrome c, cytochrome a, cytochrome a_3 and three coupling sites, where phosphorylation coupled with oxidation leads to production of ATP.

(B) Three complexes. Evidently above described components of ETS occur in the mitochondria in the form of following three complexes (**Green** *et al.*, 1967; **Capaldi** *et al.*, 1982; **Weiss** *et al.*, 1987) :

(i) The NADH-dehydrogenase complex. It is the largest of the respiratory enzyme complexes, with a mass about 800,000 daltons and more than 22 polypeptide chains. It accepts electrons from NADH

and passes them through a flavin and at least five iron-sulphur centres to ubiquinone (Q) that transfer its electrons to the next complex, the b-c_1, complex. This complex spans the inner mitochondrial membrane and is able to translocate protons across it from M side to C side (Fig.10.15).

(ii) The b-c1 complex. It contains at least 8 different polypeptide chains and is thought to function as a dimer of about 500,000 daltons. Each monomer contains three haemes bound to cytochromes and iron-sulphur protein. This complex accepts electrons from ubiquinone (Q) and passes them to cytochrome c, a small peripheral membrane protein that carries its electrons to the cytochrome oxidase complex. In the topology of this complex the Q-site may be in the middle of the membrane in the hydrophobic area and the cytochrome c-site on the C side.

(iii) The cytochrome oxidase complex. It comprises at least eight different polypeptide chains and is isolated as a dimer of about 300,000 daltons; each monomer contains two cytochromes (*a*, a_3) and two copper atoms. This complex accepts electrons from cytochrome c and passes them to oxygen and is thought to traverse the mitochondrial membrane, protruding on both surfaces. Such a transmembrane orientation is associated with the vectorial transport of protons across the membrane.

The cytochrome oxidase reaction is estimated to account for 90 per cent of the total oxygen uptake in most cells. The toxicity of the poisons such as cyanide and azide is due to their ability to bind tightly to this complex and thereby block all electron transport.

(C) F_0 - F_1 complex or coupling factors. One of the main proteins in the inner mitochondrial membrane is the multisubunit **coupling factor** (Fig. 10.16), the enzyme that actually synthesizes ATP and simultaneously acting as a **proton pump**. A quite similar enzyme complex is located in the thylakoid membranes of chloroplasts and in the plasma membrane of bacterial cell. The coupling factor has two principal components :

(a) F_0-complex. It is an integral membrane complex, composed of very hydrophobic proteins—3 or 4 distinct polypeptides and one proteolipid—which together span the mitochondrial membrane. F0 - complex possesses the proton translocating mechanism. F0-complex can be extracted only with strong detergents.

(b) F_1-particle. Attached to the F0 complex is F_1 particle, a complex of five distinct polypeptides: alpha (α), beta (β), gamma (γ), delta (δ) and epsilon (ε), with the probable composition of $\alpha_3\beta_3\gamma\delta\varepsilon$. F_1 forms the knob or 'tadpole' that protrudes on the matrix side of the inner mitochondrial membrane. F_1 particle can be detached from the membrane by mechanical agitation and is water soluble. When physically separated from the membrane, F_1 particle is capable only of catalysing the hydrolysis of ATP into ADP and phosphate. Hence, it is often called the **F_1-ATPase**. However, its natural function is the synthesis of ATP.

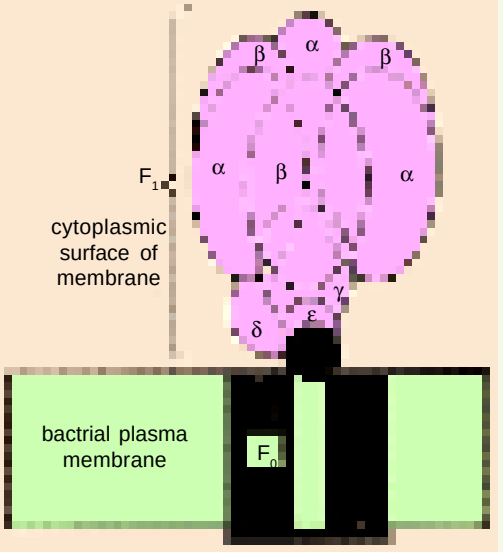


Fig. 10.16. Model of molecular structure of F_0 and F_1 partilces in the bacterial plasma membrane. The core of F_1 particle is an $\alpha_3\beta_3$ complex. The δ polypeptide of F_1 and probably the γ are involved in binding F_1 to the membrane-embedded F_0-particle. The role of ε subunit of F_1 complex is not known (after Darnell *et al.*, 1986).

(D) Redox reactions and redox couples. The movements of electrons between cellular reductants and oxidants represent a form of energy transfer in cells. A reductant (or **reducing agent**) is a substance that loses or donates electrons to another substance; the latter substance is the oxidant (or **oxidizing**

agent). Conversely, an oxidant is a substance that accepts electrons from another substance, the latter being the reductant. Reactions that involve the movement of electrons between reductants and oxidants are called **redox reactions**.

Different chemical substances have different potentials for donating or accepting electrons. The tendency of hydrogen to dissociate:

$$H_2 \rightleftharpoons 2H^+ + 2e^-$$

thereby releasing electrons, is used as a standard against which the tendencies of other substances to release or accept electrons is measured. The electron donor (*e.g.*, H_2 in the above reaction) and the electron acceptor (*e.g.*, $2H^+$ in the above reaction) are called a **redox couple** or **half cell**. The tendency of any chemical substance to lose or gain electrons is called the **redox potential** and is measured in volts(V). Measurements are made using an electrode that has been standardized against the $H_2 - 2H^+$ couple whose redox potential is set at 0.0 under standard conditions (pH 0.0, 1M(H^+), 25° C and 1 atmosphere pressure). This potential is noted by the symbol *Eo*. For biochemical reactions which normally occur at pH 7.0, the redox potential of the H_2–$2H^+$ couple is –0.421V; standard redox potentials at pH7.0 are noted by the symbol *E′o*.

Any substance with a more positive *E′o* value than another has the potential for oxidizing that substance (*i.e.*, removing electrons from the substance with the more negative *E′o* value). The greater the difference in redox potentials, the greater the energy changes involved. The change in standard free energy changes, $\Delta G^{o\prime}$ is related to *E′o* as follows:

$$\Delta G^{o\prime} = n F \Delta E' o$$

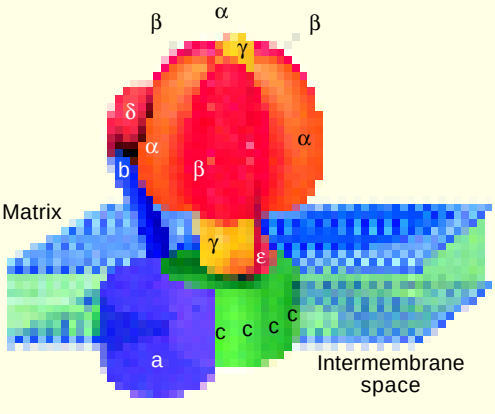
where *n* is the number of electrons exchanged per molecule, *F* is the **Faraday** (96,406 J/V), and *E′o* is the difference in redox potential between the more positive and more negative members of the redox couple. For example, the oxidized form of cytochrome *c* (*E′o* value = $^+$0.254 V) can oxidize the reduced form of cytochrome *b* (*E′o* value = + 0.030V) by removal of two electrons. The difference between the redox potentials of the two is:

$+0.254 - (+0.030) = +0.224$ V, therefore,

$\Delta G^{o\prime} = -2\,(96,406\ \text{J/V})\,(0.224\ \text{V})$

$= -43.19$ kJ (per mole of each cytochrome).

(E) ATP synthesis. The potentials drop in three large steps, one across each major respiratory enzyme complex. The change in redox potential between any two electron carriers is directly proportional to the free energy released by an electron transfer between them. Each complex acts as an **energy-conversion-device** to harness this free-energy change, pumping H^+ across the inner membrane to create an electrochemical proton gradient as electrons pass through. The energy conversion mechanism underlying oxidative phosphorylation requires that each protein complex be inserted across the inner mitochondrial membrane in a fixed orientation, so that all protons are pumped in the same direction out of the matrix space. Such a **vectorial organization** of membrane proteins has been experimentally proved.



The structure of the ATP synthase.

Just as a flow of water from a higher to a lower level can be utilized to turn a water wheel or a hydroelectric turbine, the energy released by the flow of the protons down the gradient is utilized in the synthesis of ATP (see **Reid** and **Leech**, 1980). Similarly, resultant electrochemical proton gradi-

ent is harnessed to make ATP by F_0 - F_1 complex (acting as ATP synthetase and proton pump), through which the protons flow back into the matrix (Fig. 10.17). The ATP synthetase is a reversible coupling device that normally converts a back-flow of protons into ATP phosphate-bond energy, but it can also hydrolyze ATP (into ADP and phosphate) to pump protons in the opposite direction, if the electrochemical proton gradient is reduced.

The chemiosmotic theory. Several hypotheses have been proposed to explain the mechanism of electrochemical link between respiration and phosphorylation inside inner mitochondrial membrane. Most accepted one is that of **Mitchell's chemiosmotic coupling theory**, proposed in 1967. According to this theory, the inner membrane of the mitochondria acts as a transducer converting the energy which is provided by an electrochemical gradient, into the chemical energy of ATP. In this model (Fig.10.18A), the membrane is **impermeable** to both H^+ and OH^- ions. For this reason, if pH differences are established across the membrane, they act as energy-rich gradients. The electron transport system is organized in "**redox loops**" within the membrane, and the electrons are passed from one carrier to another on the respiratory chain. At the same time, protons (H^+) are ejected toward the cytoplasmic side (C side), while OH^- remain on matrix side. This vectorial movement of protons creates a difference in pH (*i.e.*, lower pH on the C side and higher on the M side), which results in an electrical potential (Fig. 10.18B).

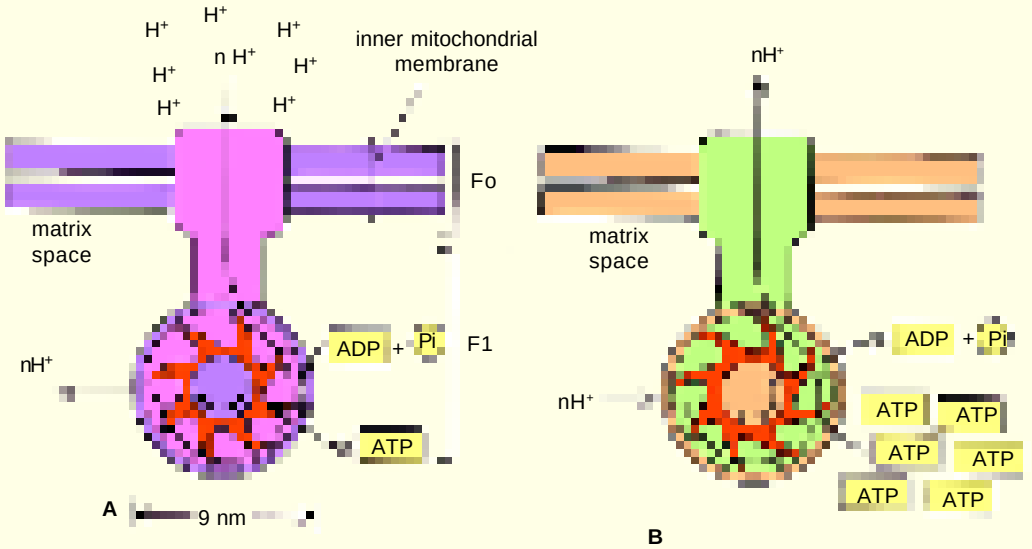


Fig. 10.17. ATP synthetase (F_0-F_1 ATPase) is a reversible coupling device that interconverts the energies of the electrochemical proton gradient and chemical bonds. The ATP synthetase can either synthesize ATP by harnessing the proton motive force (A) or pump protons against their electrochemical gradient by hydrolyzing ATP (B). The direction of operation at any given instant depends on the net free-energy change for the coupled processes of proton translocation across the membrane and the synthesis of ATP from ADP and Pi (after Albert *et al.*, 1989).

Calling Δ pH the **pH gradient** and Δψ (delta psi) the resulting **electrical gradient** in volts, the energy produced Δ P is the **proton motive force**:

$$\Delta P = \Delta\psi + 2.3\ RT/F\ \Delta pH$$

where R is the universal gas constant, T the absolute temperature, and F the Faraday constant.

The chemiosmotic theory postulates that the primary transformations occurring in the respiratory chain guide the **osmotic work** needed to accumulate ions. The energy generated by electron transport is conserved in the energy-rich form of a H^+ ion gradient. This gradient provides the driving force for the inward transport of phosphate and potential for generating ATP. ADP is brought into matrix in exchange for ATP, *i.e.*, cotransport. As indicated in Figure 10.18C, this gradient through the action of

the proton pump of the F_0-F_1, drives the oxidative phosphorylation of ADP to form ATP by which mechanism free energy is conserved :

$$ADP + Pi \rightleftharpoons ATP + H_2O$$

In this reaction H_2O is also formed because of the dehydration, which leads to the removal of H^+ and OH^- ions.

In recent years, much experimental evidence has supported the validity of Mithchell's chemiosmotic theory. However, there is some dispute over the number of H^+ ions translocated out during two-electron transportation from NADH to oxygen. According to **Lehninger** and **Brand** (1979) these may be 9 to 12 in number instead of 6 as claimed by **Mitchell** (1967). However, **Mitchell** and **Moyle** (1979) have reasserted their original claim of 6 H^+ translocation.

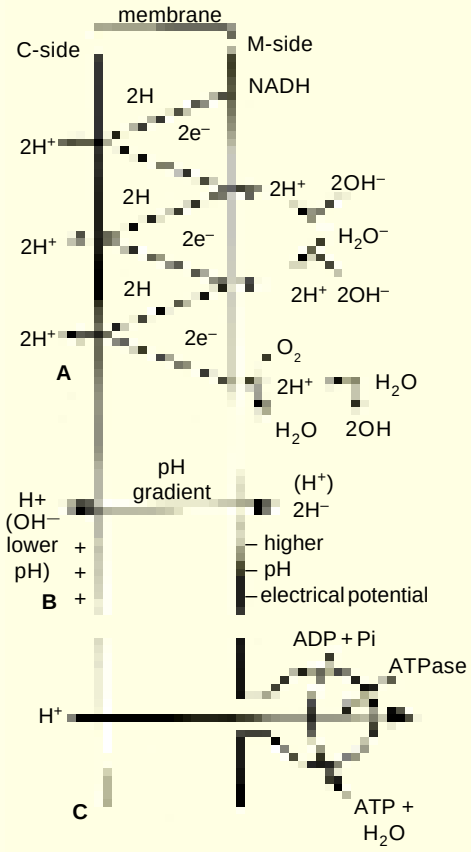


Fig. 10.18. Diagram explaining chemiosmotic coupling according to Mitchell. A—During electron transport H^+ ions are driven to the C side of inner mitochondrial membrane; B—This process produces a pH gradient and an electrical potential across the membrane; C—this gradient drives the proton pump of the ATPase and ATP is synthesized from ADP and Pi (after De Robertis and De Robrtis Jr., 1987).

(F) Energetics of glucose oxidation. Of the 686,000 calories contained in a mole of glucose, less than 10 per cent (*i.e.*, 58,000 calories) can be released by anaerobic glycolysis. The cell is able to store only 45 per cent of the chemical energy liberated by the combustion of glucose in the form of ATP (*i.e.*, only 36 ATP molecules). The rest of the energy is dissipated as heat or used for other cell functions.

At this stage, let us do stocktaking of ATP generation during aerobic respiration of one mole of glucose. We have seen that glycolysis and Krebs cycle can each generate 2 molecules of ATP per molecule of glucose by substrate level phosphorylation (total 4 ATP molecules). In addition 10NADH (*i.e.*, 2 NADH in glycolysis, 2NADH in oxidative phosphorylation and 6NADH in Krebs cycle) and 2$FADH_2$ are produced which are equivalent to 34 ATP molecules. Thus, a total of 38 ATP molecules are produced per glucose molecule oxidized. However, in most eukaryotic cells 2 molecules of ATP are used in the transportation of 2 mole of NADH produced during glycolysis into the mitochondrion (*via* the malate shuttle) for their further oxidation (*via* ETS). Hence, the net gain of ATP is 38–2=36 molecules; since one high energy phosphate bond is equal to 36.8 kJ; so 36ATP = 1325 kJ or 36,000 calories:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Inogranic phosphate}}{36Pi} + 36ADP + 6O_2 + 6H_2O \rightarrow 6CO_2 + 36\,ATP + 12H_2O \quad (360{,}000 \text{ calories or } 1325 \text{ kJ})$$

The P/O ratio. In the electron tranport chain, since the formation of ATP occurs in three steps, the equation can be written as follows:

$$NADH + H^+ + 3ADP + 3\,Pi + 1/2\,O_2 \rightarrow NAD^+ + 4H_2O + 3ATP$$

One way of indicating the ATP yield from oxidative phosphorylation is the **P/O ratio**, which is expressed as the moles of inorganic phosphate (Pi) used per oxygen atom consumed. Thus, in above equation the P/O ratio is 3 because 3Pi and 1/2 O_2 are used. On the other hand, when a substrate is oxidized *via* a flavoprotein-linked dehydrogenase, only 2 mol of ATP are formed , *i.e.*, P/O ratio is 2.

β-OXIDATION OF FATTY ACIDS

In the mitochondria of all cells, enzymes in the outer (*e.g.*, thiokinase or acyl - CoA synthetase) and inner (*e.g.*, carnitine) membrane mediate the movement of free fatty acids derived from fat molecules into the mitochondrial matrix. In the matrix, each fatty acid molecule exists in the form of "active fatty acid" or "fatty acyl CoA" and is broken down completely by a cycle of reactions, called **β-oxidation** that trims two carbons (*i.e.*, one acetyl group) at a time from its carboxyl end (β-end), generating one molecule of acetyl - CoA in each turn of cycle (Fig. 10.19). The acetyl- CoA produced is fed into Krebs cycle to be further oxidized.

Energetics of fatty acid oxidation. During β-oxidation, two ATP molecules are utilized for the activation of a fatty acid (*e.g.*, 16-carbon containing palmitic acid); thus, one ATP is used by acyl-CoA synthetase outside the mitochondria; another ATP (*i.e.*, GTP) is used by mitochondrial acyl-CoA synthetase. During oxidation of a fatty acid, water is added and 4 hydrogen atoms are removed, forming one $FADH_2$ molecule and one NADH molecule, from 2 carbon atoms nearest to CoA. When electrons of $FADH_2$ and NADH are passed through ETS, they release 5ATP molecules for each of the first 7 acetyl - CoA molecules formed by β-oxidation of palmitic acid, *i.e.*, $7 \times 5 = 35$ ATP molecules. β - oxidation of palmitic acid produces in total 8 mol of acetyl - CoA, each of which on oxidation by Krebs cycle produces 12ATP molecules; thus, making $8 \times 12 = 96$ATP molecules *via* this route. By deducting 2ATP used for initial activation of the fatty acid, a net gain of 129ATP (*i.e.*, $35 + 96 - 2 = 129$) is achieved. In terms of energy 129 ATP molecules contain 4747 kJ (129×36.8 kJ). As the free energy of combustion of palmitic acid is 9791 kJ/mol, the process of β-oxidation captures as ATP molecules on the border of 48 per cent of the total energy of combustion of the fatty acid (see **Mayes** and **Granner**, 1985).

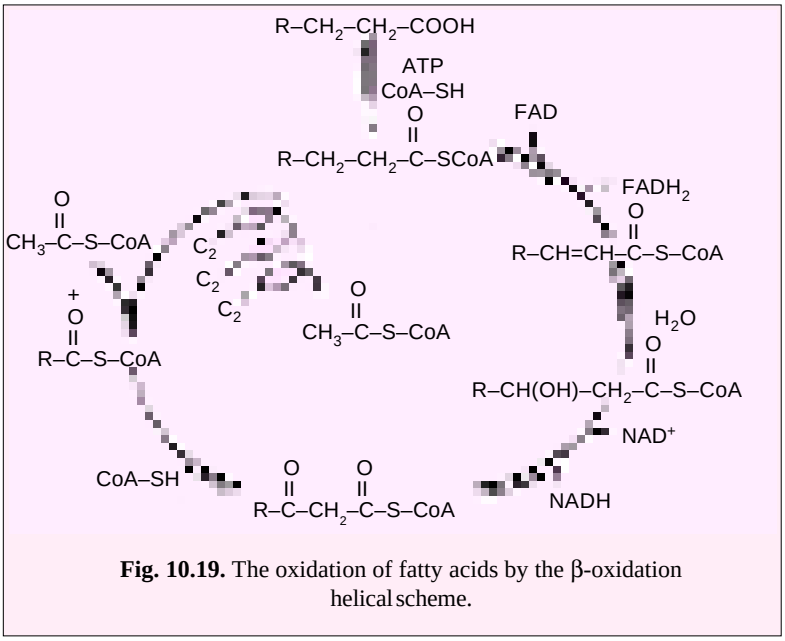


Fig. 10.19. The oxidation of fatty acids by the β-oxidation helical scheme.

OXIDATION OF PROTEINS

Before proteins can be introduced into the mainstream of metabolism (catabolism) they must be split into amino acids. The process is accomplished by protease enzymes similar to the process occurs in digestion. Each peptide bond is severed with the introduction of a water molecule, a hydrolytic

reaction. Next, nitrogen is removed from amino acids by any one following two processes: oxidative deamination and transamination.

During **oxidative deamination,** the amino group of the amino acid is split off from the rest of the molecule, forming ammonia (NH_3). The remainder of the amino acid then enters the main metabolic stream as a keto acid. Water is required for this process and two hydrogens are removed by coenzyme NAD. The ammonia formed during deamination may be immediately excrerted or organized into another molecule before excretion; for example, in human being the ammonia is converted into urea molecules by the liver cells before being sent *via* the blood to the kidney. The energy derived from oxidative deamination depends upon the amino acid involved. For example, oxidative deamination of glutamic acid involves its conversion into α-ketoglutaric acid, which is oxidized by the Krebs cycle:

$$\text{Glutamic acid (amino acid)} \xrightarrow[H_2O \quad NH_3]{NAD \rightarrow NADH_2} \alpha\text{-Ketoglutaric acid (keto acid)}$$

In this case, high-energy phosphates would be created by the transfer of hydrogen from $NADH_2$ (formed in deamination) through the cytochrome system.

Transamination reaction consists of an amino group being shifted from one molecule to another in exchange for an oxygen. For example, due to transamination amino acid glutamic acid being converted into α-ketoglutaric acid, as is shown by following reaction:

$$\underset{\text{(amino acid)}}{\text{Glutamic acid}} + \underset{\text{(keto acid)}}{\text{Oxaloacetic acid}} \longrightarrow \underset{\text{(keto acid)}}{\alpha\text{-Ketoglutaric acid}} + \underset{\text{(amino acid)}}{\text{Aspartic acid}}$$

In this case, the amino group is not lost completely but is transferred to one of the substrates of Krebs cycle. Oxaloacetic acid loses its oxygen and picks up the NH_2 group and becomes the amino acid, aspartic acid. Thus, one amino acid is converted into keto acid, while another keto acid is transformed into an amino acid. The usual purpose of such reactions is to maintain a particular balance among amino acids and substrates rather than providing grist for the metabolic mill.

Some amino acids such as alanine, cysteine, glycine, hydroxyproline, serine and threonine undergo enzymatic reaction to become pyruvic acid which enters mitochondria and is changed into acetyl-CoA and oxidized by Krebs cycle. Some other amino acids such as phenylalanine, tyrosine, tryptophan, lysine and leucine form acetyl-CoA directly without first forming the pyruvic acid.

OTHER FUNCTIONS OF MITOCHONDRIA

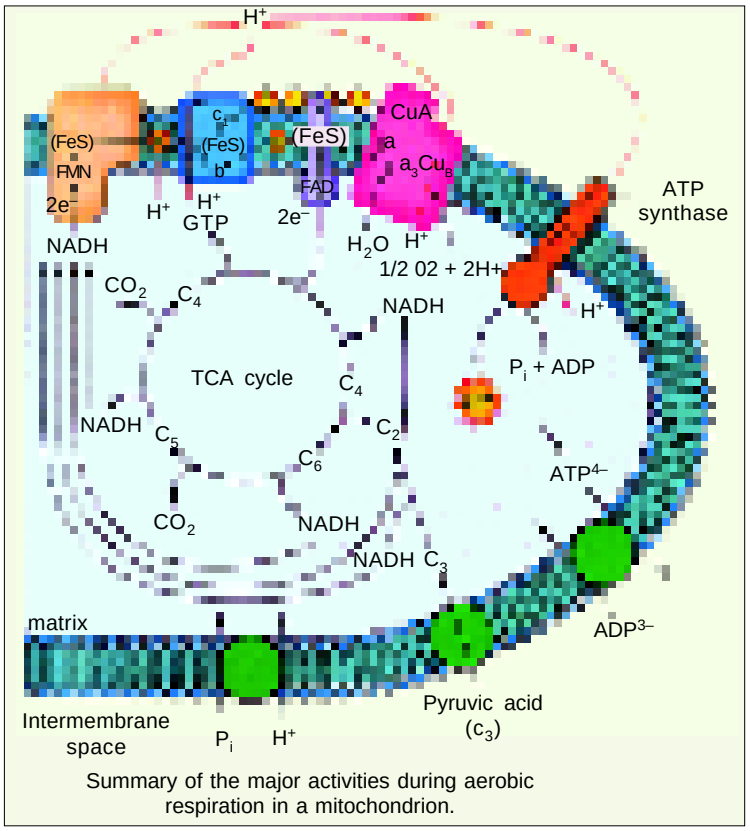
Besides the ATP production, mitochondria serve the following important functions in animals:

1. Heat production or thermiogenesis. As we have already discussed earlier that only 45 per cent of the energy released during the oxidation of glucose is captured in the form of ATP, the rest 55 per cent is either lost as heat or used to regulate body temperature of warm-blooded animals. In some mammals, especially young animals and hibernating species, there is a specialized tissue called **brown fat**. This tissue, typically located between the shoulder blades, is especially important in temperature regulation; it produces large quantities of body heat necessary for arousal from hibernation. The colour of brown fat comes from its high concentration of mitochondria, which are sparse in ordinary fat cells. The mitochondria appear to catalyze electron transport in the usual way but are much less efficient at producing ATP. Hence, a higher than usual fraction of the oxidatively released energy is converted directly to heat (called **non-shivering thermiogenesis)**.

2. Biosynthetic or anabolic activities. Mitochondria also perform certain biosynthetic or anabolic functions. Mitochondria contain DNA and the machinery needed for protein synthesis. Therefore, they can make less than a dozen different proteins. The proteins so far identified are subunits of the ATPase, portions of the reductase responsible for transfer of electrons from CoQ to the iron of Cyt c, and three of the seven subunits in cytochrome oxidase. Altogether, no more than 5–10 per cent of mitochondrial

components can be attributed to mitochondrial genes.

Some biosynthetic functions of mitochondria are of primary benefit to the rest of the cell. For example, the synthesis of **haeme** (needed for cytochromes, myoglobin and haemoglobin) begins with a mitochondrial reaction catalyzed by the enzyme, delta or δ-aminolevulinic acid synthetase. Likewise, some of the early steps in the conversion of cholesterol to steroid hormones in the adrenal cortex are also catalyzed by mitochondrial enzymes.



Summary of the major activities during aerobic respiration in a mitochondrion.

3. Accumulation of Ca^{2+} and phosphate. In the mitochondria of **osteoblasts** present in tissues undergoing calcification large amount of Ca^{2+} and phosphate (PO_4^{-}) tend to accumulate. In them microcrystalline, electrone-dense deposits may become visible. Sometimes, the mitochondria assume storage function, *e.g.*, the mitochondria of ovum store large amounts of yolk proteins and transform into yolk platelets.

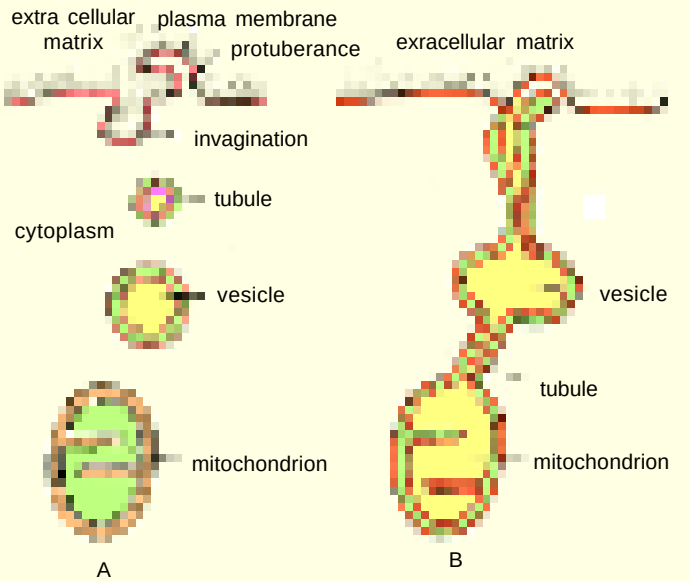


Fig. 10.20. Hypothetical diagrams showing the origin of mitochondria from plasma membrane.

BIOGENESIS OF MITOCHONDRIA

Regarding the origin of the mitochondria, several hypotheses have been postulated which are as follows:

1. "de novo" origin. According to this hypothesis, the mitochondria are originated "de novo" (L. anew) from the simple building blocks such as amino acids and lipids. But, there is no direct evidence in suppport of "de novo" hypothesis for the origin of the mitochondria therefore, it is discarded now.

2. Origin from the endoplasmic reticulum or plasma

membrane. According to **Morrison** (1966) the new mitochondria might have been originated from the endoplasmic reticulum or plasma membrane(Fig.10.20). This hypothesis also could not provide direct evidences, therefore, it is not well accepted at present time.

3. Origin by division of pre-existing mitochondria. The electron microscopic and radio-autographic observations of the culture cells have shown clearly that the new mitochondria are originated by the growth and division of pre-existing mitochondria. On average, each mitochondrion must double in mass and then divide in half once in each cell generation. Mitochondria are distributed between the daughter cells during mitosis and their number increase during interphase. Electron microscopic studies of *Neurospora crassa* (**Luck**, 1963) and HeLa cells (**Attardi** *et al.*, 1975) have suggested that organelle division begins by an inward furrowing of the inner membrane, as occurs in cell division in many bacteria (Fig. 10.21). After elongating, one or more centrally located cristae form a partition by growing across the matrix and fusing with the opposite inner membrane. This separates the matrix into two compartments. The outer membrane then invaginates at the partition plane, constricting until there is membrane fusion between the two inner membrane walls. Thus, two separable daughter mitochondria are formed.

Mitochondria as semiautonomous organelles. Recently the study of mitochondrial and chloroplast biogenesis became of great interest because it was demonstrated that these organelles contain DNA as well as ribosomes and are able to synthesize proteins. The term **semiautonomous organelles** was applied to the two structures in the recognition of these findings. This term also indicated that the biogenesis was highly dependent on the nuclear genome and the biosynthetic activity of the ground cytoplasm. It is well established now that the mitochondrial mass grows by the integrated activity of both genetic systems, which cooperate in time and space to synthesize the main components. The mitochondrial DNA codes for the mitochondrial, ribosomal and transfer RNA and for a few proteins of the inner membrane. Most of the proteins of the mitochondrion, however, result from the activity of the nuclear genes and are synthesized on ribosomes of the cytosol (cytoplasmic matrix). The cooperation of two genomes has been greatly clarified by studies on the molecular assembly of cytochrome oxidase (**Saltzgaber** *et al.*, 1977). This cytochrome, as studied in *Saccharomyces cerevisiae* is made up of seven polypeptide subunits for a combined molecular weight of 139,000 daltons. Three of the polypeptides are coded by mt DNA and assembled on mitochondrial ribosomes. They are very hydrophobic and high in molecular weight (23,000 – 40,000 daltons). The remaining four subunits are coded by nuclear DNA and made on cytoplasmic ribosomes. These are hydrophilic polypeptides of lower molecular weight (4500–14,000 daltons).

Mitochondrial DNA. Mitochondrial DNA (mt DNA) molecule is relatively small, simple, double-stranded and except for the DNA of some algae and protozoans, it is circular. The size of mitochondrial genome is very much large in plants than in animals. Thus, mt DNA varies in length from about 5 µm in most animal species to 30 µm or so in higher plants. The mt DNA is localized in the matrix and is probably attached to the inner membrane at the point where DNA duplication starts. This duplication is under nuclear control and the enzymes used (*i.e.*, polymerases) are imported from the cytosol.

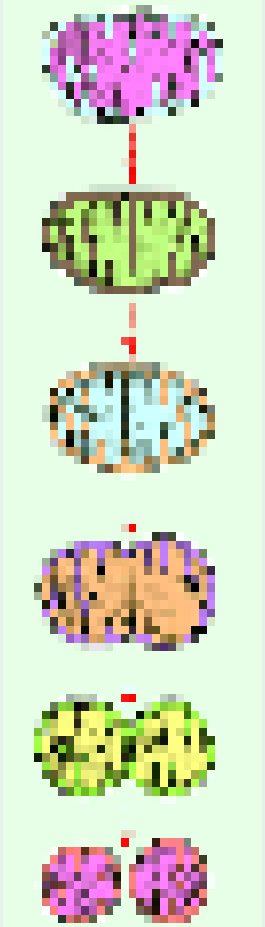


Fig. 10.21. Fission of a mitochondrion by partition formation (after Thorpe, 1984).

Mitochondrial ribosomes. Mitochondria contain ribosomes (called **mitoribosomes**) and polyribosomes. In yeast and *Neurospora*, ribosomes have been ascribed to a 70S class similar to that of bacteria ; in mammalian cells, however, mitoribosomes are smaller and have a total sedimentation coefficient of 55S, with subunits of 35S and 25S (**Attardi** *et al*., 1971). In mitochondria, ribosomes appear to be tightly associated with the inner membrane.

Mitochondrial protein synthesis. As already described, mitochondira can synthesize about 12 different proteins, which are incorporated into the inner mitochondrial membrane. These proteins are very hydrophobic (*i.e*., they are proteolipids). Thus, on the mitoribosomes are made the following proteins : three largest subunits of cytochrome oxidase (Fig. 10.6), one protein subunit of the cytochrome b-c_1 complex, four subunits of ATPase and a few hydrophobic proteins. One of the best known differences between the two mechanisms of protein synthesis (*i.e.*, in the cytosol and in the mitochondrial matrix) is in the effect of some inhibitors. The mitochondrial protein synthesis is inhibited by **chloramphenicol**, while synthesis in the cytosol (cytoplasmic matrix) is not affected by this drug. In contrast, **cycloheximide** has the reverse effect.

Import mechanism of mitochondrial proteins. Most mitochondrial proteins are coded by nuclear genes and are synthesized on free ribosomes in the cytosol (cytoplasmic matrix). The import of these polypeptides involves similar mechanism both in mitochondria, and chloroplasts. The transport processes involved have been most extensively studied in mitochondria, especially in yeasts (**Attardi** and **Schatz**, 1988). A protein is translocated into the mitochondrial matrix space by passing through sites of adhesion between the outer and inner membrane, called **contact sites**. Translocation is driven by both ATP hydrolysis and the electrochemical gradient across the inner membrane, and the transported protein is unfolded as it crosses the mitochondrial membranes. Only proteins that contain a specific **signal peptide** are translocated into mitochondria and chloroplasts. The signal peptide is usually located at the amino terminus and is cleaved off after import (Fig. 10.23A). Transport to the inner mitochondrial membrane can occur as a second step if a **hydrophobic signal peptide** is also present in the imported protein; this second signal peptide is unmarked when the first signal peptide is cleared (Fig. 10.23B). In the case of chloroplasts, import from the stroma into the thylakoid likewise requires a second signal peptide.

Mitochondrial lipid biosynthesis. The biogenesis of new mitochondria and chloroplasts requires lipids in addition to nucleic acids and proteins. Chloroplasts tend to make the lipids they require. For example, in spinach leaves, all cellular fatty acid synthesis takes place in the chloroplast. The major glycolipids of the chloroplast are also synthesized locally.

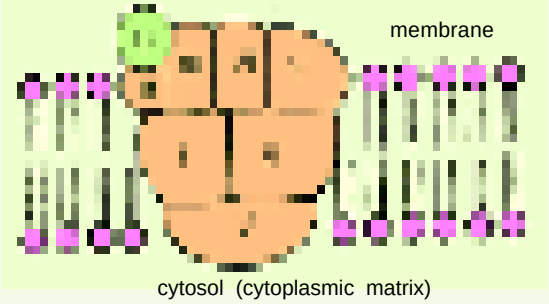


Fig. 10.22. Model of cytochrome oxidase of yeast showing topographical relationship of its seven subunits and its relation with cytochrome c (after Thorpe, 1984).

Mitochondria, on the other hand, import most of their lipids. In animal cells the phospholipids — **phosphatidyl-choline** and **phosphatidyl-serine**—are synthesized in the ER and then transferred to the outer membrane of mitochondria. The transfer reactions are believed to be mediated by **phospholipid exchange proteins**; the imported lipids then move into the inner membrane, presumably at contact sites. Inside mitochondria, some of the imported phospholipids are decarboxylated and converted into **cardiolipin** (diphosphatidyl glycerol). Cardiolipin is a " double" phospholipid that contains four fatty - acid tails; it is found mainly in the inner mitochondrial membrane, where it constitutes about 20 per cent of the total lipids.

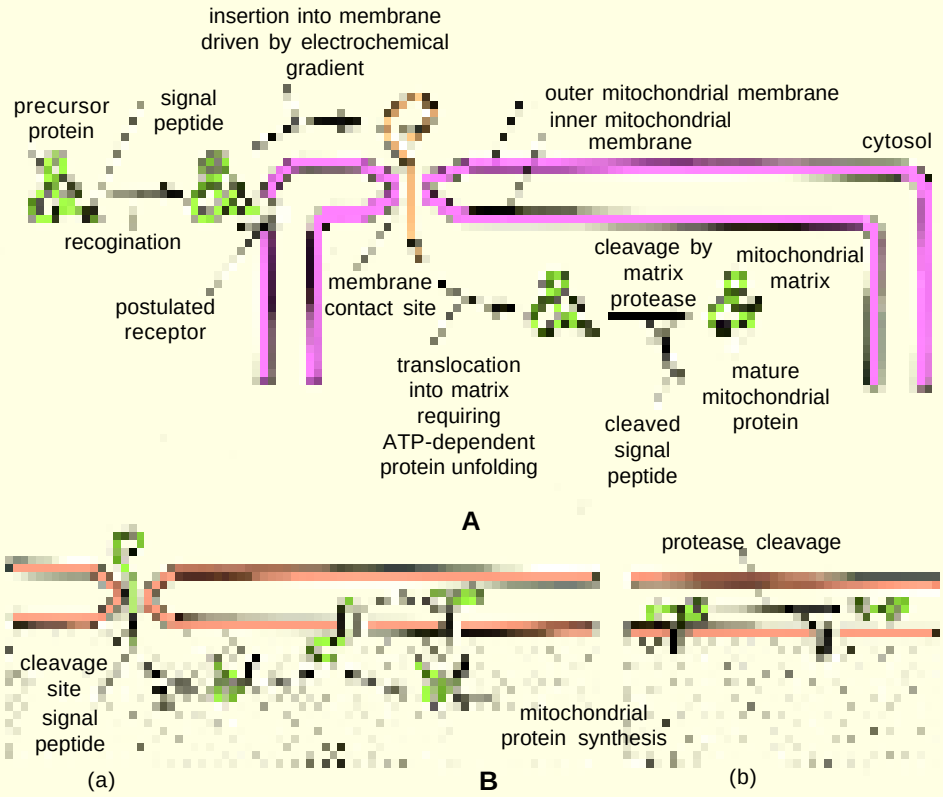


Fig. 10.23. A—Protein import by mitochondrial matrix through single signal; B—Import of proteins from the cytosol (cytoplasmic matrix) to the mitochondrial intermembrane space or inner membrane through two (or multiple) signals (after Albert *et al*; 1989).

Prokryotic Origin or Symbiont Hypothesis

Early cytologists such as **Altmann** and **Schimber** (1890) have suggested the possibility of origin of the mitochondria from the prokaryotic cells. According to their hypothesis, the mitochondria and chloroplasts may be considered as intra-cellular parasites of the cells which have entered in the cytoplasm of eukaryotic cells in early evolutionary days, and have maintained the symbiotic relations with the eukaryotic cells. The mitochondira are supposed to be derived from the bacterial cells (purple bacteria) while chloroplasts are supposed to be originated from the blue green algae (see **Margulis**, 1981). Due to these reasons **Altmann** suggested the name "**bioblasts**" to the mitochondria and he also hinted about their self-duplicating nature.

Recent cytological findings have also suggested many homologies between the mitochondria and the bacterial cells. The similarities between the two can be summarised as follows :

1. Similarity in inner mitochondrial membrane and bacterial plasma membrane. (i) In the mitochondria the enzymes of the respiratory chain are localized on the inner mitochondrial membrane like the bacteria in which they remain localized in the plasma membrane. The bacterial plasma membrane resembles with the inner mitochondrial membrane in certain respects.

(ii) The plasma membrane of certain bacterial cells gives out finger-like projections in the cytoplasm known as mesosomes. The mesosomes can be compared with mitochondrial crests. **Salton** (1962) has reported respiratory chain enzymes in the mesosomes.

(iii) Because the outer mitochondrial membrane resembles with the plasma membrane, therefore, it may be assumed that the mitochondrial matrix and the inner mitochondrial membrane represent the symbiont which might be enclosed by the membrane of the cellular origin (outer mitochondrial membrane).

2. Similarity in DNA molecule. The DNA molecule of the mitochondria is circular like the DNA molecule of the bacterial cells. Further the replication process of the mitochondrial DNA is also similar to bacterial DNA.

3. Similarity in ribosomes. The mitochondrial ribosomes are small in size and resemble the ribosomes of the bacteria.

4. Similarity in the process of protein synthesis. The process of protein synthesis of both mitochondria and bacteria is fundamentally same because in both, the process of protein synthesis can be inhibited by same inhibitor known as chloramphenicol.

Further, the mitochondria for the process of protein synthesis depend partially on the mitochondrial matrix and DNA and partially on the nucleus and cytoplasm of the eukaryotic cells. This shows the symbiotic nature of the mitochondria.

Due to the above-mentioned similarities between the bacteria and mitochondria, the symbiont hypothesis postulated that the host cell (eukaryotic cell) represented an

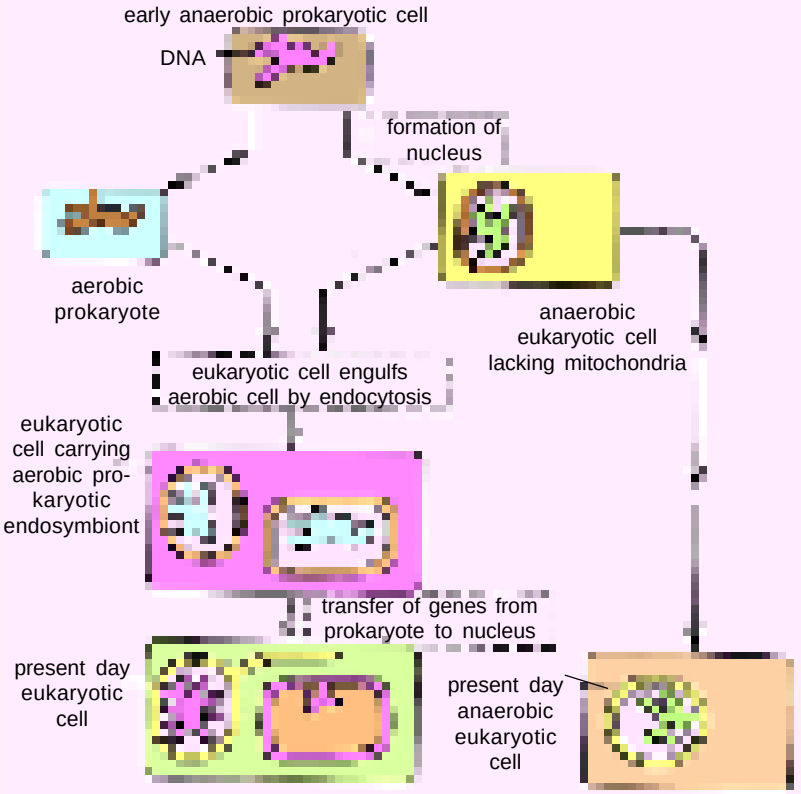
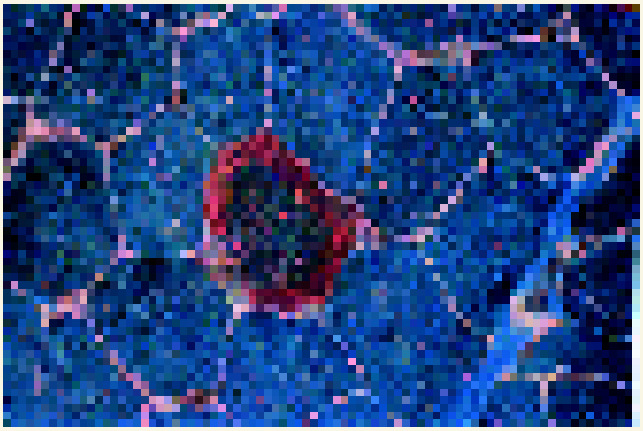


Fig. 10.24. Symbiotic origin of mitochondria and chloroplast (after Alberts *et al*., 1989).



These degenerating muscle fibers are from a biospy of a patient and show accumulations of red staining "blotches" just beneath the cell's plasma membrane, which are due to abnormal proliferation of mitochondria.

anaerobic organism which derives the required energy from the oxidations of food by the process of glycolysis. While the mitochondria represent the symbionts which respire **aerobically** and contain the enzymes of **Krebs cycle** and **respiratory chain**. The symbionts seem to be capable to get the energy by **oxidative phosphorylation** from the partially oxidised food (pyruvic acid) of the host cell.

REVISION QUESTIONS

1. What are the characteristic structural features of mitochondira that aid in their identification ?
2. Why are mitochondria termed as the “power houses” of the eukaryotic cells ?
3. What is the major function of mitochondria ?
4. What differences exist in structure and function between the inner and outer membranes of the mitochondria ?
5. Would you expect plant cells to have the Embden-Meyerof glycolytic pathway ? Explain your answer.
6. What is the significance of acetyl-coenzyme A and where does it come from ?
7. What is an oxidative decarboxylation ? Give an example.
8. Of what value to the cell is a cyclic process such as the Krebs cycle ?
9. Define the terms redox reactions and redox potential. Explain Mitchell's chemiosmotic coupling theory.
10. Define the following : electron transport; respiratory chain; oxidative phosphorylation; cytochrome oxidase. Summarize various schemes for coupling electron transport to ADP phosphorylation.
11. What is a fermentation, and why is it needed by some cells ?
12. What is the special function of brown fat mitochondria and how is it carried out ?
13. Describe the β-oxidation of fats.
14. Describe the biogenesis of the mitochondria.
15. Explain why are the mitochondria considered as semiautonomous organelles.
16. Describe the energetics of glucose oxidation and compare it with that of fat oxidation.
17. Give a short account of symbiotic origin of mitochondria and chloroplasts.
18. Compare the functions of mitochondria and chloroplasts.