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SUBTOPIC NAME	**Protein targeting to Sub Cellular Organelles (Mitochondria, Chloroplast, Peroxisome and Nucleus)**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**Protein targeting to Sub Cellular Organelles**

Protein Targeting: 2. Sorting to organelles.

Objectives:

To acquaint the students about:

i) Protein Targeting to mitochondria: Matrix, inner membrane, intermembrane space and outer membrane;

ii) Protein Targeting to chloroplast: Stroma and thylakoid

iii) Protein Targeting to peroxisomes

iv) Protein Targeting to nucleus

Targeting for MITOCHONDRIA matrix

Requisites:

- **Matrix-targeting sequence** (MTS): MTS is the signal sequence for targeting proteins to mitochondria.

  It is composed of 20-50 amino acids, and is rich in positively charged basic amino acids (Arginine and Lysine), hydroxylated ones (Serine and Threonine), but lack negatively charged amino acids (aspartate and glutamate).

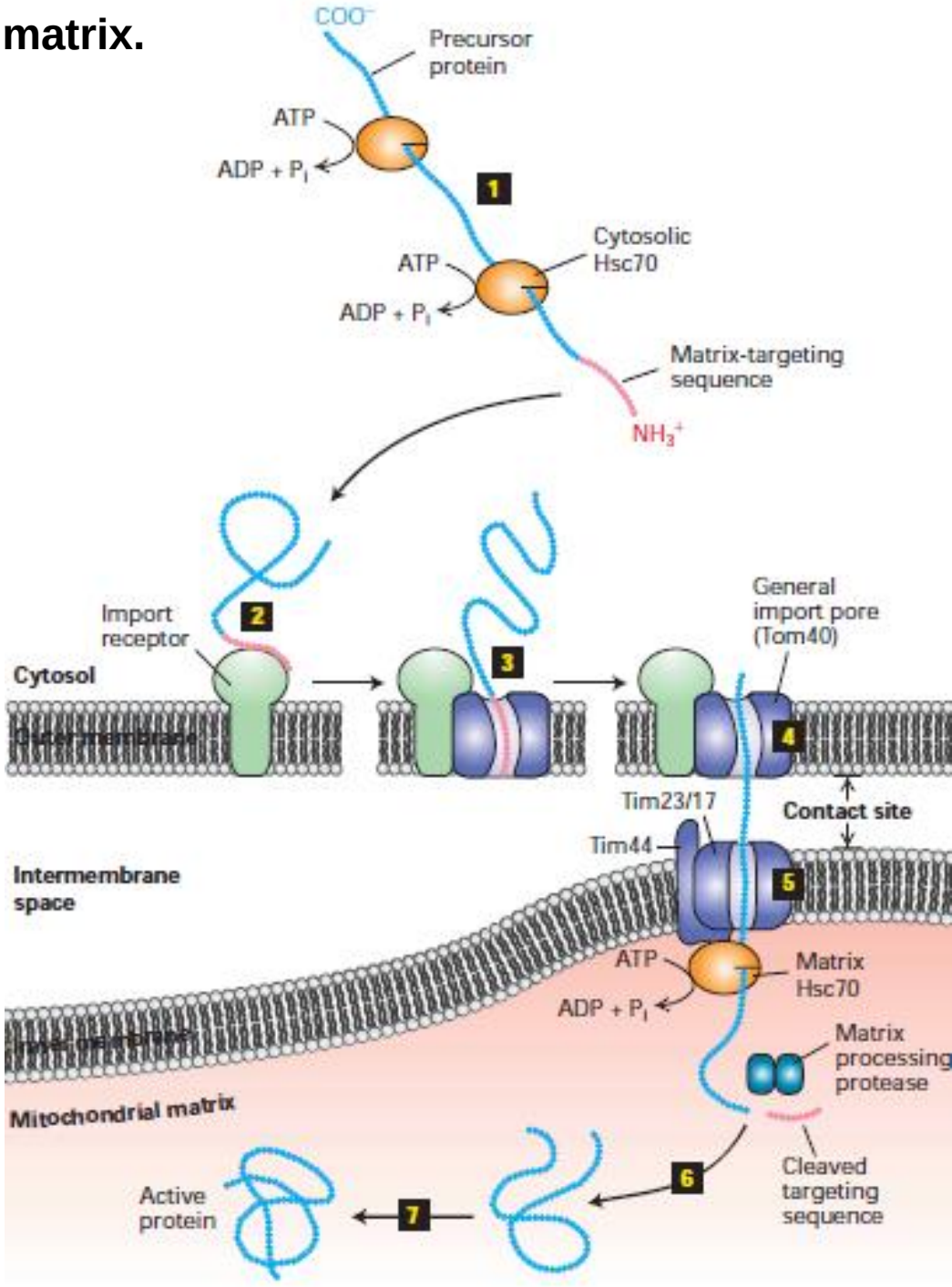
- Receptor: Import receptor

- Translocon: General import pore (Tom40), Tim23/17 and Tim44, passive channel)

- Energy requirement: ATP hydrolysis

Protein import into the mitochondrial matrix.

Precursor proteins synthesized on cytosolic ribosomes are maintained in unfolded or partially folded state by chaperones as Hsc70 (step **1**). After a precursor protein binds to an import receptor (step **2**), it is transferred into general import pore (step **3**). The translocating protein then moves through this channel and an adjacent channel in the inner membrane (steps **4**, **5**). Translocation occurs at rare "contact sites" where inner and outer membranes appear to touch. Binding of translocating protein by the matrix chaperone Hsc70 and subsequent ATP hydrolysis by Hsc70 helps drive import into the matrix. Once the uptake-targeting sequence is removed by a matrix protease and Hsc70 is released from the newly imported protein (step **6**), it folds into mature, active conformation within matrix (step **7**).



Mitochondrial Inner Membrane protein targeting

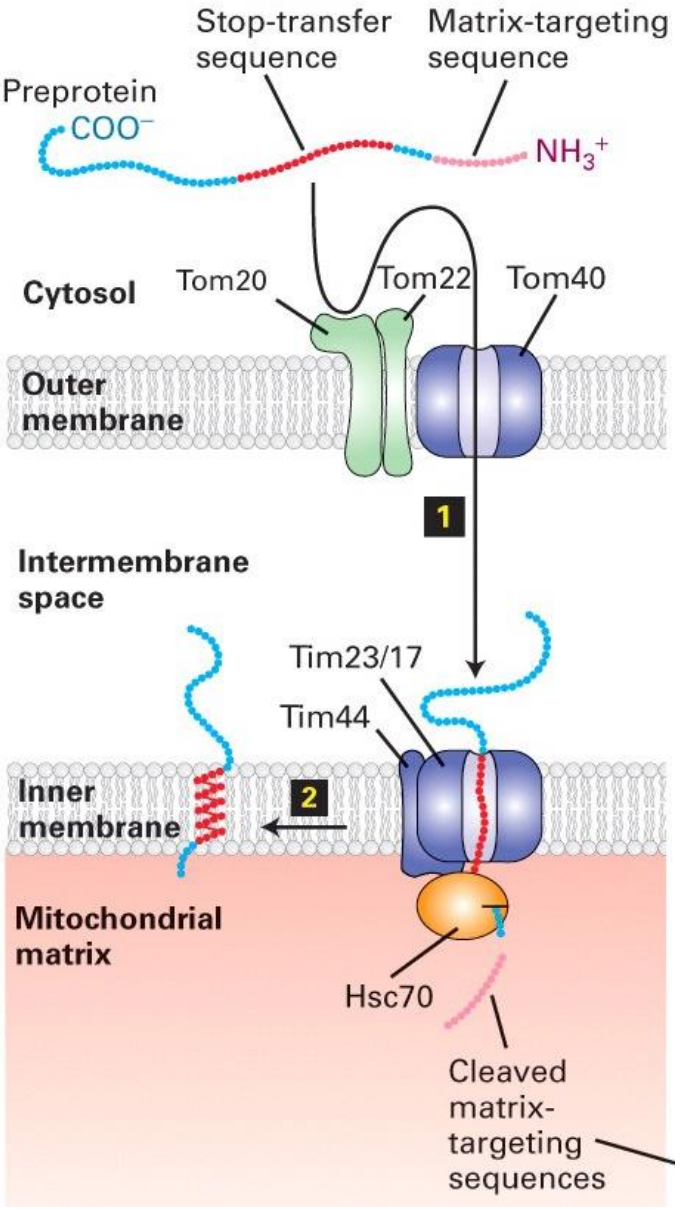
Energy comes from proton motive force

Three pathways:

-**Pathway A** uses the same machinery used for targeting of matrix proteins.

-Tom20/22 recognize their matrix targeting sequence.

- e.g. cytochrome oxidase subunit Cox Va.



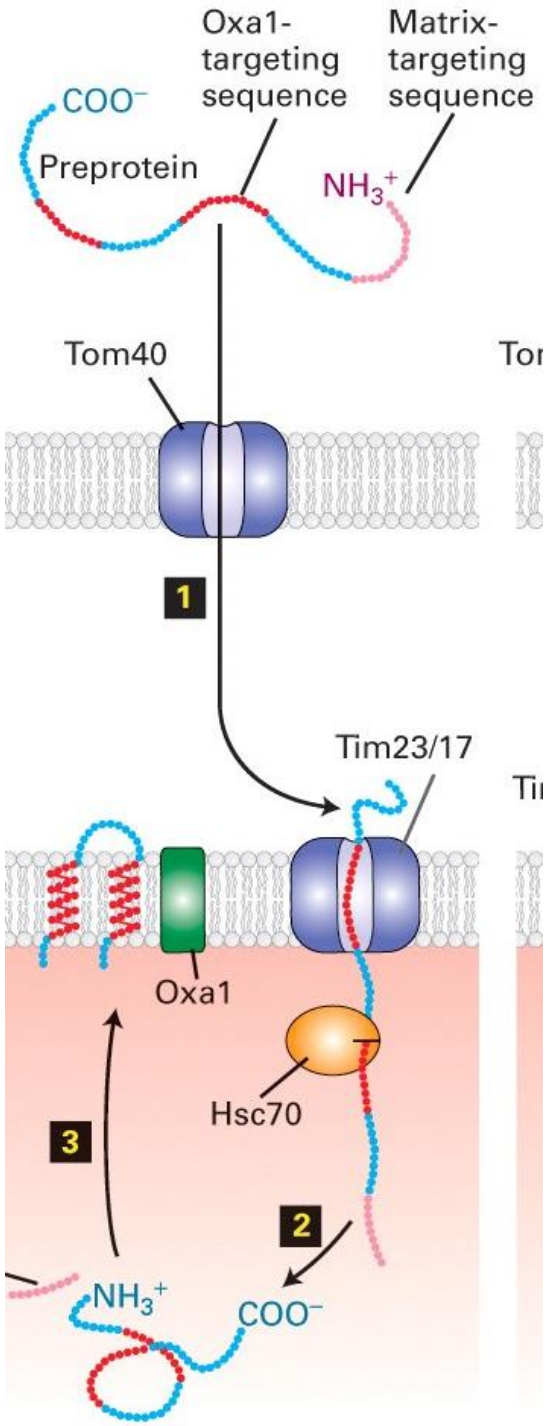
Mitochondrial Inner Membrane protein targeting contd.

Precursors of **pathway B** contain both a matrix targeting sequence and internal hydrophobic domains recognized by an inner-membrane protein named Oxa1.
-e.g. ATP synthase subunit 9

-Insertion of these proteins in Inner membrane require interaction with Oxa-1.

-Proteins delivered by both A and B pathways contain N-terminal MTS recognized by Tom20/22 import receptor in OM, and use Tim23/17 inner membrane channel.

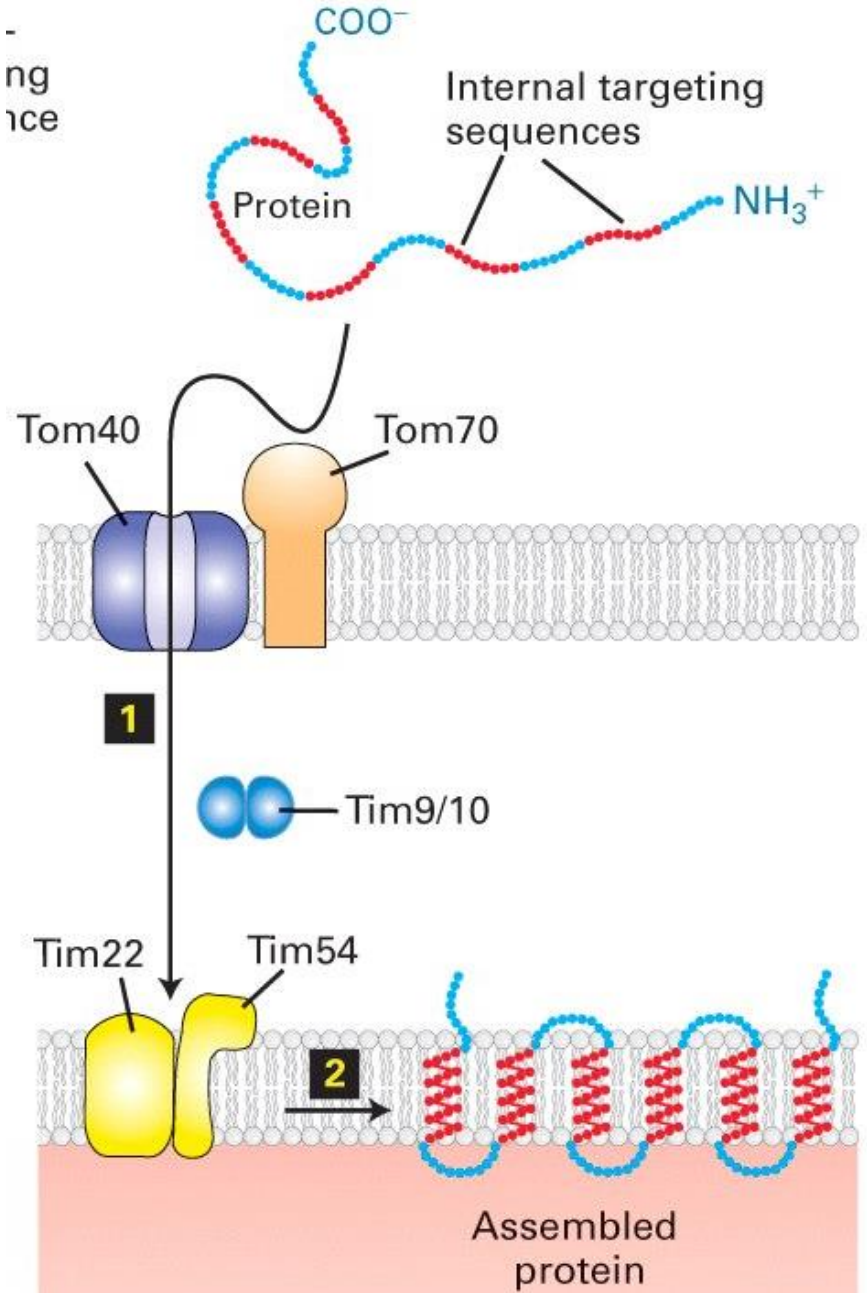
-Pathway A and B differ in that in the entire precursor protein is delivered to the matrix and then redirected to inner membrane in pathway B.



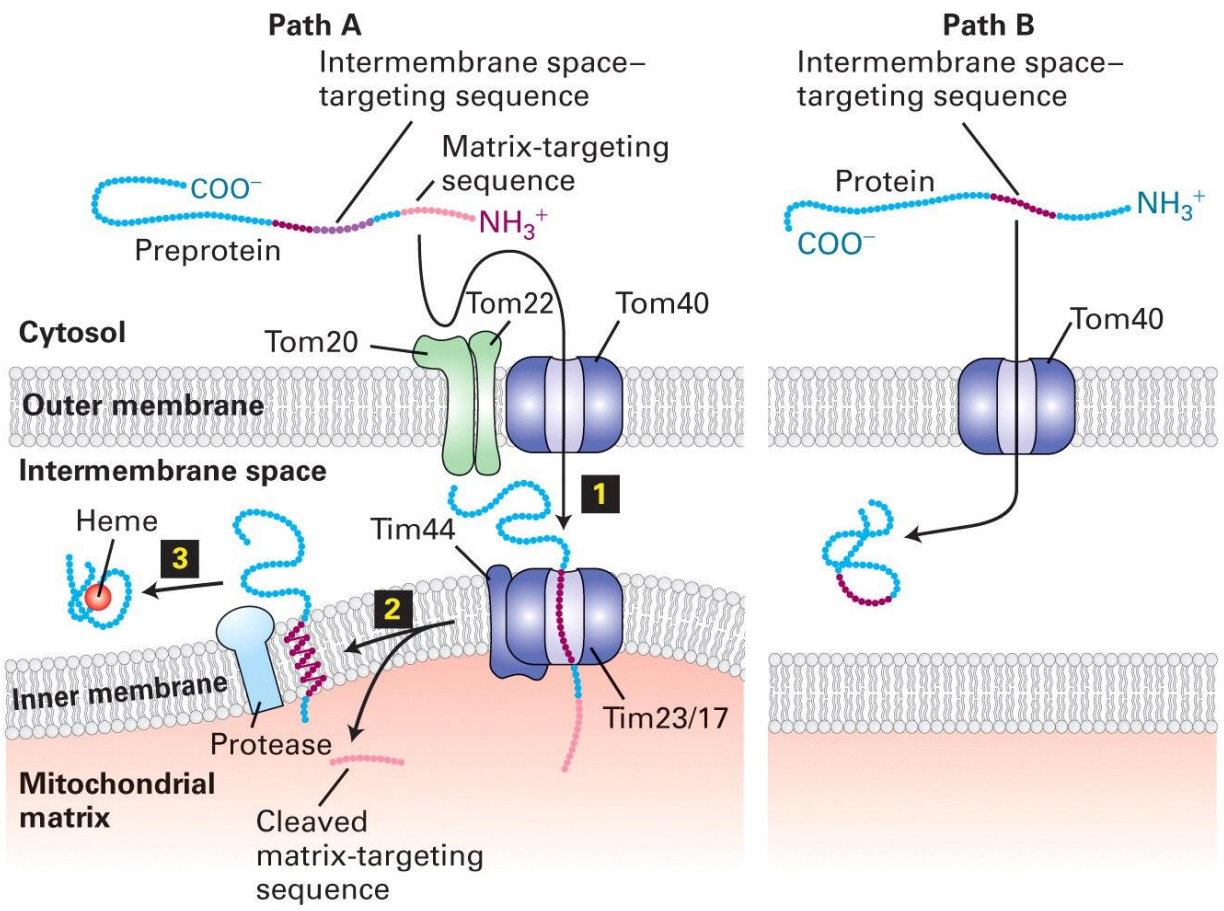
Mitochondrial Inner Membrane protein targeting

Pathway C is used by multi-pass proteins containing multiple internal mitochondrial targeting sequences recognized by Tom70.
-e.g. ADP/ATP antiporter
-No N-terminal MTS

-- Imported protein passes through gen import pore (Tom40)
-Tim 9 andTim 10 protein complex in IM space act as chaperones to guide imported proteins to Tim22/54 complex in IMM.
-- Tim22/54 complex responsible for incorporating multiple hydrophobic segments of imported protein into IMM.



Intermembrane-space protein targeting

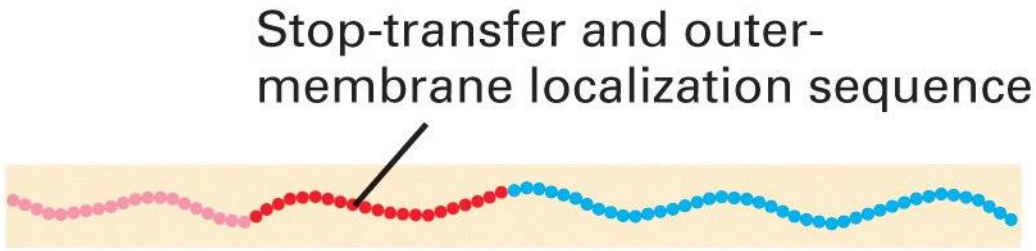


Path A is similar to pathway A for delivery to the inner membrane, but with intermembrane space-targeting sequence that are recognized and cleaved by an inner-membrane protease. E.g. *cytochrome b2*

Path B delivers protein to intermembrane space directly by Tom40. e.g. *cyt c heme lyase*

Outer-membrane protein targeting

e.g. for mitochondrial porin (P70)

Imported protein	Location of imported protein	Locations of targeting sequences in preprotein
Porin (P70)	Outer membrane	

- Sequence context: N-terminal short matrix-targeting sequence (MTS) followed by a long stretch of hydrophobic amino acids.
- Sequence will not be cleaved after translocation.

CHLOROPLAST stromal protein targeting

- Import process is similar to mitochondria matrix protein import but import proteins are different.
- Chloroplast stroma does not have proton motive force, so the energy of stromal import comes solely from ATP hydrolysis.
- Signal Sequence for chloroplast targeting is **Stromal Import Sequence**, that is generally rich in Serine, Threonine and small hydrophobic residues, but poor in Glutamate and Aspartate.

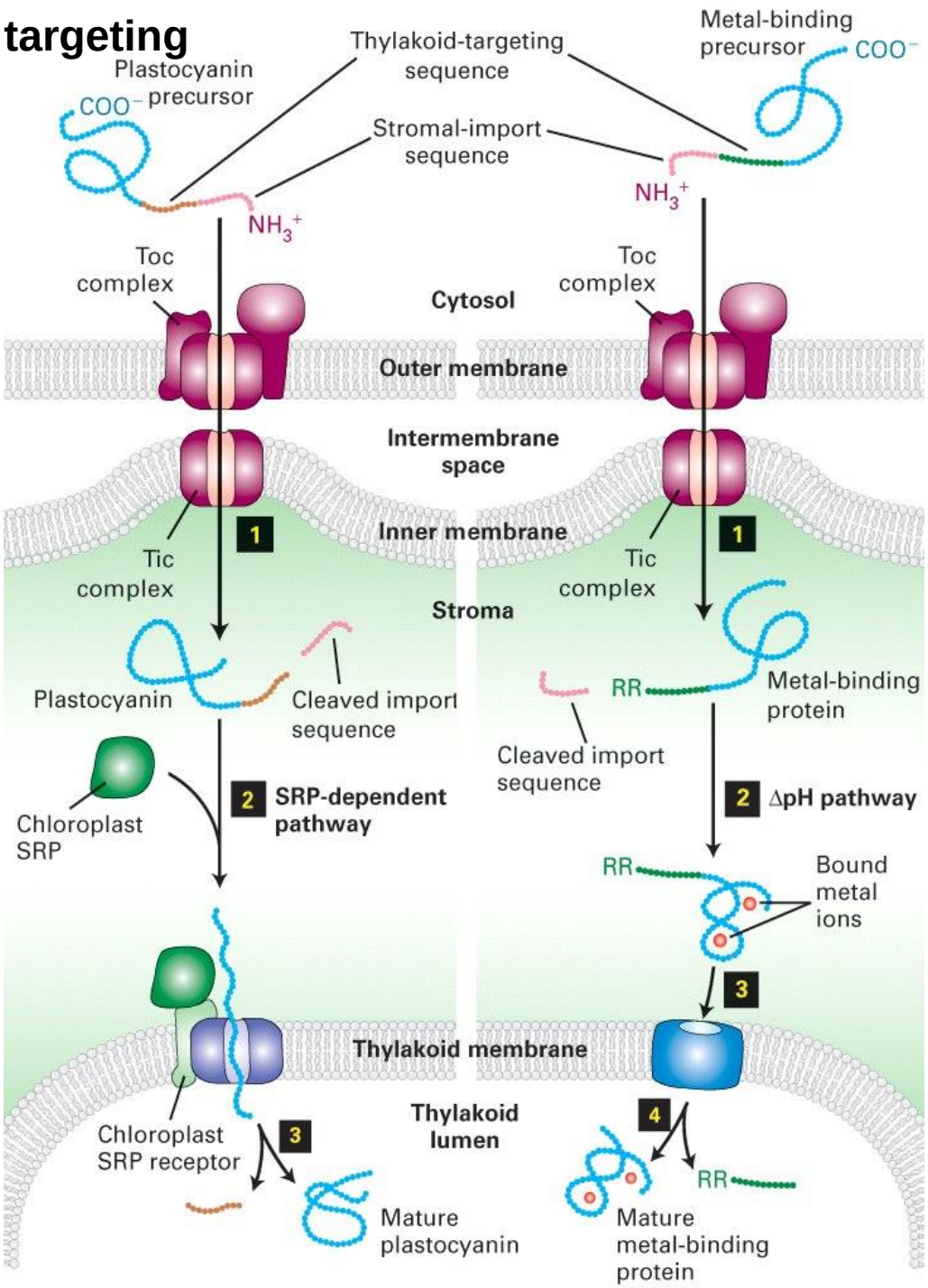
Thylakoid protein targeting

- Protein targeting to thylakoids is mediated by **Thylakoid-Targeting sequence** and occurs via four different pathways:
  -SRP-dependent pathway
  -SecA-related pathway
  -Oxa1 related protein pathway
  -ΔpH pathway

Thylakoid protein targeting

SRP-dependent pathway: is observed for transport of plastocyanin (involved in photo-phosphorylation) to thylakoid lumen.

*Proteins kept in unfolded state in stroma by a set of chaperones



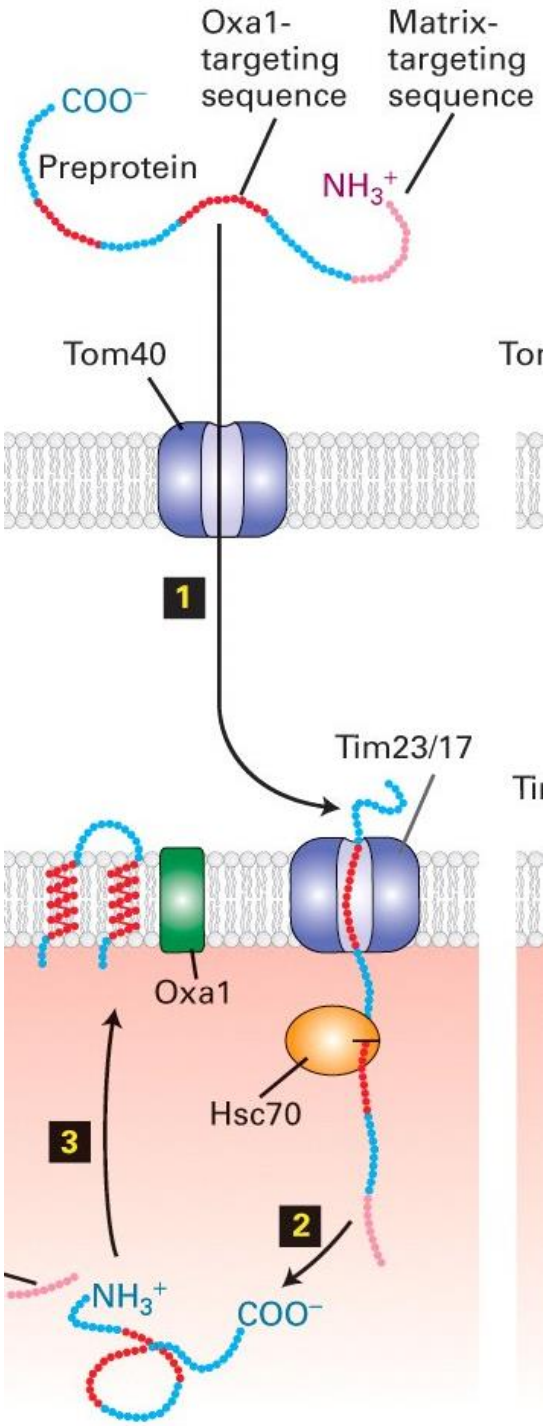
ΔpH pathway: unfolded **metal binding proteins** are transported to the stroma first, then protein folds and binds cofactor.

Then a set of thylakoid-membrane proteins will transport them to thylakoid lumen (process powered by pH gradient across thylakoid membrane)

Thylakoid-targeting sequence contain two closely spaced Arginine residues that are crucial for recognition.

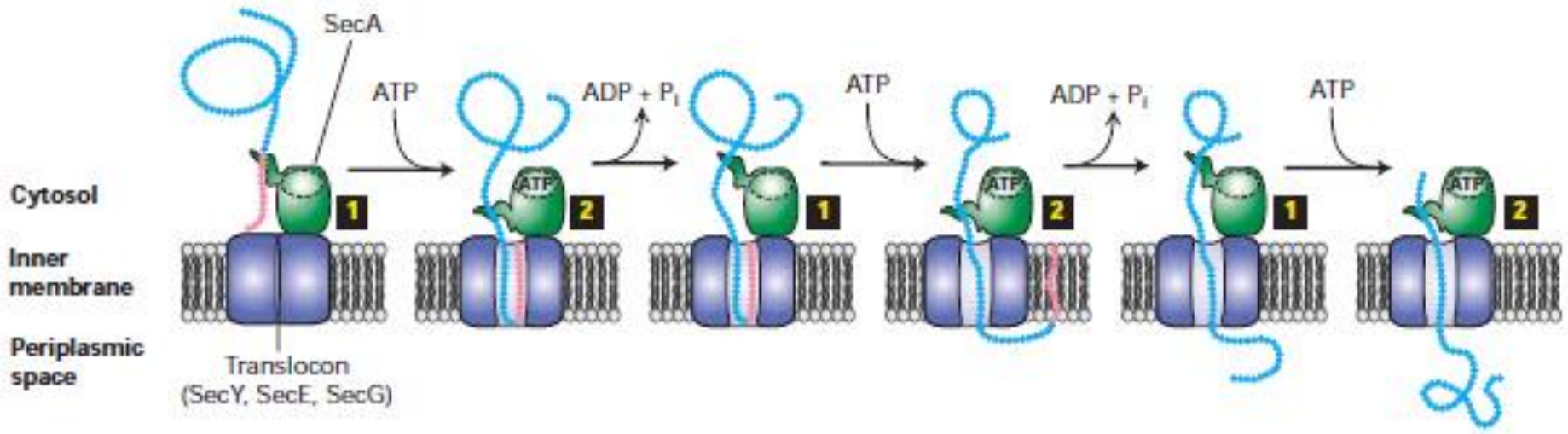
Oxal related protein pathway

Some proteins are inserted into the thylakoid membrane by this pathway.



SecA-related protein pathway

This pathway transport proteins into thylakoid lumen and in gram negative bacteria.

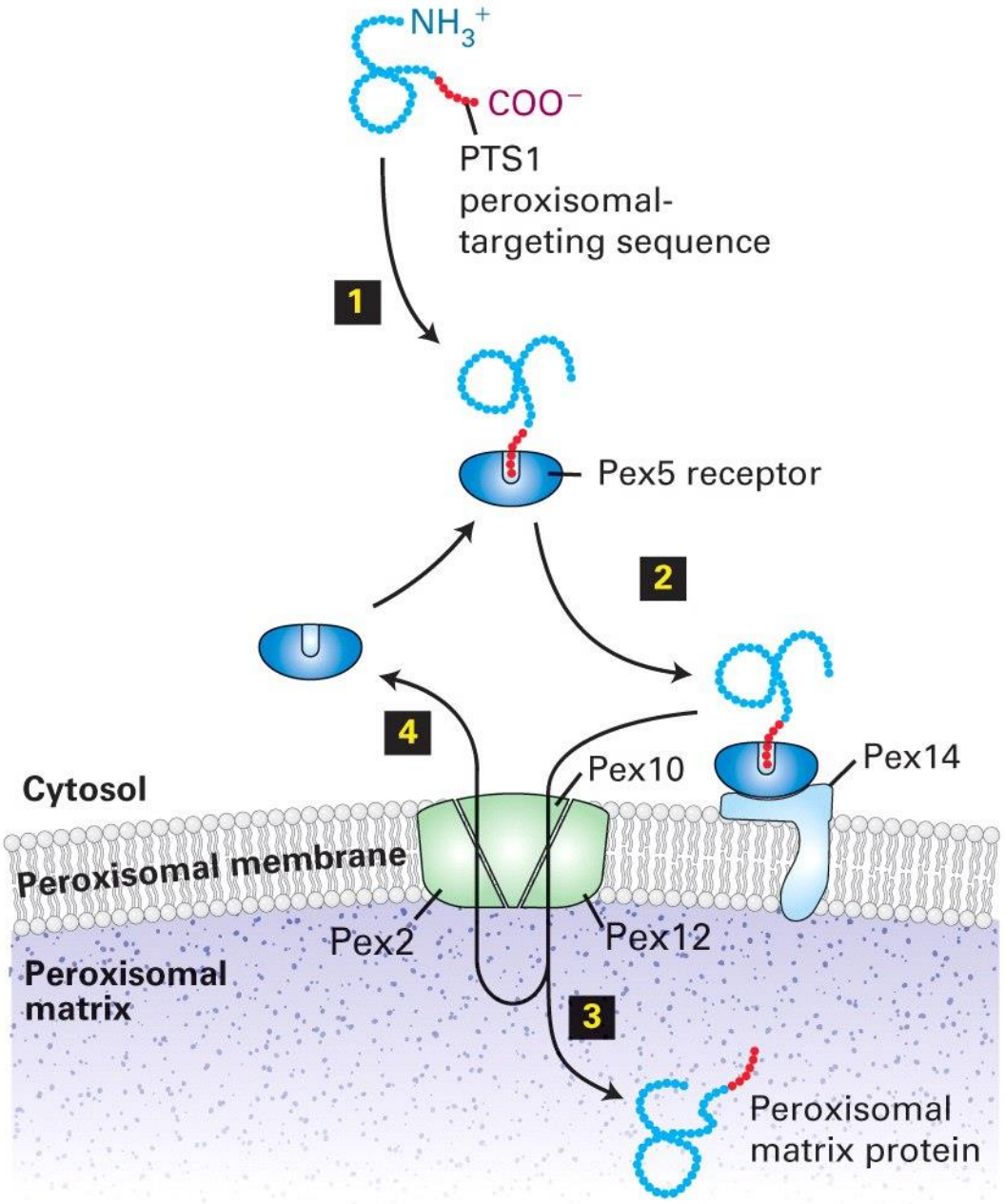


Post-translational translocation across inner membrane in gram-negative bacteria. The bacterial inner membrane contains a translocon channel composed of three subunits that are homologous to the components of the eukaryotic Sec61 complex. Translocation of polypeptides from the cytosol to the periplasmic space is powered by SecA, a cytosolic ATPase that binds to the translocon and to the translocating polypeptide. In the model shown here, binding and hydrolysis of ATP cause conformational changes in SecA that push the bound polypeptide segment through the channel (steps 1, 2). Repetition of this cycle results in movement of the polypeptide through the channel in one direction. Current evidence indicates that the N-terminal signal sequence moves from the channel into the bilayer but at some point is cleaved by a signal peptidase, so that the mature polypeptide enters the periplasmic space.

PROTEIN TARGETING TO PEROXISOME

- Peroxisome Targeting Sequence: **PTS1** (SKL or Ser-Lys-Leu) near the C-terminus is required for peroxisome targeting, it will not be cleaved after transport
- ATP hydrolysis is required.
- Receptor: Pex5, Pex14
- Translocon: Pex10, Pex12
- Folded proteins can be translocated.
- Few peroxisome matrix proteins as thiolase-synthesised as precursor with N-terminal **PTS2**.

Import mechanism – same.



Uptake Targeting Sequences That Direct Proteins from the Cytosol to Organelles

Target Organelle	Location of Sequence Within Protein	Removal of Sequence	Nature of Sequence
Endoplasmic reticulum (lumen)	N-terminus	Yes	Core of 6–12 hydrophobic amino acids, often preceded by one or more basic amino acids (Arg, Lys)
Mitochondrion (matrix)	N-terminus	Yes	Amphipathic helix, 20–50 residues in length, with Arg and Lys residues on one side and hydrophobic residues on the other
Chloroplast (stroma)	N-terminus	Yes	No common motifs; generally rich in Ser, Thr, and small hydrophobic residues and poor in Glu and Asp
Peroxisome (matrix)	C-terminus (most proteins); N-terminus (few proteins)	No	PTS1 signal (Ser-Lys-Leu) at extreme C-terminus; PTS2 signal at N-terminus

DISCLAIMER:

All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline.

References

- Lodish, Harvey F. *Molecular Cell Biology.* 5th ed. New York: W.H. Freeman, 2003.

- Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. New York: Garland Science, 2002.