



# CYTOLOGY

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Sheet

Slides

Number

12

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# Mitochondria

## the structure of the mitochondria

mitochondria do exist in nearly all eukaryotic cells and function as machines that generate energy by the breakdown of carbohydrates (by glycolysis and the citric acid cycle) and fatty acids (by beta oxidation and citric acid cycle).

Mitochondria are composed of two membranes: **the outer membrane** and the **inner membrane** that are separated from each other by **intermembrane space**. Also, the intermembrane space is similar in composition to the cytosol. In addition, the inner membrane is rich with proteins (>70%) of its contents, which are involved in oxidative phosphorylation as well as in the transport of metabolites.

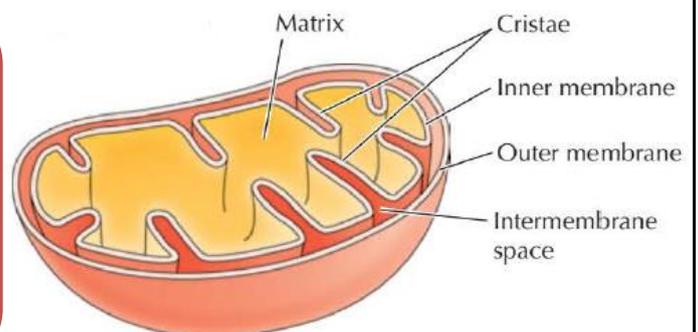
the inner membrane is **impermeable** to most ions and small molecules (why?) to maintain proton gradient that drives oxidative phosphorylation that occurs in the inner membrane, while the outer membrane is highly **permeable** to small molecules(why?) because it contains channel proteins which allow the simple diffusion of small molecules called **porins**. Each of the two membranes is a phospholipid bilayer.

The inner membrane forms folds that are called **cristae** that increase the surface area. Another compartment of mitochondria is the **mitochondrial matrix** which is enclosed by the inner membrane & Contains the mitochondrial genetic system and the enzymes responsible for Krebs cycle.

### NOTE

The intermembrane space is divided into:

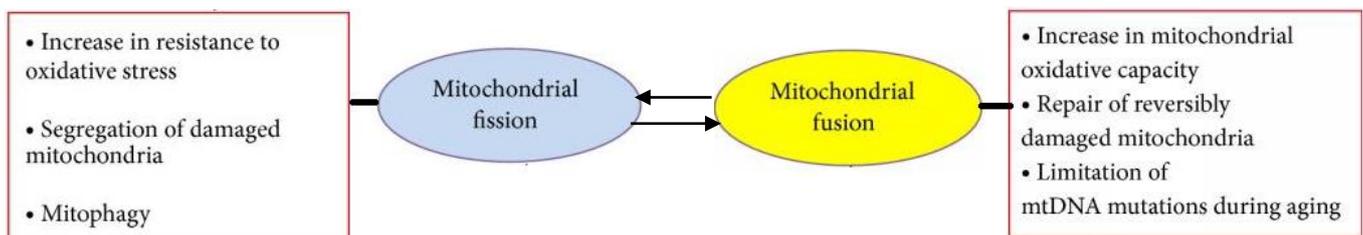
- 1) The intracristal space.  
The space between cristae.
- 2) The peripheral space  
The space between the two membranes.



## Mitochondrial fusion and fission

✓ Mitochondria are dynamic organelles (they divide and fuse with each other) (why?)

1) To exchange genetic material    2) to regulate autophagy    3) for cell survival





How can the final destination of the mitochondrial proteins be determined?

Simply, by targeting sequence which are amino terminal presequence of 15-55 amino acids that direct the import of the protein either to the matrix or to the inner membrane. (Proteins can be targeted to the inner membrane not only by presequences, but also by other signals).

And this Presequence is composed of:

Hydrophilic and hydrophobic Amino acids- in which the hydrophilic amino acids are positively charged (basic amino acids) -that can form a helix (amphipathic helix). Therefore, the presequence is positively charged.

- ✓ Remember: Most mitochondrial proteins are translated on FREE cytosolic ribosomes (by nuclear DNA) and imported into the organelle.
- ✓ No modifications for mitochondrial proteins happen in the mitochondria.

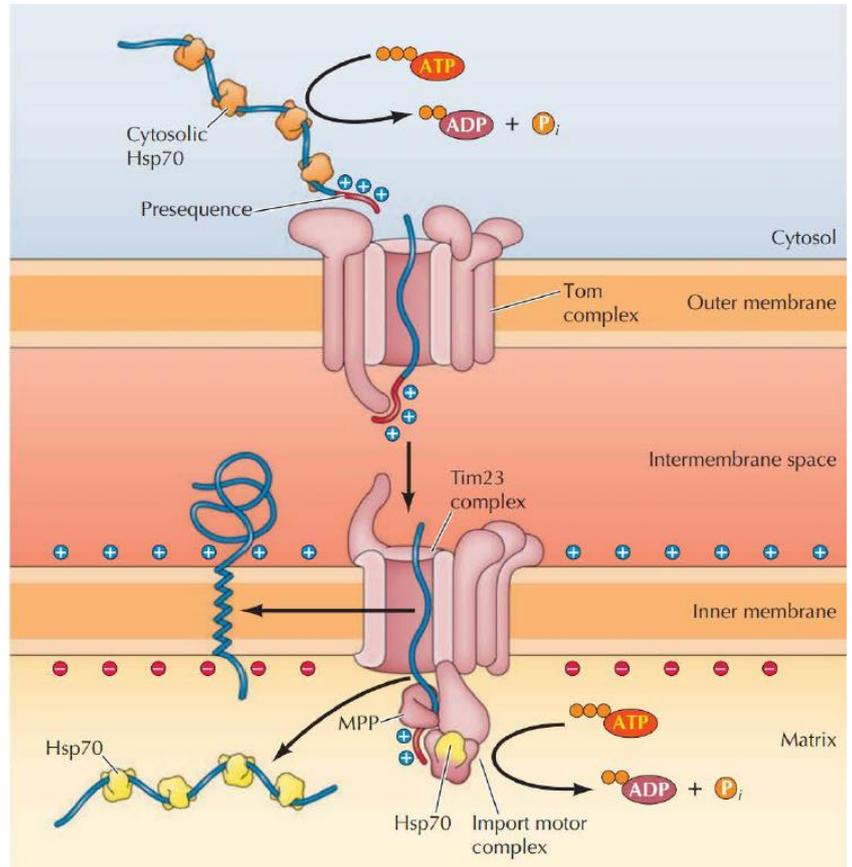
### Targeting proteins that spans the Inner membrane once and matrix proteins

- ❖ Proteins destined for the inner membrane or the mitochondrial matrix have a presequence in their **amino** terminus.
1. First of all, this positive presequence will be recognized by an outer membrane protein called Tom complex (**t**ranslocase of the **o**uter **m**embrane) which is a protein complex that direct translocation across the outer membrane. On the cytosolic side, Hsp70 chaperones both maintain proteins in a partially unfolded state and pull them to the Tom complex.
  2. Then, after the protein is transferred to the intermembrane space, it will be recognized again by another complex in the inner membrane called Tim23 (which is one of the two different **t**ranslocases of the **i**nnner **m**embrane). however, Translocation of proteins containing presequences through Tim23 requires the electrochemical potential established across the inner mitochondrial membrane during electron transport chain and oxidative phosphorylation (how?)
    - as protons are pumped from the matrix to the intermembrane space in oxidative phosphorylation, the matrix becomes negatively charged relative to the intermembrane space. Thus, electrochemical potential is established across the inner membrane.....So, the +ve presequene will attract to the -ve matrix.
  3. In the matrix, an import motor complex containing an Hsp70 chaperone uses ATP to drive translocation of the protein across the inner membrane. This complex is made of more than one protein, one of these proteins is the **m**atrix **p**rocessing **p**eptidase (MPP).

- Next MPP cleaves the presequence (although the presequence can form a helix, it is not a part of the final protein).

If the protein is destined for the inner membrane, once we reach the hydrophobic amino acids that can form a helix, the protein is going to fold and to be pushed into the inner membrane.

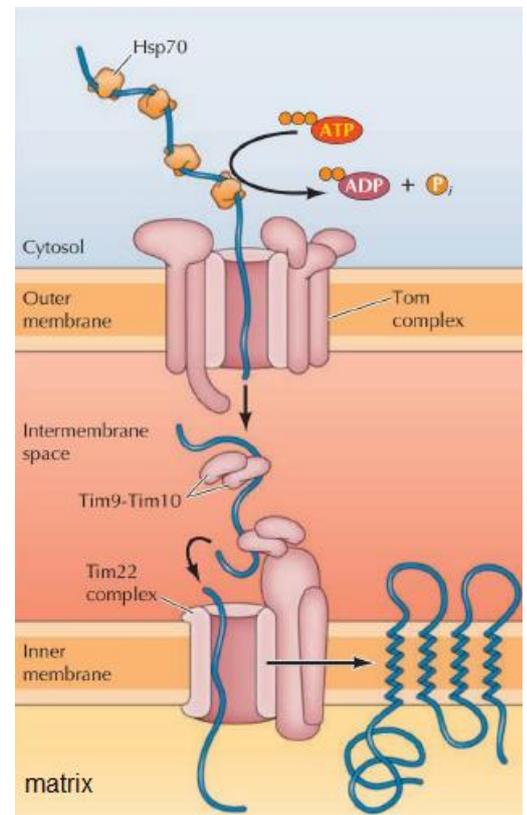
If the protein is soluble (destined for the matrix), it is going to enter the inner membrane completely and Hsp70 chaperons will assist its folding.



### Proteins in the inner membrane that spans the membrane multiple times

- ✓ Many proteins in the inner membrane are multiple-pass transmembrane proteins (function?) that serve as transporters to exchange nucleotides and ions between the mitochondria and the cytosol.
- ✓ These proteins do not contain presequences but instead have multiple internal mitochondrial import signals.

- 1) First, these protein crosses the outer membrane through Tom complex (also Hsp70 chaperons on the cytosolic side maintain the protein in an unfolded state)
- 2) Then, the protein is recognized by Tim9 & Tim10 chaperons (instead of being transferred to Tim23)
- 3) Tim9 & Tim10 pull the protein to the second translocase of the inner membrane (Tim22).
- 4) Now the protein is inside Tim, once we reach a stretch of hydrophobic amino acids, the protein is going to form the first helix and it will be pushed into the inner membrane.



- 5) Internal transmembrane sequences halt translocation, and the protein is transferred laterally into the inner membrane... this process will continue until we reach the last helix.

NOTE

In this case, there are internal stop transfer sequences because of the presence of more than one helix.

### Targeting proteins to the outer membrane

1) Outer membrane proteins (beta barrel protein).

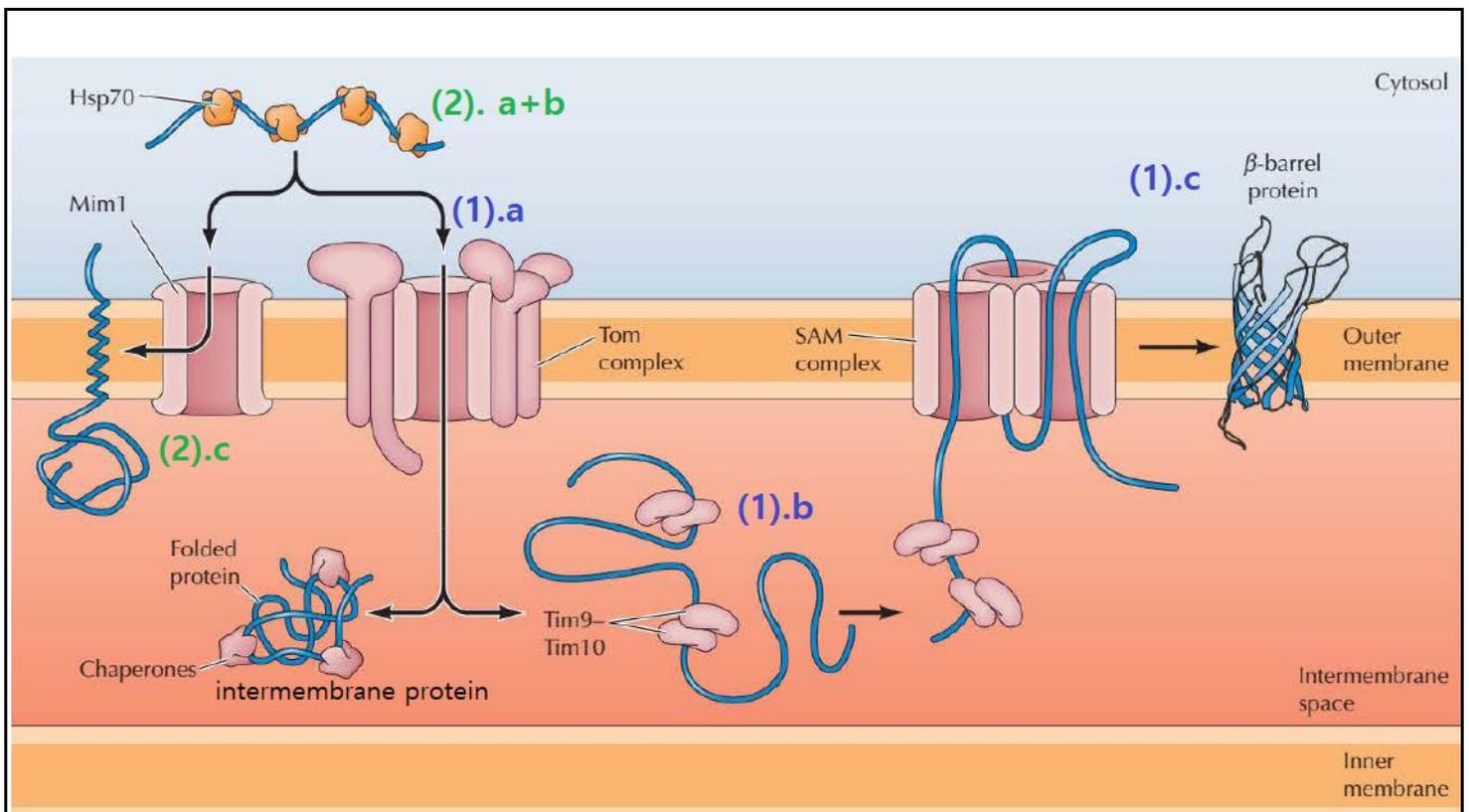
- Many outer membrane proteins are beta-barrel proteins (e.g., porins).
- No presequence.
- Steps of translocating these proteins to the outer membrane:
  - a) First, the protein will pass through the Tom complex into the intermembrane space (the cytosolic Hsp70 chaperons will hold the protein in an unfolded state). see (1).a in the figure
  - b) Then, the mobile Tim9 & Tim10 chaperons will pull the protein to a special machinery called SAM (sorting and assembly machinery). (1).b in the figure
  - c) SAM -which is composed of different protein complex- will fold the protein in beta barrel sheets and insert it to the outer membrane. (1).c in the figure

2) Outer membrane proteins (alpha helix)

- No presequence.
- Steps of targeting these proteins to the outer membrane:
  - a) The cytosolic Hsp70 chaperons will hold the protein in an unfolded state.
  - b) Then, the cytosolic Hsp70 chaperons will pull the protein to another outer membrane protein called Mim1. (2).a+b
  - c) Mim1 will fold & insert the protein in the outer membrane. (2).c

### Targeting proteins to the intermembrane space

- These proteins are soluble (don't contain the hydrophobic stretches that can form a helix)
- Most of the soluble proteins (intermembrane proteins) contain a stretch of cysteine (cysteine rich sequences)
- The intermembrane proteins are targeted to the intermembrane space by cysteine rich sequences (not by the presequence).
- Steps of targeting these proteins to the intermembrane space:
  - a) First, the protein will pass through the Tom complex into the intermembrane space (the cytosolic Hsp70 chaperons will hold the protein in an unfolded state)
  - b) After the protein exits the Tom complex, it will be recognized by specific intermembrane chaperones that facilitate its folding.



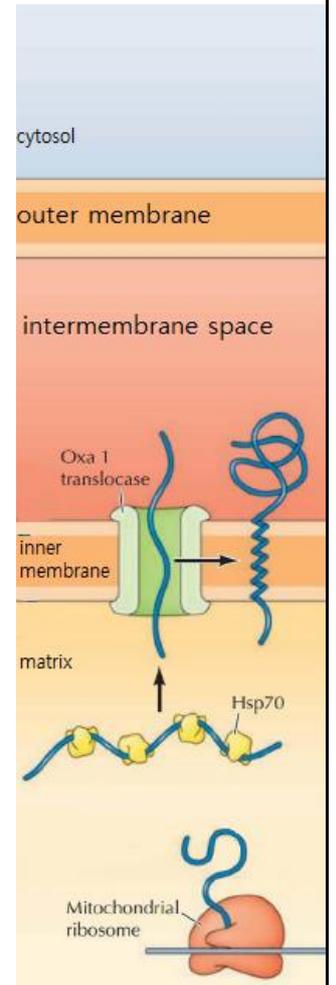
### Targeting proteins that are encoded by the mitochondrial DNA to the inner membrane

- ✓ some inner membrane proteins are encoded by the mitochondrial genome.
- ✓ These proteins are synthesized on ribosomes within the mitochondrial matrix.
- ✓ Steps of targeting these proteins:
  - a) After the synthesis of the protein inside the matrix, Hsp70 chaperons bound to it and pull it to Oxa1 translocase.
  - b) Oxa1 translocase recognizes the protein and inserts it to the inner membrane. (Oxa1 translocase recognizes proteins that are synthesized by the mitochondrial DNA).

**NOTE**

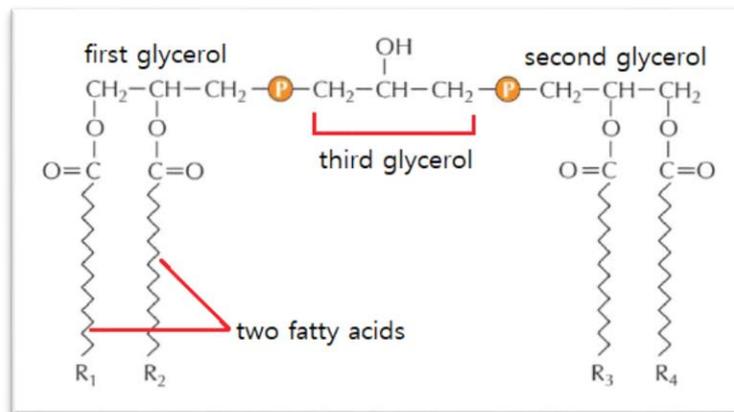
#### The function of chaperons:

- If the protein is soluble, they help the protein in folding.
- If the protein is not soluble, they hide it from the aqueous environment.
  - Imagine a membrane protein that contains hydrophobic amino acids, this protein will be unstable if it is exposed to the aqueous environment. Thus, the chaperons will hide these hydrophobic amino acids until the protein is transferred to a translocase and inserted in the membrane.



## Mitochondrial phospholipid

- ✓ Most of the lipids of mitochondrial membrane are imported from the cytosol. For example, In animal cells, **sphingolipids**, **cholesterol**, **phosphatidylcholine**, **phosphatidylinositol**, and **phosphatidylserine** are synthesized in the ER and transported to mitochondria, and then the mitochondria synthesize **phosphatidylethanolamine** from **phosphatidylserine**.
- ✓ Also, mitochondria contain a special type of phospholipids that exists in the inner membrane ONLY, called cardiolipin.
- ✓ Cardiolipins are synthesized in the mitochondria.
- ✓ **The structure of cardiolipin:**  
**Two phosphatidic acid molecules** connect with a **glycerol** (third one), this glycerol is connected to the first phosphate through carbon number 1, and to the other phosphate through carbon number 3. Thus, the third glycerol is like a junction that connects the two phosphatidic acid molecules.



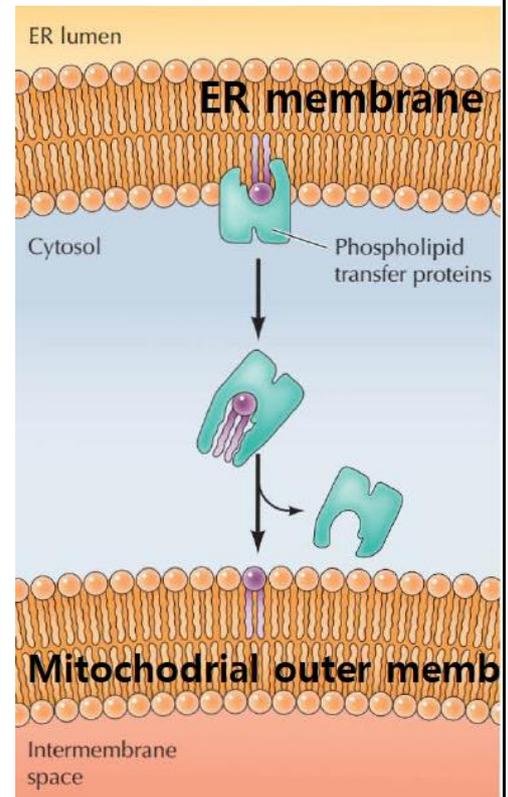
- **The function of cardiolipins:**

Cardiolipin is localized to the inner membrane of mitochondria, where it acts to improve the efficiency of oxidative phosphorylation through affecting the proton transports. (As you remember in oxidative phosphorylation, there are proton pumps that make the proton gradient). So, this type of phospholipids will help in creating this proton gradient.

### **The transfer of lipids between the ER and mitochondria**

- ❖ Lipids are exchanged between the mitochondrial outer and inner membranes at sites of contact between them (the cristae may touch the outer membrane)
- ❖ The transfer of lipids between the ER and mitochondria takes place at sites of close contact between the ER and mitochondrial membranes and it is mediated by phospholipid transfer proteins (how?).

1. First, a phospholipid transfer protein extract a single phospholipid molecule from the membrane of the ER.
2. The lipid(hydrophobic) is transported through the aqueous environment of the cytosol (how?)
  - By binding to a hydrophobic binding site of the protein, so the lipid becomes buried in the hydrophobic binding site.
3. The lipid is released when the complex reaches a new membrane (mitochondrial outer membrane).



#### NOTE

(Cardio=heart)

But this phospholipid doesn't present in heart cells only, instead it is present in the inner membrane of the mitochondria of all cells.

#### Why "cardio"?

We may think of the heart because its cells are very active, so they need lots of energy. Therefore, they include large number of mitochondria inside them

### Mitochondrial Diseases

- They are mostly rare genetic diseases (caused by mutations in mitochondrial DNA) that affect the proteins encoded by the mitochondrial DNA.
- Defects of Mitochondrial DNA (mtDNA) are disorders associated with the dysfunction of the respiratory chain because all 13 subunits encoded by mtDNA are subunits of respiratory chain complexes.
- These diseases are inherited from **Mothers** (Maternal inheritance), because almost all mitochondria of fertilized eggs come from the cytoplasm of the ovum(oocyte) rather than the sperm.

- Diseases due to **point mutation** are transmitted by maternal inheritance as mentioned above.
- These diseases are mostly related to metabolism, because proteins which are encoded by the mitochondrial DNA are enzymes involved in metabolism especially in oxidative phosphorylation, electron transport chain, and ATP synthesis.
- One main syndrome is **myoclonic epilepsy and ragged red fiber disease** (MERRF), which can be caused by a mutation in one of the mitochondrial transfer RNA genes required for synthesis of mitochondrial proteins responsible for electron transport chain and synthesis of ATP.

**Other syndromes include:**

- 1- Lactic acidosis and stroke-like episodes (MELAS).
- 2- Leber's hereditary optic neuropathy (LHON).
- 3- Neurogenic atrophy, ataxia and retinitis pigmentosa (NARP).

**+ Leber's Hereditary optic neuropathy (LHON)**

- It is a rare inherited disease that results in blinding because of degeneration of the optic nerve.
  - Vision lost is the only manifestation that occurs between 15-35
- Females are affected **less** frequently than males.
- Only about 10% of females and 50% of males possessing a mutation suffer vision loss (not all individuals with mutation develop the disease).
- Males never transmit LHON to their offspring.
- Mutations **reduce** the efficiency of oxidative phosphorylation and ATP generation.
  - This disease can be caused by mutations in mitochondrial genes that encode components of the electron transport chain.



Credit: Lohm Eye Society Sweden

### Short quiz from Obada Froukh :

**1) The binding site of phospholipid transfer proteins is :**

- A) hydrophilic.
- B) hydrophobic.
- C) amphipathic.
- D) soluble for ions.
- E) It differs from many types of these proteins.

**2) In the electron microscope, a researcher found a polypeptide moving in the inter-membrane space, so the conclusion about signal sequence of this polypeptide is:**

- A) cystine-rich sequences.
- B) presequences from 15 to 55 amino acids.
- C) multiple internal mitochondrial import signals.
- D) all of the above could be a signal sequences of this polypeptide.
- E) None of the above.

**3) In importing of polypeptide having presequences to the mitochondrion, the energy carrier molecule that been used is:**

- A) GTP.
- B) ATP.
- C) electrochemical potential.
- D) A+B.
- E) This process doesn't need an energy carrier molecule.

**4) A polypeptide considers to be beta barrel protein in the outer membrane, so it must pass through:**

- A) Mim1 .
- B) sorting and assembly machinery.
- C) Tom complex.
- D) Tim23 .
- E) B+C.

Answers :

- 1) B (direct question).
- 2) D (many students read the question fast and when they see that the polypeptide in the intermembrane space, they will choose (A) , but actually all polypeptides that have one of all signal sequences mentioned in the question can be seen in the intermembrane space . But If the question mentions a protein moving in the intermembrane space, the answer will be (A).
- 3) B (direct question from the figure).
- 4) E .