Mitochondria: Function

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Lesson: Mitochondria: Function
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Mitochondria: Function

Protein Import into mitochondria

As already mentioned, mitochondria have their own genetic system, ribosomes and t-RNAs and can synthesize their own proteins. Despite this, they are dependent on nuclear DNA for about 99% of their 1000 proteins. The mitochondrial proteins are synthesized at the cytosolic ribosomes and are then imported into the mitochondria. The presence of two membranes make protein import into mitochondria a complex process where proteins are destined for four different locations: matrix, inner membrane, intermembrane space and the outer membrane.

Protein import into mitochondrial matrix

The proteins that are destined for matrix have an N-terminal **pre-sequence**. The pre-sequence is characterized by the presence of 20-35 positively charged amino acids. The pre-sequence is recognized by the receptors of the translocons present on the outer mitochondrial membrane known as the *t*ranslocons of the *o*uter *m*embrane (Tom) which are complex of different proteins and named according to their molecular weight. Similar translocons are also found on the inner membrane known as *t*ranslocons of the *i*nner *m*embrane (Tim).

A protein that is to be translocated into the mitochondria should be atleast partially folded which is achieved by binding of the protein with molecular chaperones like Hsp70 (Fig. 1). The presequence first binds to the ***import receptors:*** Tom20 and then is transferred to Tom5. After this the protein is imported through ***general import pore***, Tom40 channel into the intermembrane space. From here, the protein is passed to Tim23 complex. Tim44 is present on the matrix side of the membrane and has bound mitochondrial Hsp70 which hydrolyses ATP and pulls the protein into the matrix. The presequence of the imported proteins are cleaved by **Matrix Processing Peptidases (MPP**) and are folded by mitochondrial Hsp70. The protein import requires H+ electrochemical gradient or proton motive force (pmf) that is generated by movement of H+ across the inner membrane.

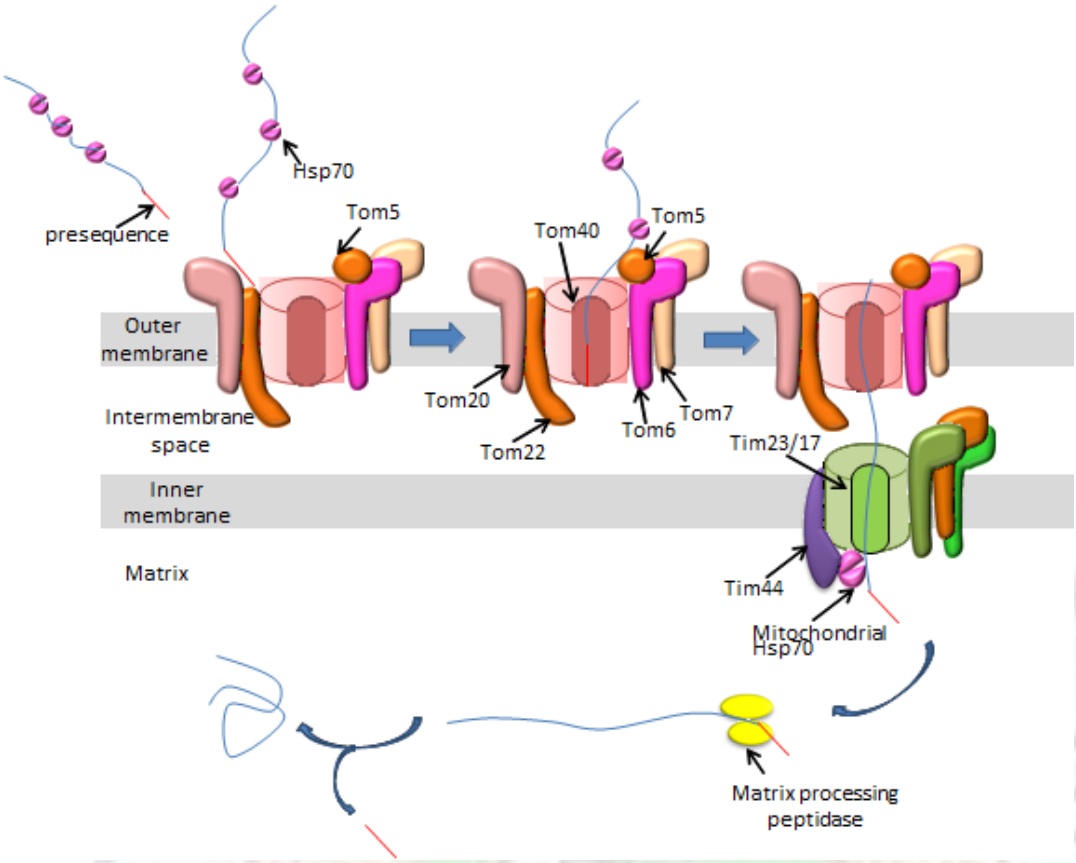

Fig. 1: Mechanism of the movement of protein into the matrix. The proteins destined for matrix have an N-terminal presequence consists of positively charged amino acids and is maintained in partially unfolded state by Hsp70. **Source: Author**

Protein import into mitochondrial inner membrane

Atleast three different pathways target proteins into inner mitochondrial membrane.

1. Some multipass proteins like ADP/ATP antiporter that are destined for inner mitochondrial membrane lack N-terminal presequence but have **multiple internal mitochondrial import signals**. Like proteins destined for matrix, these proteins are also maintained in partially folded by Hsp70. However these proteins also require additional Hsp90 (Fig. 2a). These proteins interact with an additional Tom70 receptor and then are translocated to Tom40 channel. In intermembrane space, mobile components of Tim (Tim9 and Tim10) also known as "Tiny Tims" direct them to Tim54 and then to the Tim22 import pore. Inside the import pore, the further protein translocation is hampered by **hydrophobic internal stop transfer sequences** and the protein is laterally moved into the inner mitochondrial membrane.

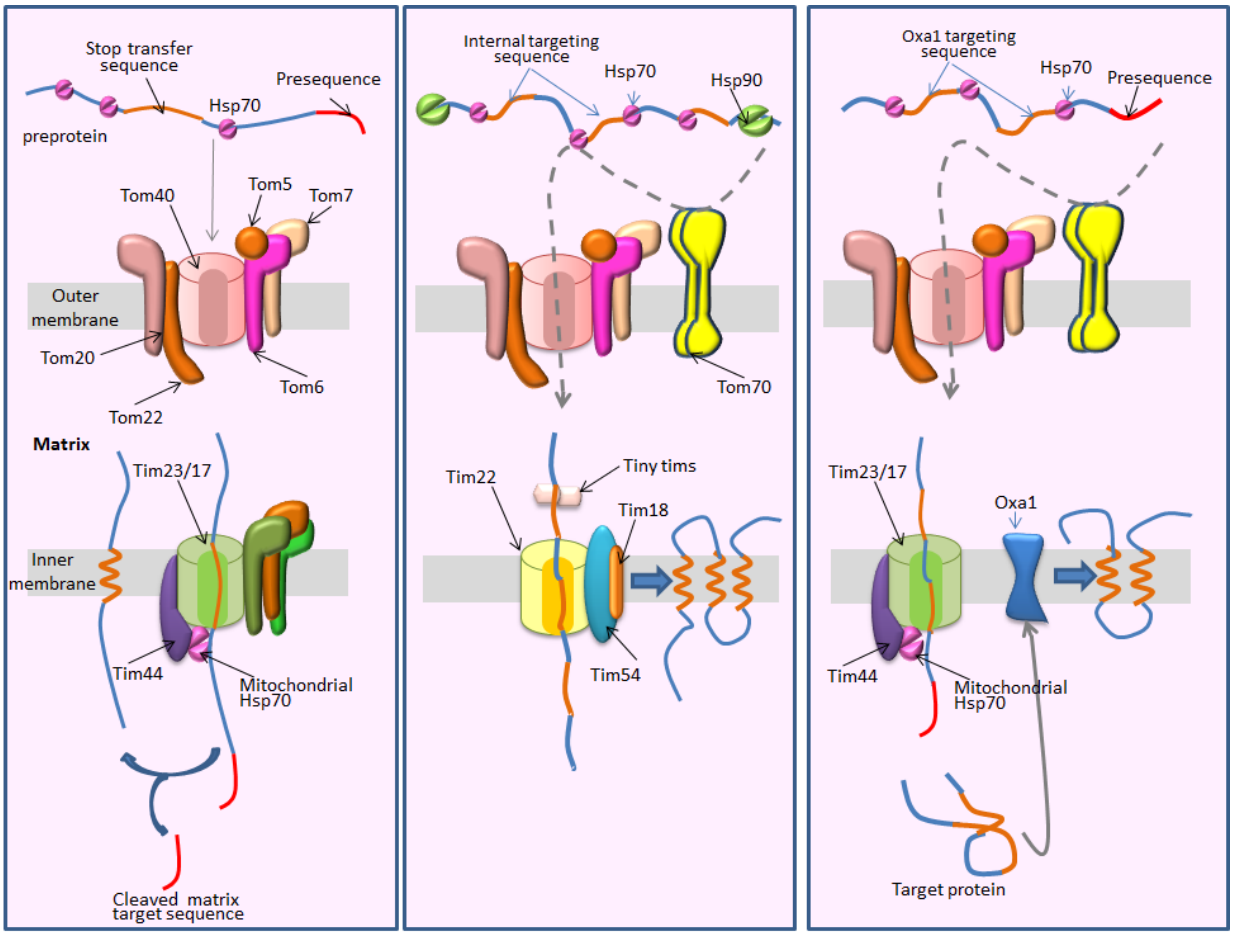

Fig. 2: Three different pathways are required for targeting proteins into inner mitochondrial membrane. For details see text. **Source: Author**

2. The proteins that have N-terminal presequence can also be targeted into the inner mitochondrial membrane. The initial mechanism of import is same as found in those proteins that are destined for the matrix. However, some proteins contain additional second sorting signal (e.g. some subunits of ATP synthase) which is exposed after the cleavage of presequence by Matrix Processing Peptidases (MPP). This second sorting signal guides the protein to Oxa1 translocation pore, from where it is laterally passed into the inner membrane (Fig. 2b). Studies have suggested that Oxa1 is related to a bacterial protein that is required for insertion of certain membrane proteins again suggesting endosymbiotic origin of mitochondria. Oxa1 is also involved in inserting those proteins into mitochondrial inner membrane that are encoded by mtDNA.
3. Other proteins with N-terminal presequence do not first enter into the matrix (like Cytochrome oxidase subunit). Rather, the presequence is cleaved while the protein is still inside the Tim23 channel (Fig. 2c) exposing the hydrophobic stop transfer

sequence, which translocates the protein laterally into the inner mitochondrial membrane.

Protein import into mitochondrial intermembrane space

Most proteins destined for mitochondrial intermembrane space contain two N-terminal sequences. The mechanism of import is same like found in the transport of proteins into the inner membrane where the presequence is cleaved while the protein is still inside the Tim23 channel exposing the hydrophobic stop transfer sequence. The protein is translocated laterally into the inner mitochondrial membrane where an inner membrane protease cleaves the hydrophobic sequence releasing it into the intermembrane space (Fig. 3a).

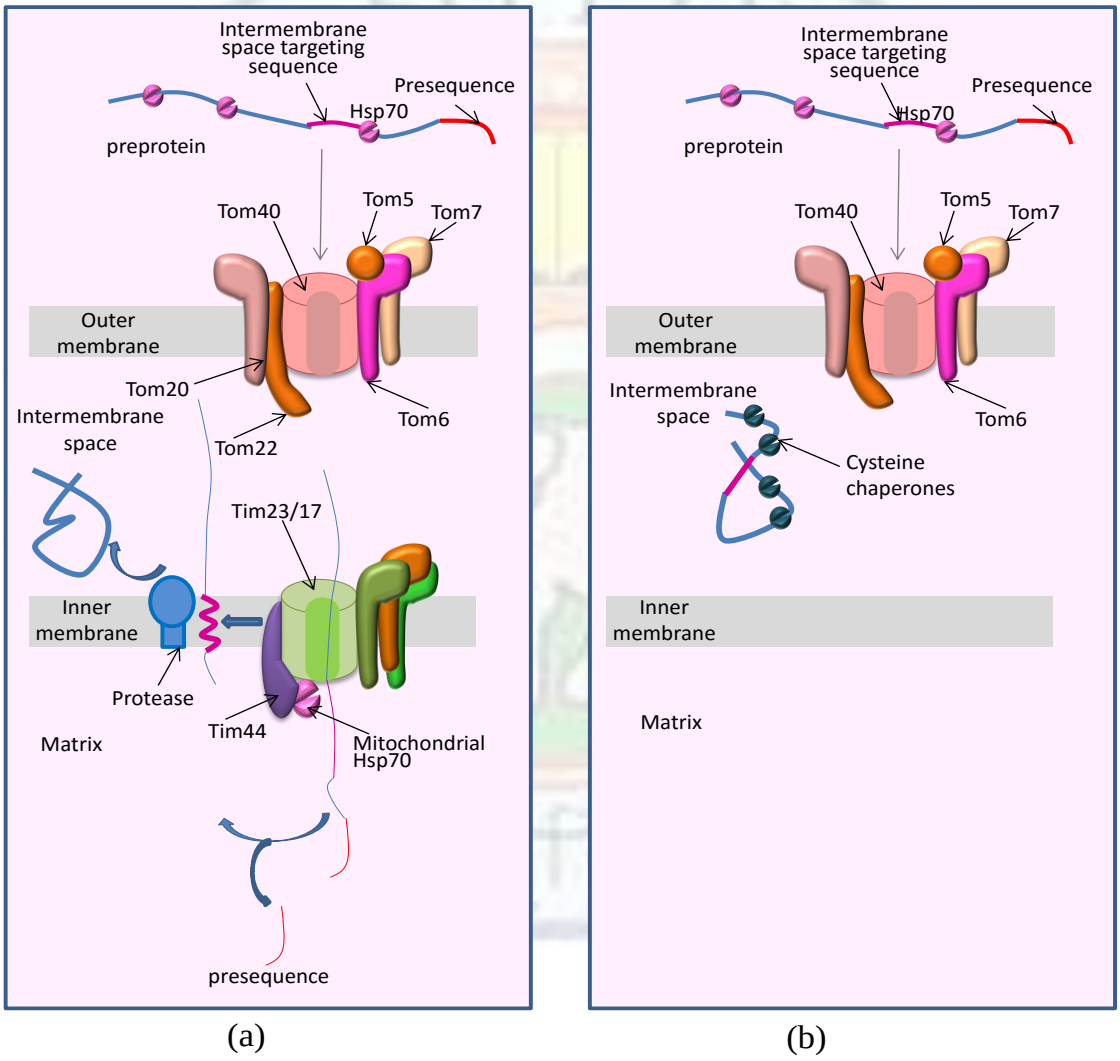

Fig. 3: Two mechanisms for import proteins into the intermembrane space (refer text for details). **Source: Author**

Some proteins are recognized by cysteine-chaperones in the intermembrane space preventing its translocation further. Such proteins remain in the intermembrane space (Fig. 3b).

Protein import into mitochondrial outer membrane

The proteins destined for outer mitochondrial membrane have **long hydrophobic sequences** which function as **Stop-transfer** and **outermembrane localization sequence**. It has also been proposed that the outer membrane proteins like porins first pass into the intermembrane space through Tom40 channel where they are recognized by "Tiny Tims". The proteins are then passed to a second translocon known as **Sorting and Assembly machinery (SAM)** which inserts them into the outer membrane.

Energy for protein translocation

Hsp70 chaperones are key molecules in importing the proteins into the mitochondria. Hsp70 use energy from ATP hydrolysis to bind and translocate proteins. Mitochondrial Hsp70 bind with Tim44 acts as a molecular motor that use energy from ATP hydrolysis to pull proteins into the matrix. Continued protein translocation into mitochondria also requires proton motive force (pmf) across the inner mitochondrial membrane. However, it is not fully understood how the pmf helps in protein translocation.

Functions of mitochondria

1. Mitochondria are known as the "Power House" of the cell and therefore the key function of mitochondria is electron transport and oxidative phosphorylation to produce ATP.
2. Fatty acid oxidation and citric acid cycle take place in the mitochondrial matrix.
3. Mitochondria are the sites of synthesis of certain amino acids and the heme groups.
4. Mitochondria also play an important role in regulating the Ca^{2+} concentration of the cytosol.
5. Mitochondria regulates the process of programmed cell death or apoptosis by releasing cytochrome c which forms apoptosomes (intrinsic pathway of apoptosis).

Citric Acid Cycle

Citric Acid Cycle or tricarboxylic acid (TCA) cycle or Krebs cycle was proposed in 1937 by Sir Hans Krebs. It is a universal oxidative degradation that is found both in the prokaryotes and eukaryotes. Apart from this, TCA cycle also generates many important biosynthetic precursors and therefore it is amphibolic (both catabolic and anabolic).

The cycle takes place in the mitochondrial matrix where pyruvate is first converted into acetyl CoA. Acetyl CoA the common product generated by the breakdown of carbohydrate,

amino acids and fatty acids. The acetyl CoA enters into the cycle by reacting with oxaloacetate forming citrate. The overall process results in the production of CO_2, 3NADH, $FADH_2$ and GTP. Out of the products generated, NADH and $FADH_2$ are important as they result in the generation of ATP (through electron transport and oxidative phosphorylation). There are eight enzymes of the cycle: citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl CoA synthetase, succinate dehydrogenase, fumarase and malate dehydrogenase which function in a cyclic manner to regenerate oxaloacetate (Fig. 4).

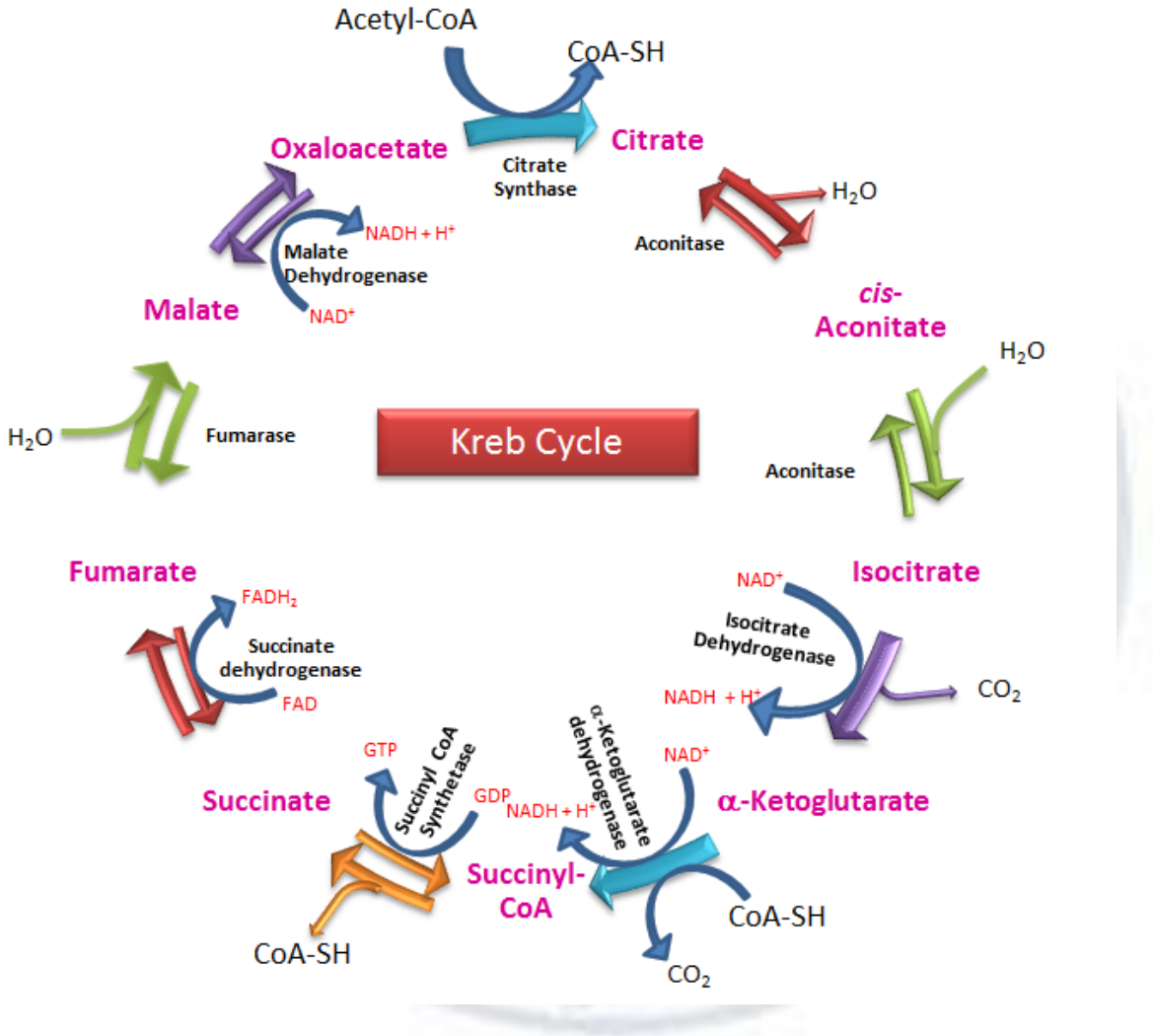

Fig. 4: Citric Acid Cycle or tricarboxylic acid (TCA) cycle that takes place into the mitochondrial matrix. **Source: Author**

TCA cycle is a central metabolic cycle that generates metabolites/intermediates that are used by other pathways. Those reactions that use these metabolites/intermediates are known as **cataplerotic reactions**. The pathways like gluconeogenesis (uses oxaloacetate

as starting material), amino acid biosynthesis (uses oxaloacetate and α-ketoglutarate) and lipid biosynthesis (uses acetyl CoA obtained from the breakdown of citrate) use metabolites/intermediates from TCA cycle. These reactions thus prevent accumulation of TCA cycle metabolites/intermediates in mitochondrial matrix. There should be a balance in the use up and generation of the TCA cycle metabolites/intermediates. So the reactions replenish the metabolites/intermediates also operate and are known as **anaplerotic reactions**. The pathways like oxidation of fatty acids (generates succinyl CoA) and breakdown of some amino acids (generates succinyl CoA, oxaloacetate and α-ketoglutarate) replenish metabolites/intermediates of TCA cycle.

Electron transport chain

In electron transport chain the free energy of electron transfer from NADH and $FADH_2$ to various intermediate electron acceptors and donors is coupled to the production of ATP. O_2 acts as the final acceptor of electrons. In an electron transport chain, a series of four protein complexes (Complex I- Complex IV) are present in the inner mitochondrial membrane through which the electrons are passed. The various components of electron transfer are arranged from lower to higher standard reduction potential.

Electron carriers found in the inner mitochondrial membrane

Five types of electron carriers are found in the inner mitochondrial membrane which in association with other components form four protein complexes. These electron carriers are flavoproteins, iron-sulfur proteins, ubiquinone, cytochromes and copper atoms. The electron carriers are prosthetic group except ubiquinone.

1. **Flavoproteins:** Flavoproteins consist of prosthetic group like flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) which is derived from vitamin B2 or riboflavin. The important flavoproteins found in mitochondria are NADH dehydrogenase and succinate dehydrogenase.
2. **Iron-sulfur proteins:** Iron-sulfur clusters are the prothetic groups in iron-sulfur proteins and were first discovered by Helmut Beinert. There are four main types of iron-sulfur clusters designated as [Fe-S], [2Fe-2S], [4Fe-4S] and [3Fe-4S]. [2Fe-2S] and [4Fe-4S] are linked to protein cysteine sulfhydryl groups.
3. **Cytochromes**: Cytochromes are heme proteins whose function was first elucidated by David Keilin in 1925. Cytochromes contain porphyrin ring and their heme groups alternate between Fe (II) and Fe (III) during electron transport. The electron-transport chain contains three distinct cytochrome types—a, b, and c.
4. **Copper atoms**: These are part of Complex IV and alternate between the Cu^{++} and Cu^{+} states.

Components of electron transport chain

1. **Complex I (NADH: Coenzyme Q Oxidoreductase/ NADH dehydrogenase)**

The Complex I is the largest protein component (~900 kD and 46 subunits) of the inner mitochondrial membrane and takes up electron from NADH and passes them to Coenzyme Q (CoQ) (Fig. 5). Out of 46 subunits, 7 are encoded by mtDNA and 39 by nuclear DNA. The complex consists of one molecule of FMN (Flavin Mononucleotide) and 5-6 molecules of Fe-S clusters. Coenzyme Q (ubiquinone) is lipid soluble and is found in the inner mitochondrial membrane.

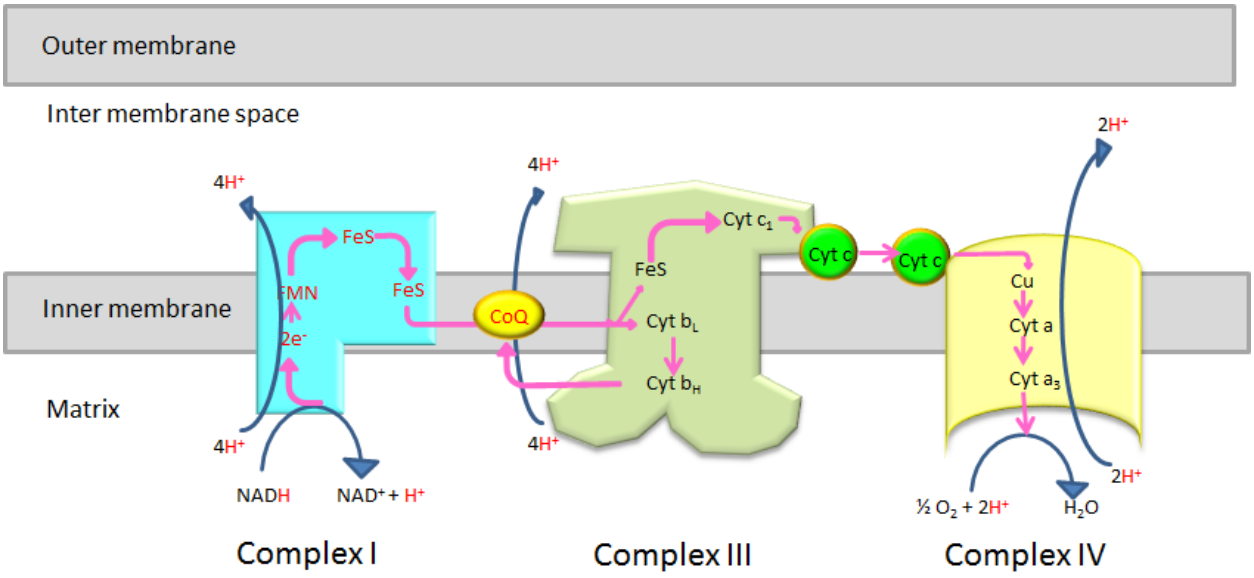

Fig. 5: Electron transport chain: the figure shows the movement of electrons from NADH (at complex I) to O_2 through various electron acceptors. **Source: Author**

2. Complex II (Succinate: Coenzyme Q Oxidoreductase/ Succinate dehydrogenase)

Complex II is of the size 127 kD and consists of 4 subunits. All 4 subunits are encoded by nuclear DNA. It passes electrons from succinate to CoQ (Fig. 6). The complex consists of one FAD which is covalently bound, three Fe-S clusters (2Fe-2S, 4Fe-4S and 3Fe-4S) and one cytochrome b_{560}.

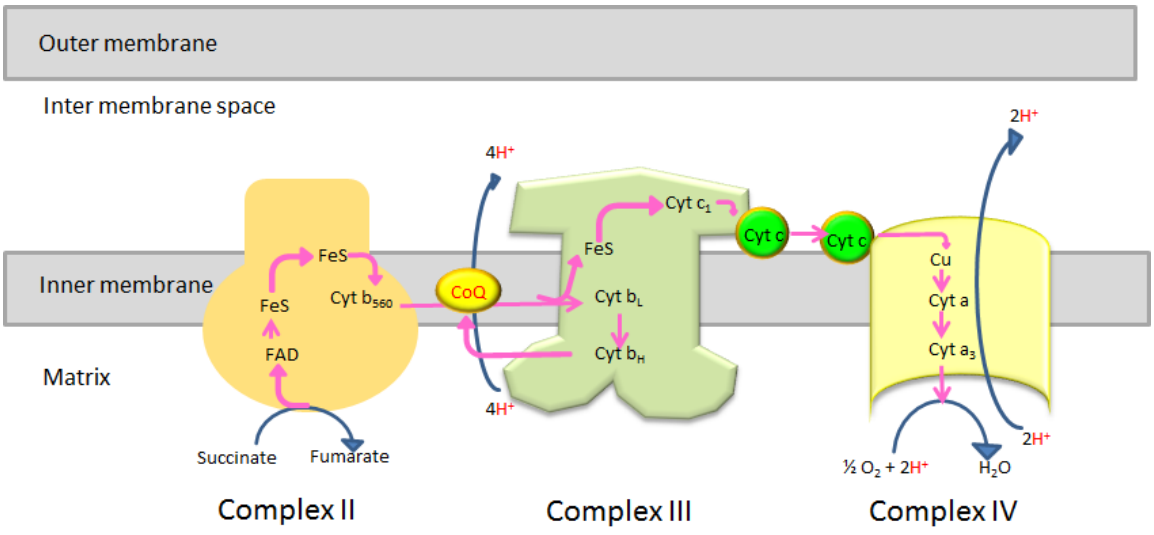

Fig. 6: Electron transport chain: the figure shows the movement of electrons from succinate (at complex II) to O_2 through various electron acceptors. **Source: Author**

3. Complex III (Coenzyme Q: Cytochrome c Oxidoreductase/Cytochrome bc_1 Complex)

Complex III is of the size 243 kD and consists of 11 subunits. Out of 11 subunits, 1 is encoded by mtDNA and 10 by nuclear DNA. It passes electrons from CoQ to cytochrome c. The complex consists of cytochrome b_L (Heme $b_L/b_{566,}$ L denotes low potential; previously known as b_T; absorbs maximally at 566 nm), cytochrome b_H (Heme $b_H/b_{562,}$ H denotes high potential; previously known as b_K; absorbs maximally at 562 nm), cytochrome c_1 (Heme c_1) and Fe-S cluster. Cytochrome c is a mobile peripheral membrane protein found on the outer face of the inner mitochondrial membrane. It carries electrons from Complex III and passes to Complex IV and therefore it keeps on shuttling between these two complexes.

4. Complex IV (Cytochrome c oxidase/COX)

Complex IV is of the size nearly 200 kD and consists of 13 subunits. Out of 13 subunits, 3 is encoded by mtDNA and 10 by nuclear DNA It is the terminal complex which passes electron to O_2 generating H_2O. The complex consists of cytochrome a (Heme a), cytochrome a_3 (Heme a_3) and two copper containing centers (Cu_A and Cu_B). Cytochrome a_3 and Cu_B form a complex which binds O_2 which is reduced to H_2O.

During electron transport process there is subsequent pumping of H^+ from matrix to the intermembrane space (Fig. 9 and 10). Four H^+ are pumped each through Complex I and Complex III (the H^+ are actually pumped by CoQ and not Complex III); and two H^+ pumped through Complex IV making a total of 10 H^+ pumped per pair of electrons through NADH.

From $FADH_2$, total of 6 H^+ are pumped (Four H^+ through Complex III and two H^+ through Complex IV). The overall reaction is:

$NADH + H^+ + 1/2\ O_2 \longrightarrow NAD^+ + H_2O$ ---------- (1)

$FADH_2 + 1/2\ O_2 \longrightarrow FAD + H_2O$ ---------- (2)

The $\Delta G^{o'}$ values First reaction is -52.6 kcal/mol and second reaction is -43.4 kcal/mol.

As it is clear from the $\Delta G^{o'}$ values that oxidation of one molecule of either NADH or $FADH_2$ is highly exergonic and can lead to synthesis of several ATP molecules. However, by electron transport, this free energy is released in installments and stored as proton motif force (pmf).

Do you know??

Many inhibitors of electron transport chain are known which are used to study the sequential steps of the electron transport chain. Some of these inhibitors are rotenone, cyanide, antimycin and amytal. The inhibitors that inhibit electron transport chain also affect protein translocation into the mitochondria. Treatment with these inhibitors prevent protein translocation even in the presence of ATP and chaperone proteins.

Oxidative Phosphorylation

The electron transport process results in the generation of free energy which is conserved by pumping H^+ into the intermembrane space. The energy generated by translocation of H^+ back to the matrix is harnessed by Complex V (also known as proton translocating ATP synthase) to produce ATP (Fig. 7).

Mitochondria: Function

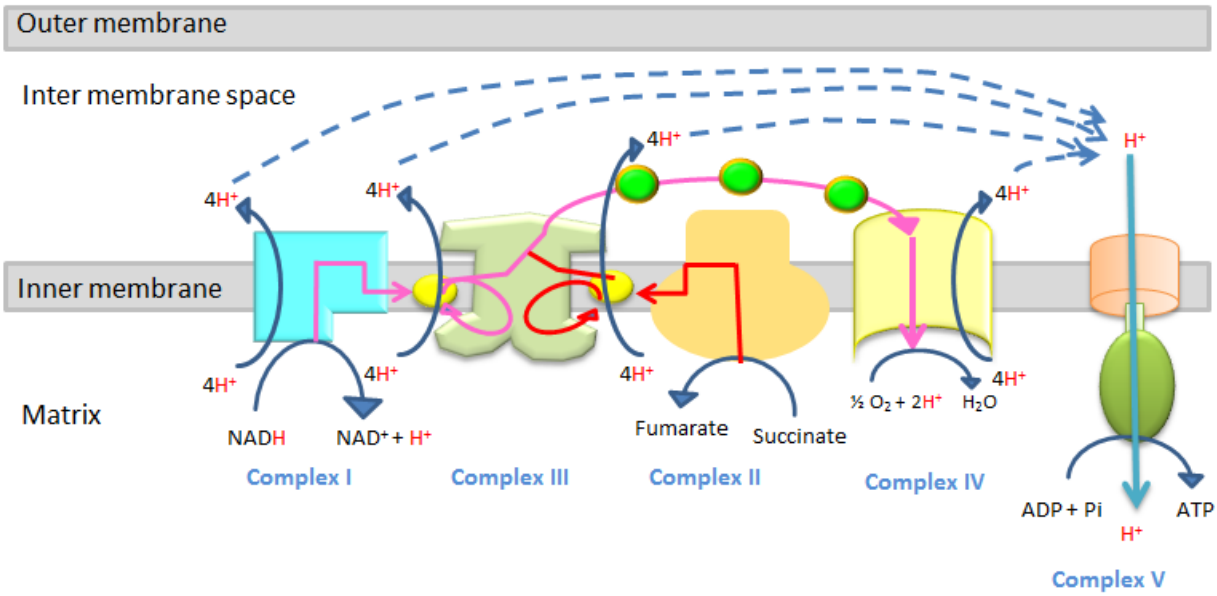

Fig. 7: The generation of proton-motif force and production of ATP by ATP synthase also known as complex V. **Source: Author**

A number of researchers have tried to work out how electron transport is coupled with the synthesis of ATP. Different mechanisms and models were proposed like:

1. **Chemical coupling hypothesis:** The Chemical coupling hypothesis was proposed by Edward Slater in 1953. According to this hypothesis, the process of electron transport generates reactive intermediates. These intermediates are subsequently broken down to yield free energy which is used to drive ATP synthesis.

2. **Chemiosmotic hypothesis:** The Chemiosmotic hypothesis was proposed by Peter Mitchell in 1961. According to this hypothesis, the movement of electron generates free energy which is used to pump H^+ from matrix to the intermembrane space creating an electrochemical H^+ gradient. This electrochemical H^+ gradient is subsequently used by ATP synthase to produce ATP. This hypothesis gained much attention and was also able to gather experimental evidence. Therefore this has been an important model which is now widely accepted.

3. **Conformational coupling hypothesis:** The Conformational coupling hypothesis was proposed by Paul Boyer in 1964. According to this hypothesis, the proteins of the inner mitochondrial membrane are coupled with ATP synthase. These proteins adopt a high energy conformational states during electron transport. When these proteins return back to the ground or deactivated conformation they result in ATP synthesis through ATP synthase. The hypothesis was found to be partially correct as we now know that conformational changes do take place during ATP synthesis.

Mitochondria: Function

Generation of proton gradient

As mentioned earlier, the free energy of electron transfer is used to pump protons from matrix to the intermembrane space resulting in creation of H^+ gradient across the inner mitochondrial membrane. The H^+ gradient has two components:

1. **pH gradient (ΔpH):** Difference in the concentration of H^+ across the inner mitochondrial membrane.
2. **Electric potential or voltage (Ψ):** The voltage component is the result of the separation of the charged particles across the inner mitochondrial membrane.

The H^+ gradient is therefore electrochemical in nature. The two components of H^+ gradient are combined and is known as **proton-motif force or pmf** (**Δp**) which is measured in millivolts.

$\Delta p = \Psi - 2.3 (RT/F) \Delta pH$ or $\Delta p = \Psi - 59 \Delta pH$ (as 2.3 (RT/F) = 59mV)

where *R* is the gas constant, *T* is the temperature (in degrees Kelvin), *F* is the Faraday constant, Ψ is the transmembrane electric potential.

Measurements done by various researchers suggest that transmembrane electric potential (Ψ) is **-**160mV and ΔpH ~ 1, making Δp= -220mV.

Do you know??

About 30.5 kJ of standard free energy is required for synthesis of 1 mol of ATP.

Mechanism of ATP synthesis

ATP is synthesized by F_1F_0-ATPase using energy from the H^+ translocation. The mechanism of ATP synthesis resembles the conformational coupling hypothesis proposed by Boyer. In 1994, the research group of John Walker provided a detailed atomic model of the F_1 head which provided important structural evidence to support the conformational changes that take place in F_1 during ATP synthesis. The F_1 consists of three catalytically active sites present *on β* subunit with different binding affinities to the substrates (ADP and P_i). These catalytic sites exist in three distinct conformational states denoted by:

1. **O or Open conformational state** which has very low affinity for substrate and is catalytically not active
2. **L or Loose conformational state** which binds substrates loosely

3. **T or Tight conformational state** which binds substrates tightly and is catalytically active. The phosphoanhydride bond formation between ADP and P_i takes place in this conformational state.

The substrates bind to the L state loosely (Fig. 8). The conformational changes driven by the free energy of H^+ translocation converts L state to T state. The changes also take place in two other subunits converting O state to L state and T state to O state. The ATP formation takes place in T state. Again there is change in conformation due to H^+ translocation converting T state to O state. Since O state cannot binds the ligand, ATP is released from O state. This process continues as long as H^+ translocation takes place.

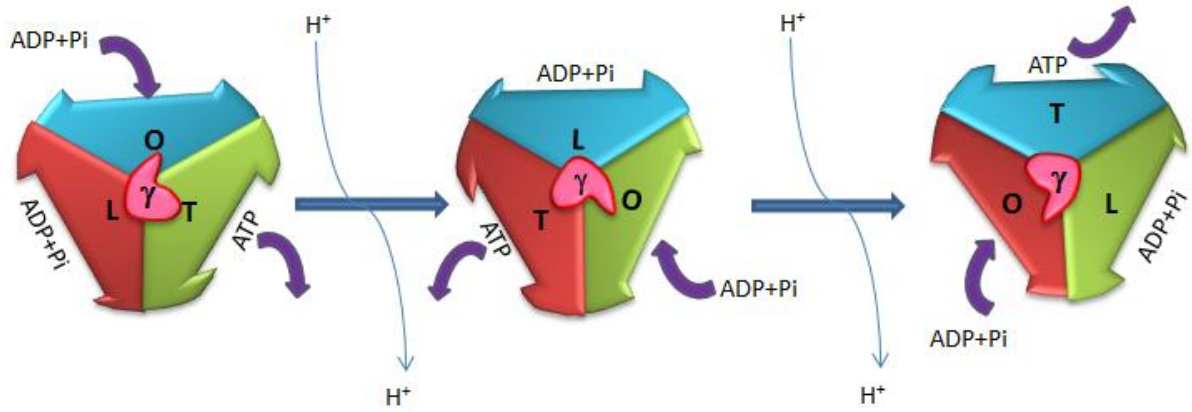

Fig. 8: The mechanism of ATP synthesis by F_1. The catalytic β subunit undergoes conformational changes upon mediated by the rotation of *c* subunit. **Source: Author**

The conformational changes in *β* subunits take place due to H^+ translocation through a channel found between *a* subunit and *c* subunit which forms rotating ring. H+ translocation causes rotation of *c* subunit and since the γ subunit of F_1 is tightly attached to the *c* ring, rotation of *c* ring results in the rotation of the γ subunit. This rotation of γ subunit produces the conformational changes in the catalytic site of the *β* subunits. It has been proposed that the rotation of γ subunit by 120° results in the corresponding rotation of the γ subunit by 120° resulting in the production of one ATP molecule. Therefore a complete rotation of the *c* ring by 360° generates three molecules of ATP.

Apoptosis

Mitochondria also plays important role in regulating apoptosis or programmed cell death in mammalian cells by a pathway known as intrinsic pathway of apoptosis. The intrinsic pathway gets activated by a number of internal stimuli like DNA damage, oxidative stress, viral infection, high cytosolic Ca^{2+} ion concentrations and hypoxia. The intrinsic pathway is activated by members of multidomain Bcl-2 family like Bax and Bak. Once activated by

death signals, Bax and Bak oligomerize at the outer mitochondrial membrane resulting in their insertion into the outer mitochondrial membrane. Once inserted into the outer membrane they assemble into a multisubunit channel which increases the permeability of the outer membrane for certain mitochondrial proteins like cytochrome c (Fig. 9). Once released into the cytosol, cytochrome c forms a part of large protein complex known as **apoptosome**. The apoptosome consists of several molecules of procaspase-9 which are activated by joining the large protein complex. Caspase-9 (which is an initiator caspase) once activated activates downstream executioner caspases which bring about cell death.

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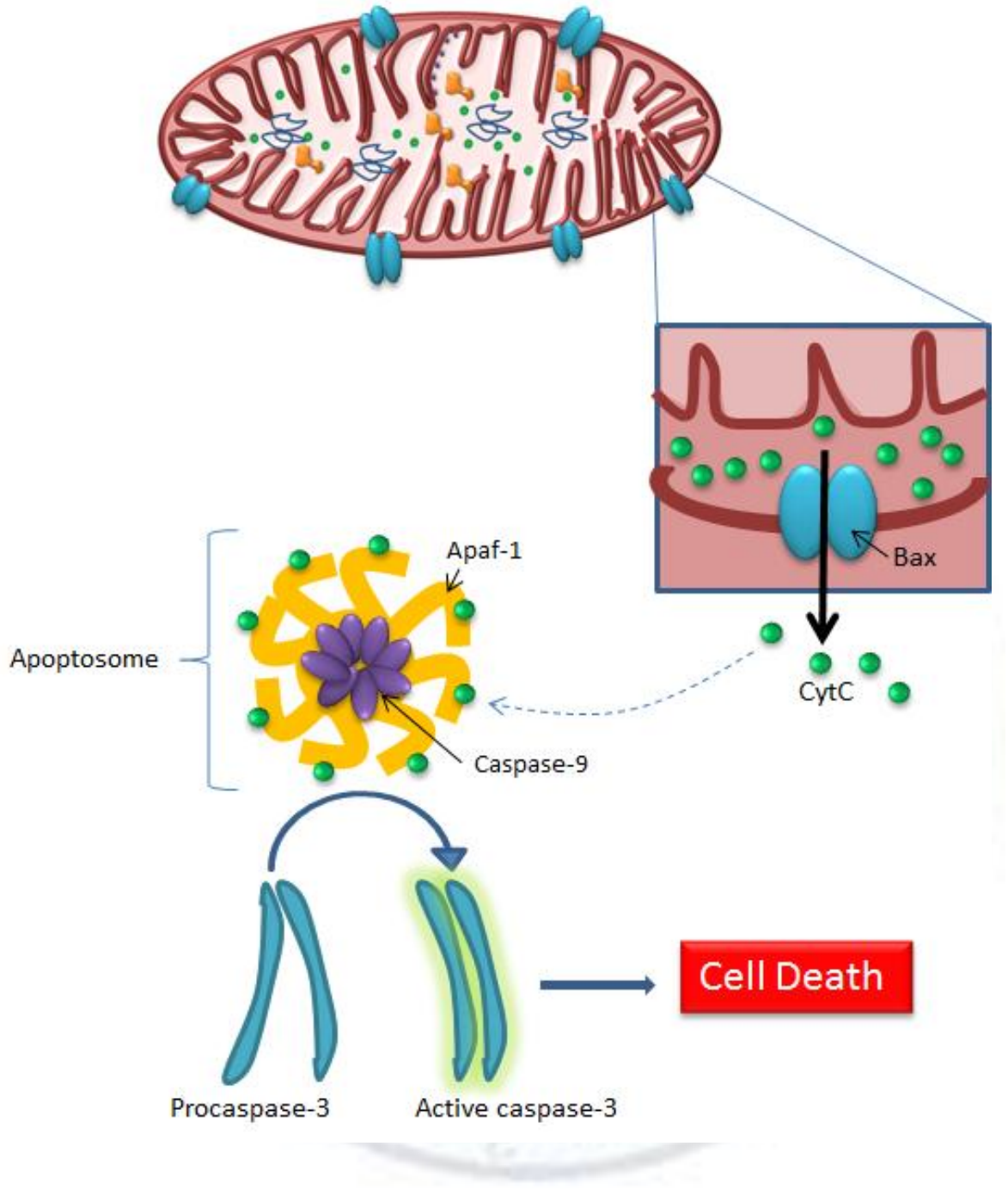

Fig. 9: Intrinsic pathway of apoptosis. The figure depicts the role of mitochondria in regulating the cell death along with members of Bcl-2 family like Bax. The cytochrome c released from the mitochondria binds and form a large complex known as apoptosome which activates downstream caspases causing cell death.

Mitochondria: Function

Do you know??

bcl-2 is a mammalian gene first identified as an oncogene responsible for human B-cell lymphoma in 1985. Mammals code for about 20 proteins like Bcl-2. Bcl-2 family can be divided into three groups: (i) Proapoptotic: promote apoptosis like Bax and Bak; (ii) Antiapoptotic: prevent apoptosis like Bcl-2 and Bcl-xL (iii) BH3-only proteins: promote apoptosis by an indirect mechanism like Bid, Bad and Bim. BH3-only proteins are important regulators of apoptosis which is executed by either activating other proapoptotic members or inactivating antiapoptotic members. It has been proposed that BH3-only proteins are absent or inhibited in healthy cells. They are activated under certain conditions of cell stress and inactivating antiapoptotic members leading to the translocation of proapoptotic members like Bax and Bak to the mitochondria.

Summary

Mitochondria have their own genetic system still they are dependent on nuclear DNA for about 99% of their 1000 proteins. The mitochondrial proteins are synthesized at the cytosolic ribosomes and are then imported into the mitochondria. The proteins that are destined for matrix have an N-terminal pre-sequence consist of 20-35 positively charged amino acids. The pre-sequence is recognized by the receptors of the translocons present on the outer mitochondrial membrane, Tom. Similar translocons are also found on the inner membrane known as Tim. The presequence of the imported proteins are cleaved by Matrix Processing Peptidases (MPP). The protein import requires electrochemical H^+ gradient. Three different pathways target proteins into inner mitochondrial membrane: (1) multipass proteins have multiple internal mitochondrial import signals which promotes passing of protein into inner membrane (2) some proteins contain additional second sorting signal, exposed after the cleavage of presequence which guides the protein to Oxa1 translocation pore, from where it is laterally passed into the inner membrane (3) In some proteins the presequence is cleaved while the protein is still inside the Tim23 channel exposing the hydrophobic stop transfer sequence, which translocates the protein laterally into the inner mitochondrial membrane. Most proteins destined for mitochondrial intermembrane space contain two N-terminal sequences. The proteins destined for outer mitochondrial membrane have long hydrophobic sequences which function as Stop-transfer and outermembrane localization sequence.

Mitochondria has many important function like ATP production, Fatty acid oxidation, citric acid cycle. They are the sites of synthesis of certain amino acids and the heme groups and play an important role in regulating the Ca^{2+} concentration of the cytosol. They also regulates the process of programmed cell death or apoptosis.

In electron transport chain the free energy of electron transfer from NADH and $FADH_2$ is coupled to the production of ATP. Five types of electron carriers are found in the inner

mitochondrial membrane arranged from lower to higher standard reduction potential: flavoproteins, iron-sulfur proteins, ubiquinone, cytochromes and copper atoms. These electrons acceptors are arranged in a series of four protein complexes (Complex I- Complex IV in the inner mitochondrial membrane. The electron transport process results in the generation of free energy which is harnessed by Complex V (also known as proton translocating ATP synthase) to produce ATP. Many different hypothesis have been proposed to explain how electron transport is coupled with the synthesis of ATP. But it is the Chemiosmotic hypothesis proposed by Peter Mitchell in 1961 that gained much attention. According to this hypothesis, the movement of electron generates free energy which is used to pump H^+ from matrix to the intermembrane space creating an electrochemical H^+ gradient. This electrochemical H^+ gradient is subsequently used by ATP synthase to produce ATP. The H^+ gradient has two components: pH gradient (ΔpH) and Electric potential or voltage (Ψ). The two components of H^+ gradient are combined and is known as proton-motif force or pmf (Δp) which is measured in millivolts.

ATP is synthesized by F_1F_0-ATPase using energy from the H^+ translocation resulting in the conformational changes in F1. F1 consists of three catalytically active sites present *on β* subunit which exist in three distinct conformational states (1) O or Open conformational state which is catalytically inactive and has very low affinity for substrate (2) L or Loose conformational state binds substrates loosely (3) T or Tight conformational state which binds substrates tightly and is catalytically active. The conformational changes driven by the free energy of H^+ translocation converts L state to T state, O state to L state and T state to O state. The ATP formation takes place in T state. H^+ translocation causes rotation of *c* subunit of F_0 and γ subunit of F_1. The rotation of γ subunit produces the conformational changes in the catalytic site of the *β*.

Mitochondria also plays important role in regulating apoptosis by a pathway known as intrinsic pathway of apoptosis. The intrinsic pathway is activated by members of Bcl-2 family like Bax and Bak which oligomerize at the outer mitochondrial membrane resulting increaseing the permeability of the outer membrane for proapoptotic cytochrome c. Cytochrome c forms a part of large protein complex known as apoptosome which consists of several molecules of procaspase-9 which once activated activates downstream executioner caspases.

Exercise/ Practice

A. Multiple choice questions:

1. The import of proteins into mitochondria takes place through

   (a) Tom40 (b) Tom5 (c) Tom20 (d) Tom22

2. Proteins destined for mitochondrial matrix should have

   (a) internal mitochondrial import signals (b) internal stop transfer sequences

   (c) presequence (d) NLS

3. Which of these is not an electron carrier found in the inner mitochondrial membrane
   (a) Flavoprotein (b) cytochromes (c) Fe-S clusters (d) Mg^{2+}
4. The complex that passes electron from NADH to Coenzyme Q is
   (a) Complex I (b) Complex II (c) Complex III (d) Complex IV
5. The largest protein component of the inner mitochondrial membrane is
   (a) Complex I (b) Complex II (c) Complex III (d) Complex IV
6. Which of the following is not a Fe-S cluster associated with ETC?
   (a) [Fe-S] (b) [2Fe-2S] (c) [4Fe-4S] (d) [3Fe-3S]
7. Which of the following is not a prosthetic group?
   (a) Flavoprotein (b) cytochromes (c) Fe-S clusters (d) ubiquinone
8. Mobile electron transfer protein found on the outer face of inner mitochondrial membrane
   (a) FMN (b) cytochrome c (c) Fe-S cluster (d) ubiquinone
9. Mitochondria is not involved in
   (a) Apoptosis (b) ATP synthesis (c) glycolysis (d) Ca^{2+} regulation
10. Sorting and Assembly machinery (SAM) is required for
    (a) protein insertion into the outer membrane (b) protein inserttion into the inner membrane (c) protein import into matrix (d) protein import into intermembrane space
11. The death signal release from mitochondria is
    (a) cytochrome c (b) ATP (c) NADPH (d) Ca^{2+}
12. Which of these play direct role in executing the intrinsic pathway of apoptosis
    (a) Bcl-2 (b) Bax (c) Bim (d) Bcl-xL
13. The process not taking place into the matrix is
    (a) fatty acid oxidation (b) TCA cycle (c) electron transport (d) none

B. Fill in the blanks:

1. Heme proteins containing porphyrin ring are _____
2. Number of ATP produced from 1 molecule of NADH are _____
3. Number of ATP produced from 1 molecule of $FADH_2$ are _____
4. Copper containing centers are the characteristic features of _____
5. The Complex I takes up electron from _____ and passes them to _____
6. Complex II passes electrons from _____ to_____
7. Complex IV is also known as _____

8. The two components of H^+ gradient are _____
9. The number of subunits in F_1 is _____
10. The rotatory motor is formed by _____ subunit of F_0.
11. The ATP formation takes place in _____ state.
12. Cytochrome c released from mitochondria forms a part of large protein complex known as _____
13. Examples of Proapoptotic Bcl-2 family members are: _____
14. Examples of Antiapoptotic Bcl-2 family members are _____

C. True/False

1. All proteins require a presequence for entry into mitochondria.
2. TCA cycle takes place in the cytosol.
3. The components of ETC are localized in the inner mitochondrial membrane.
4. The structure of F1 component is found to be homologous on different organisms.
5. ATP synthesis does not require any conformational changes
6. It is not the direct flow of electrons but the translocation of H+ that is responsible for ATP synthesis.
7. Cytochromes are found in complex I of the ETC.
8. Protein translocation into mitochondria requires H^+ gradient.
9. BH3-only proteins are antiaoptotic.

D. Expand the following

1. Pmf
2. Tom
3. Tim
4. MPP
5. SAM
6. TCA
7. COX
8. CoQ

Glossary

Anaplerotic reactions: reactions that replenish TCA cycle metabolites/intermediates

Apoptosome: large protein complex consisting of cytochrome c and procaspase-9, helps in activation of caspase-9 and downstream pathway activation

Cataplerotic reactions: reactions that use TCA cycle metabolites/intermediates

Cytochromes: heme proteins, contain porphyrin ring and play important role in electron transport. Electron-transport chain contains three distinct cytochrome types—a, b, and c.

Mitochondria: Function

Electric potential: separation of the charged particles across the inner mitochondrial membrane generates electric potential

L state: Loose conformational state which binds substrates loosely

O state: Open conformational state which has very low affinity for substrate and is catalytically not active

Pre-sequence: N-terminal sequence characterized by the presence of 20-35 positively charged amino acids, required for import into the mitochondrial matrix

T state: Tight conformational state which binds substrates tightly and is catalytically active.

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Answers

1. (a) Tom40
2. (c) presequence
3. (d) Mg^{2+}
4. (a) Complex I
5. (a) Complex I
6. (d) [3Fe-3S]
7. (d) ubiquinone
8. (b) cytochrome c
9. (c) glycolysis
10. (a) protein insertion into the outer membrane
11. (a) cytochrome c
12. (b) Bax
13. (c) electron transport

B. Fill in the blanks:

1. Cytochromes
2. 3
3. 2
4. Complex V
5. NADH, Coenzyme Q
6. Succinate, CoQ.
7. Cytochrome c oxidase/COX
8. pH gradient (ΔpH) and Electric potential or voltage (Ψ)
9. 9
10. c
11. T or tight conformational state
12. Apoptosome
13. Bax and Bak
14. Bcl-2 and Bcl-xL

Mitochondria: Function

C. True/False

1. False; presequence is always not required for translocation
2. False; TCA cycle takes place in mitochondrial matrix
3. True
4. True
5. False; ATP synthesis requires conformational changes in the β subunit of F_1
6. True
7. False; Cytochromes are not found in complex I but are found in complex II, III and IV.
8. True
9. False; they are proapoptotic

D. Expand the following

1. proton motif force
2. translocons of the outer membrane
3. translocons of the inner membrane
4. Matrix Processing Peptidases
5. Sorting and Assembly machinery
6. Tricarboxylic acid
7. Cytochrome c oxidase
8. Coenzyme Q