

CHAPTER 5

5. MITOCHONDRION STRUCTURE AND FUNCTION

5.1 Introduction

- i) Definition: Mitochondrion means thread-like granules.
- ii) Number per cell vary from several to a few thousands.

They are especially abundant in cells and parts of cells that are associated with active processes. For example, in flagellated protozoa or in mammalian sperm, mitochondria are concentrated around the base of the flagellum or flagella.

Mitochondria origin - are thought to have descended from specialized bacteria probably purple nonsulfur bacteria) that somehow survived endocytosis by another species of prokaryote or some other cell type, and became incorporated into the cytoplasm.

Arrangement of the mitochondrion within the cell: Mitochondrion often occurs in close association with the structures that either utilize the ATP they produce or provide the Mitochondrion with oxidizable substrate.

Example i) Muscle cell mitochondria are seen linked up adjacent to the fibrils that utilize the ATP.

ii) Mitochondrion are seen surrounding lipid droplets that contain fatty acids destined for mit oxidation.

5.2 Micro-anatomy of mitochondrion.

- i) Outer membrane system
- ii) Cristae (inner membrane)
- iii) Intermediate membrane space
- iv) Mitochondrial Matrix space (called Mitoplast when it includes matrix and membrane)
- v) Inner membrane knobs
- vi) Ribosome
- vii) DNA.

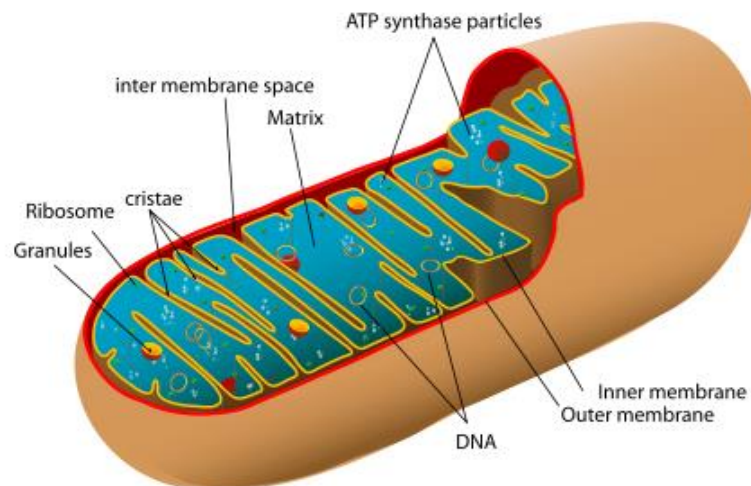


Fig. 5.1 Simplified 3-D structural model of a Mitochondria. Parts of a Mitochondria in 3-D include : i) Outer membrane ii) Cristae (inner membrane) iii) Intermediate membrane space iv) Matrix space v) Inner membrane knobs vi) Ribosome vii) DNA.

The Arrangement of the mitochondrion: Mitochondrion is often occurs in close association with the structures that either utilize the ATP they produce or provide the Mitochondrion with oxidizable substrate e.g. Muscle cell mitochondria are seen linked up adjacent to the fibrils that utilize the ATP. Mitochondrion are also seen surrounding lipid droplets that contain fatty acids destined for Mitochondrion oxidation.

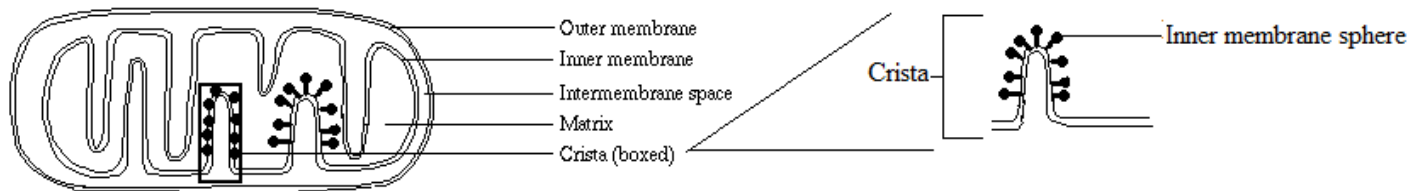


Fig. 5.2 Simplified 2-D structure of a Mitochondria

5.2.1 Outer membrane system or Outer mitochondria Membrane (OMM)

- i) It defines external boundary of the organelle.
- ii) May be involved in the biosynthesis of membrane phospholipids- evidenced by the presence of enzymes involved in lipid synthesis e.g. glycerophosphate-acyl transferase.
- iii) O M M is highly permeable to small molecules such as salts, sugar, nucleotides and co-enzymes.
- iv) Permiability of OMM allows inner membrane direct access to metabolites present in the cell sap

5.2.2 The inter membrane space

- i) separates inner and outer membrane
- ii) Assumed to be a single continuous chamber bounded by the inner and outer membrane. Evidence of continuity-small solutes penetrate between intracristal and peripheral regions.
- iii) Pediculi cristae is said to interrupt this continuity (see figure above). Pediculi cristae are small tubular channels.

5.2.3 The matrix protein system or Mitochondrial Matrix

- i) This is the interior of the mitochondrion
 - ii) Contain the following;
 - a) All the enzymes and cofactors involved in the Krebs cycle with the exception of succinate dehydrogenase.
 - b) Enzymes involved in β -oxidation. Example it contains pyruvate dehydrogenase which catalyzes the conversion of pyruvate to acetyl-coA and also enzymes involved in fatty acids β -oxidation which degrades fatty acids into acetyl-coA units that enter the Krebs cycle.
 - c) DNA, RNA, Ribosomes and other enzymes involved in nucleic acid and protein synthesis.
- The material contained in mitochondria matrix are involved in mitochondrion growth and division.

5.2.4 The inner mitochondrion membrane (IMM) system

- i) It is made up of the following ;
 - a) Cristae-which form the bulk of the inner membrane.
 - b) Inner boundary membrane (refer to the diagram)
The two seem to have continuity with each other.
- ii) Morphology consists of spherical particles that protrude from the inner side of the cristae

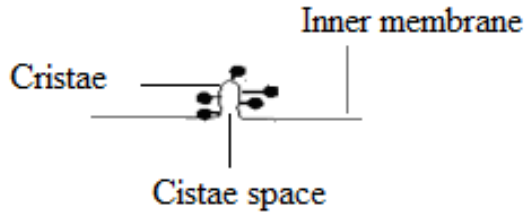


Fig. 5.3 Diagram of mitochondrion Cristae

- ii) IMM have high protein to lipid ratio and high unsaturated to saturated phospholipid ratio.
- iii) It contains large protein content, involved in electron transfer and oxidative phosphorylation.
- iv) IMM have inner membrane knobs-these knobs are spherical, 8-9 nm in diameter, protrude into the matrix and contain a protein complex which plays a key role in mitochondrion ATP formation.

5.2.5 The inner membrane knob: these are inner membrane spheres, that are part of the inner membrane and cristae

5.2.6 Mitochondrion Ribosome or mitoribosome: are coded by rRNA genes in the mtDNA. The function of mtRNA is to translate a small number (10-15) of mtDNA-encoded messenger RNAs which specify the same basic set of polypeptides. In other words it is used to synthesize mitochondrial proteins. Most protein synthesized here are integrated in the inner membrane.

5.2.7 Mitochondrion genome

- it extranuclear genome
- the size vary between species.

Table 5.1 Examples of mtDNA and their sizes

	Species	Genome size	Reference
1	Tetrahymena	46-kb	Borst 1 980
2	Animal mtDNA	16-20kb	Attardi 1985
3	Maize	570kb	Lonsdale et al1984
4	Yeast Mitochondrial Genome	75-78 kb	Leaver 1982

- The mtDNA in human
 - i) Most mtDNA is translated (no significant non-coding regions).
 - ii) The genes includes the 2 rRNA genes, 14 tRNA genes, and 12 protein-coding genes.
 - iii) Most genes lack a complete termination codon instead they end with either a T or TA following the last sense codon.
- The mtDNA in *S. cerevisiae*
 - i) The mtDNA genes are scattered with large non-coding sequences
- Transcription of RNA molecule in mammalian mtDNA is in a polycistronic RNA molecules starting at promoters all located in the gene-free region close to the origin of replication.
- In most organism mitochondrial protein synthesis machinery the RNA needed are encoded almost entirely by mtDNA but the proteins component are encoded by the nuclear DNA.
- The mtDNA code vary from species to species and from universal code. For example stop codon in universal code is used to code for tryptophan codon in mitochondria of animals, fungi, and protozoa, but not in those of higher plants.
- Functioning of mtDNA is controlled by nuclear genes, example Replication and expression of mtDNA, specification all the enzymes of the mitochondrial matrix and the assembly of the multisubunit enzyme complexes of the oxidative phosphorylation apparatus.

- The mtDNA is involved in biosynthesis of few of mitochondrion proteins, otherwise more proteins are imported from nuclear coded genes

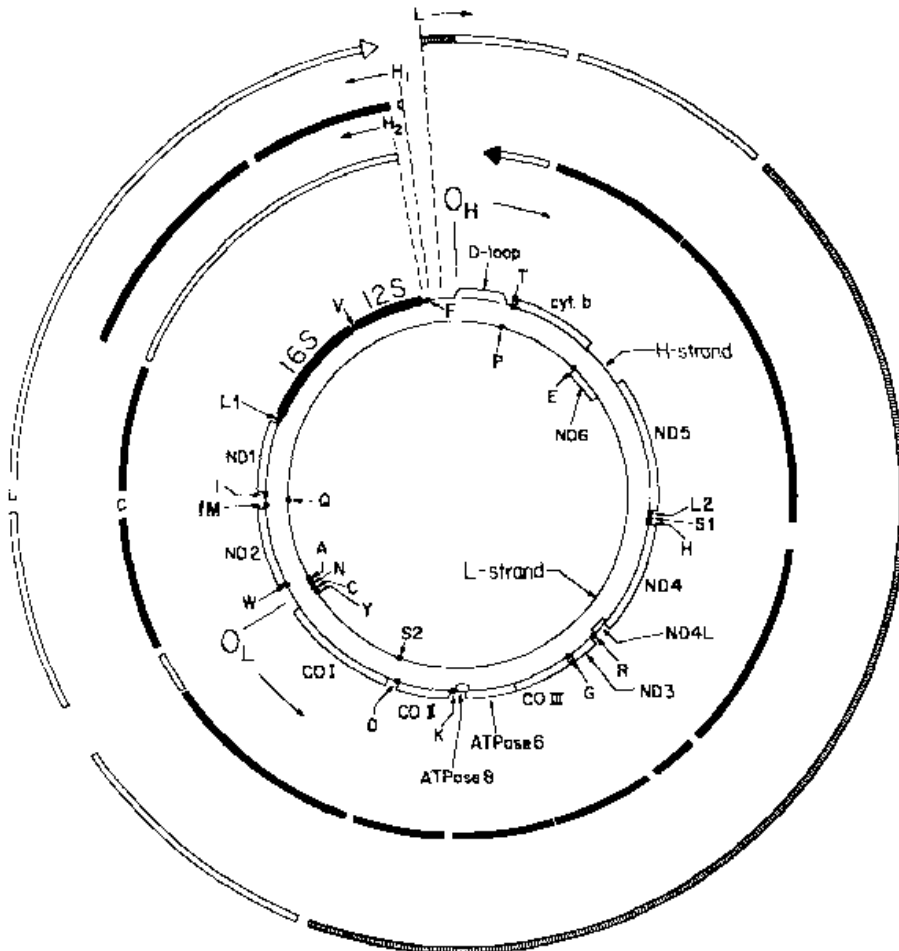


Figure 1 Genetic and transcription maps of the **human mitochondrial genome**. The two inner circles show the positions of the 2 rRNA genes (1 2S and 16S), 14 tRNA genes (black circles), and 12 reading frames transcribed from the heavy (H)-strand, and the positions of 8 tRNA genes and one reading frame transcribed from the light (L)-strand. In the outer portion of the diagram, curved black bars represent the identified functional RNA species other than tRNAs resulting from processing of the two polycistronic primary transcripts of the H-strand starting at HI (rDNA transcription unit) and H2 (total H-strand transcription unit). Cross-hatched bars represent the identified RNA species resulting from processing of the polycistronic primary transcript of the L-strand. The white bars represent unstable, presumably nonfunctional by-products. COI, COII, and COIII : subunits I, II, and III of cytochrome c oxidase; cyt. b: apocytochrome b; ATPase 6 and ATPase 8: subunits 6 and 8 of H⁺-ATPase; NDI, ND2, ND3, ND4, ND4L, ND5, and ND6: subunits of NADH dehydrogenase; OH, OL : origin of H-strand and L-strand synthesis, respectively, A: ala; R: arg; N: asn; D: asp; C: cys; Q: gin; E: glu; G: gly; H: his; I: ile; L: leu; K: Iys; fM: F-met; F: phe; P: pro; S: ser; T: thr; W: trp; Y: tyr; V: val. (Modified from Attardi 1986.)

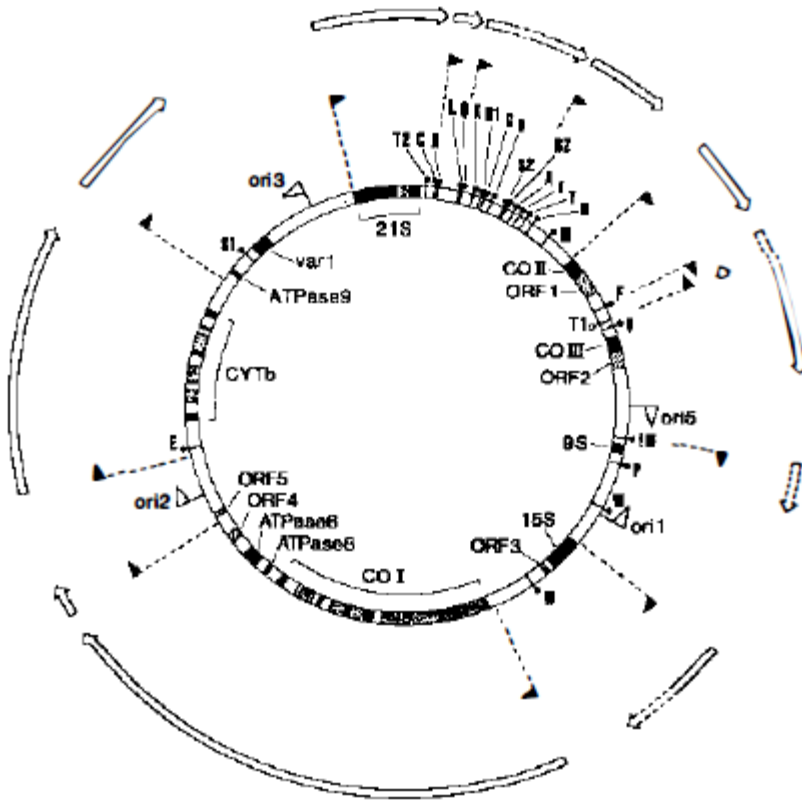


Figure 2 Maps of genes and transcription units of the mitochondrial genome from *S. cerevisiae*. In the inner map, the identified genes or their exons are represented by black boxes, the intergenic unidentified reading frames (ORF1-ORF5) by hatched boxes, and the intronic reading frames by dotted areas. The tRNA genes transcribed from the main coding strand are indicated by T, the single tRNA gene transcribed from the opposite strand (tRNATh), by f. The black flags indicate the positions of the transcription initiation sites and the direction of transcription, the white flags, the positions of the functional origins of replication (ori 1 , ori2, ori3, and ori5) and the 5' to 3' direction of the corresponding identified RNA primers. In the outer portion of the diagram, the identified primary transcripts are represented by curved open bars, with the arrow pointing towards the 3'-end and the dashed contour indicating a tentative extension of the transcript. 21S and 16S: large and small rRNAs; 9S: RNA component of mitochondrial RNase P; the specificities of the tRNA genes are indicated by the one-letter amino acid code. vor1: protein of the small ribosomal subunit; ATPase 9: subunit 9 of H⁺-ATPase; the other symbols designating the individual protein coding genes are as in Figure 1 . The positions of the genes, transcription initiation sites, origins of replication and primary transcripts were derived from the references given in the text and from Colin et al (1985), Bordonne et al (1986), Francisci et al (1987) and Backer & Getz (1987).

From Walter Neupert (1997)

5.3 General functions of Mitochondria.

- i) Site of respiratory chain- electron transfer and oxidative phosphorylation. This is done through polarizing and depolarizing of mitochondrion molecule.

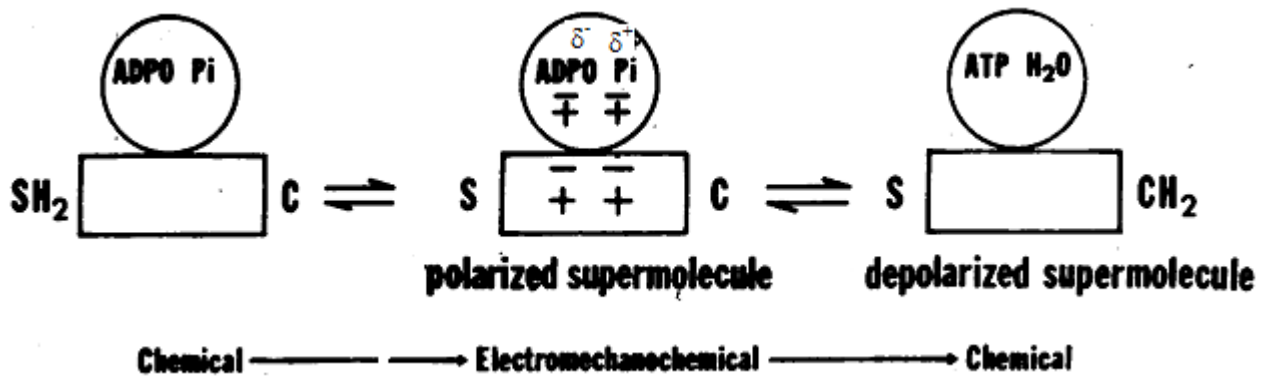


Fig. 5.4. Representation of electromechanochemical model of oxidative phosphorylation. SH₂=oxidizable substrate, C=mobile electron carrier.

- ii) Inner membrane is the site of certain enzymatic pathways (not directly related to energy metabolism) e.g. steroids metabolism pathway.
- iii) Synthesis of metabolites – Mitochondria contain proteases. In the matrix, the matrix processing protease matrix processing peptidase (MPP) cleaves the targeting sequence.
- iv) Protein sorting and targeting capabilities: take place on the outer and inner membrane to enable assembly of hydrophilic and hydrophobic membrane proteins for proper membrane topography.
- v) lipid metabolism
- vi) Free radical production,
- vii) Metal ion homeostasis.
- viii) Codes some proteins in its small genome. Translated proteins are exported and assembled in inner membrane and the remainder is translated in the cytosol and exported into the mitochondrion.

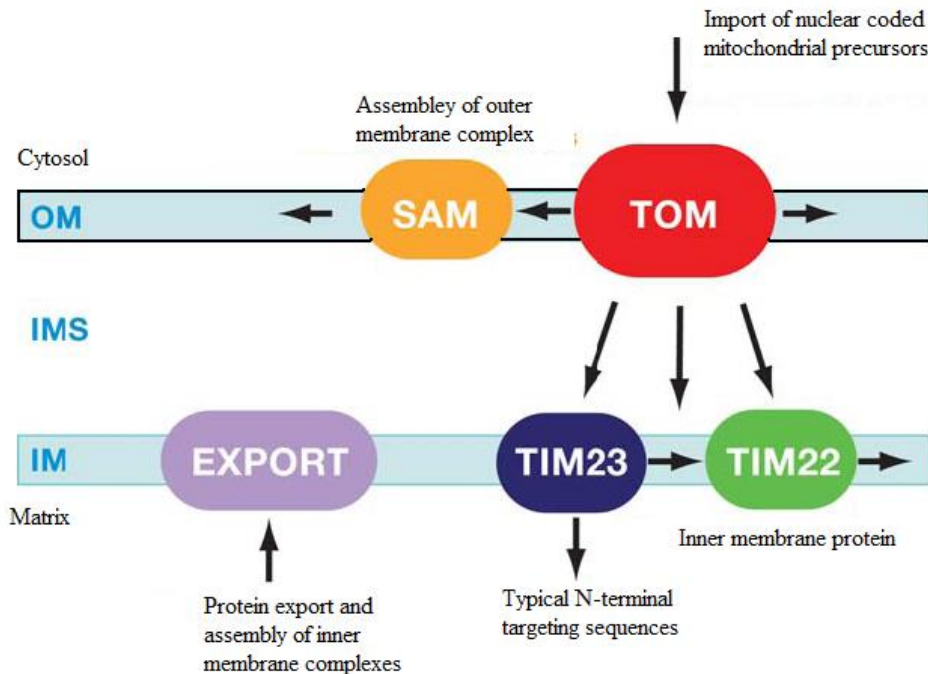


Fig. 5.5 TOM- translocase of the outer membrane- found on OM; SAM-The sorting and assembly machinery- found on OM; TIM- translocase of the inner membrane; OM – Outer membrane; IMS – intermembrane space; IM – Inner membrane

Schematic of translocation and assembly complexes in the mitochondrion. Nuclear-coded mitochondrial precursors cross the TOM complex. The SAM complex mediates the assembly of outer membrane proteins with complicated topologies. Proteins with a typical N-terminal targeting presequence are imported via the TIM23 complex, whereas inner membrane proteins such as the carrier proteins are imported via the TIM22 complex. Components mediating protein export and assembly of inner membrane complexes (designated EXPORT) include Oxa1p, Mba1p, and the complex consisting of Pnt1p, Mss2p, and Cox18p.

5.4 Biosynthesis of Mitochondrion

Mitochondrion are occur in most Eukaryotic cells. *de novo*.

In growing cells, mitochondrial membranes appear to increase in proportion to cell mass, like other cell membranes but not in accordance to Mitochondrion DNA replication.

In the same cell system (Pica-Mattocchia & Attardi 1972) , mtDNA replication occurs predominantly in the late S and G2 phases of the cell cycle

5.5 Preparing Mitochondria from Rat Liver

5.5.1 Rationale

There several reasons why liver is a convenient source for functional intact mitochondria. The major one is that, animal tissue and liver in particular is more readily homogenized than plant tissue because there are no cell walls. One can quickly and easily obtain a substantial quantity of liver mitochondria within less than an hour's preparation time. Although male rats and female rats can be used with equal success, for serious studies we usually males are preferred to avoid complications due to the estrous cycle. A good weight range for the animal is 200 - 250 gms, perhaps a bit larger. A liver from this size animal yields up to two ml or so of concentrated mitochondria. Since the liver is responsible for detoxification processes including metabolism of anesthetic agents, and because both an anesthetic and its metabolites can affect liver function, it is preferable from an experimental viewpoint to avoid using anesthetic or tranquilizing drugs. Animals will be sedated using isoflurane then decapitate them using a rat guillotine. Isoflurane is short acting and has not appeared to compromise mitochondria function.

5.5.2 Protocol

- i) Open up the animal with a medial incision (not a "medical" incision) from groin to sternum. Nb: adjective "medial" is an anatomical term meaning vertical, up the middle.
- ii) First separate the skin then the underlying muscle and peritoneum, revealing the liver. The liver is brown, large, and almost unmistakable.
- iii) Quickly get into the rat
- iv) Cuts through the sternum
If this done within 2-3 min of decapitation and opening the chest, the heart can be seen still beating.
- v) Chill the liver immediately by pouring a generous amount (100 ml) of ice-cold 0.85% NaCl into the peritoneal cavity.
- vi) Remove the liver in pieces or intact by cutting it off at the base
- vii) Drop it into a second beaker of ice-cold saline solution to continue to reduce the temperature.
- viii) Subsequent steps in the isolation should all be done at ice-bucket temperature.

- ix) Divide the tissue from one 200-225 gm rat into two equal portions, each weighing 3 to 5 grams (it is convenient).
- x) Homogenize the tissue using a homogenizer e.g "Tissuemizer" or any homogenizer.

NB: a) Ratio of medium to tissue is important

b) Fast and prolonged or overly vigorous homogenization can be damaging to mitochondria.

c) Great success has been achieved by draining the tissue then mincing it in a 50 ml plastic disposable beaker, followed by addition of 20 ml homogenizing medium (0.25M sucrose, 5 mM HEPES buffer, and 1 mM EDTA, pH 7.2). After mixing to suspend the mince homogenize at a setting of '40' for 10 sec.

- xi) Top off the homogenate with medium to fill each tube.

- xi) Remove large cell and tissue fragments and cell nuclei (the 'nuclear pellet') by centrifuging the homogenate at 500 x g for 10 minutes.

- xii) Pour the supernatant into a clean centrifuge tube, and without topping off we centrifuge at 9400 x g for 10 min bring down the mitochondrial pellet. Care must be taken to avoid pouring off the loose the mitochondrial pellet. Intact mitochondria tend to sediment more quickly than damaged mitochondria.

- xiii) Wipe off the white foamy material near the top of the tube that consists of lipids using a lab wiper. Mixing of lipids with the mitochondria suspension will cause some degree of uncoupling (loss of ability to maintain respiratory control).

- xiv) Use a pasteur pipet to remove the last bit of liquid

- xv) Use a glass rod to stir the remaining pellet into a smooth paste.

NB: Do NOT add buffer at all. The more liquid that remains with the pellet, the more difficult it is to homogenize all of the particles.

- xvi) Keep the centrifuge tube on ice while stirring, and try not to introduce air into the suspension.

Mitochondria keep best when concentrated, to minimize exposure to oxygen. They remain dormant until diluted into an oxygen-rich respiration medium.

- xvii) Transfer the paste into an eppendorf tube, avoiding air bubbles by careful pipetting with a micropipettor set to, say, 100 μ l or so. The suspensions are very viscous. Care must be taken to allow pressure to equalize after drawing up suspension, otherwise it may shoot up into the pipettor. Use of a pasteur pipet at this point results in loss of much of the pellet, since the material readily sticks to glass. Once transferred to an eppendorf tube the preparation (which must be stored on ice) is ready for use.