

Biogenesis: Post-translational targeting



Compartmentalization of eukaryotic cells



- Each eukaryotic cell is subdivided into functionally distinct, membrane-bound compartments - organelles
- Each compartment has its own distinct set of proteins = functions
- A complex distribution system moves proteins from the place of synthesis to its proper destination

Protein targeting



- Protein has to be correctly localized to perform proper function
- Location, location, location!
 - Receptors – plasma membrane
 - DNA polymerase – nucleus
 - Catalase – peroxisomes
 - Insulin – outside

Protein targeting



- Targeting of the protein to the subcellular compartment (organelle) starts as early as the beginning of translation (synthesis)
- The information about the destination is encoded in the coding sequence



Sorting of nuclear- encoded proteins in eukaryotic cells



- All proteins begin to be synthesized on cytosolic ribosomes
- Sorting or translocation can occur
 - Co-translational
 - Post-translational
- If the protein is cytosolic the synthesis will be finished on free ribosomes and peptide released into the cytosol

Sorting of nuclear- encoded proteins in eukaryotic cells



- If the protein is destined for nucleus, mitochondria or peroxisomes the synthesis is also finished on cytoplasmic ribosomes and the peptide is released to the cytosol (to be sorted later or post-translationally)

Sorting of nuclear- encoded proteins in eukaryotic cells



- If the protein is going to be secreted from the cell or it destined for the membranes the ribosome with the nascent peptide is targeted to the ER (ER becomes rough) and sorting is done during translation (co-translationally)

Post-translational translocation



- Proteins that are going to **nucleus, mitochondria, chloroplasts and peroxisomes**
- Proteins that are going to cytosolic leaflet of the membrane
- Proteins that are going out of bacteria

How do proteins know where to go?



- The information about the destination is encoded in the coding sequence
- Targeting signal (sequence) – a delivery address
- Present in the precursors of all proteins except those remaining in the cytosol (no need for targeting)

Targeting signal



- **Characteristic for the destination not the protein**
- Part of the polypeptide
 - Can be cleaved later by signal peptidase or remain permanent part of protein
- Can be located on N-, C-terminus or in the middle of the protein

Targeting signal



- Mechanism of targeting - directs protein to the organelle by binding to specific receptor on the **destination** organelle
- In case of double membrane organelles transfer requires two signals and two receptors

Translocation - what is needed



- Targeting signal
- Specific receptor
- A **channel (translocon)** or two (for double membrane organelles) to translocate (cross the organelle's membrane)
- ATP to provide energy
- Chaperones

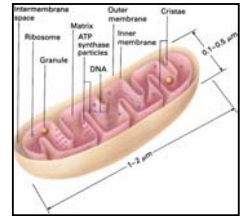
Transport of the new protein into mitochondria

- Most mitochondrial proteins are encoded by nuclear DNA
 - Only very few are encoded by mitochondrial DNA and synthesized on mitochondrial ribosomes



Mitochondria

- Mitochondrial proteins are directed into several location
 - Outer membrane
 - Inner membrane
 - Intermembrane space
 - Matrix



What is required for protein translocation into mitochondria?

- Mitochondrial targeting signal
 - or two if the protein crosses both membranes
- Receptors on the mitochondrial membrane(s)
- One or two translocation channels
- Energy
- Chaperones



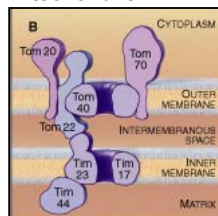
Mitochondrial targeting signal

- Usually located at N-terminus of precursor polypeptide
- Usually removed in mitochondrial matrix



Receptor/translocation channels in mitochondria

- Tom – translocase of the outer mitochondrial membrane
- Tim - translocase of the inner mitochondrial membrane



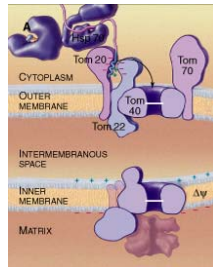
Transport of new proteins into mitochondria (1. Delivery)

- Mitochondrial proteins are synthesized in cytosol as precursors
- Bind to cytosolic chaperones to keep them unfolded until they ready to be translocated
 - Hsp70 and MSF (mitochondrial-import stimulation factor)
 - Energy from ATP



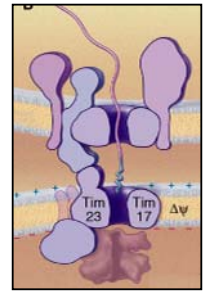
Transport of new proteins into mitochondria (1. Delivery)

- Most precursor proteins bind to Hsp70
- Targeting signal binds to a channel - linked receptors (Tom 20 and Tom 22) in the outer mitochondrial membrane



2. Translocation across outer membrane

- Tom 20 and Tom 22 receptors are linked to Tom 40 (an outer membrane translocon)
- Protein passes through Tom 40
- Energy provided by hydrolysis of ATP by Hsp70

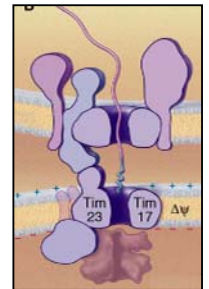


2. Translocation across outer membrane

- Some outer membrane proteins insert themselves in the membrane while in transit
- Intermembrane space proteins remain there and fold
- Protein destined to matrix passes through Tom 40 and then Tim (inner membrane translocon)

3. Translocation to mitochondrial matrix

- Occurs only at contact points (both membranes are nearby)
- Tom and Tim come together (electrostatic interactions)

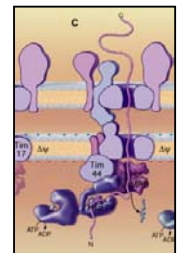


3. Translocation to mitochondrial matrix

- Tim 23 and Tim 17 form a translocon
- Energy for translocation
 - ATP hydrolysis by matrix chaperones
 - Transmembrane potential across inner membrane

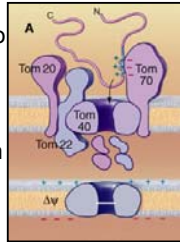
In the mitochondrial matrix

- After crossing both membranes the matrix-targeting sequence is cleaved by matrix protease
- A protein binds to **matrix** Hsp70
 - Prevents premature folding
- Final folding



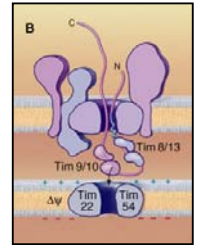
Another 3. Translocation with insertion into inner membrane

- Internal targeting sequence
- Usually precursor proteins bind to MSF chaperones
- The complex binds to a set of receptors Tom 5 and/or Tom 70 that guide protein to translocation channel (Tom 40)



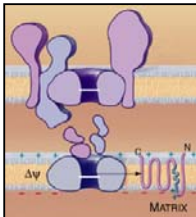
Another 3. Translocation with insertion into inner membrane

- “tiny” Tims – chaperones in the intermembrane space – direct protein to inner membrane translocon Tim 22 and Tim 54
- Energy
 - Transmembrane potential



Another 3. Translocation with insertion into inner membrane

- Insertion into inner membrane directed by transmembrane potential (+ and -)



4. Insertion of mitochondrial DNA encoded proteins

- Insertion into inner membrane
- Oxa 1p, process similar to bacterial insertion



Peroxisomes

- Single membrane organelle
- Matrix contains oxidative enzymes
 - Lipid oxidation without ATP production
- Proteins encoded by nuclear DNA (all have to be imported)



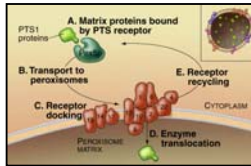
Transport into peroxisomes

- Proteins are synthesized and fully folded in cytosol
- **Fully functional, fully folded protein is transported!**
- Import requires ATP hydrolysis
- Peroxisome targeting sequences
 - PTS1 on C-terminus, very conserved
 - PTS2 on N-terminus, just few proteins



Transport into peroxisomes

- Peroxins - peroxisome transport receptors
- Bind to proteins with PTS1 and dock to the translocation channel
- The complex is transported through the membrane
- Protein is released
- Peroxin is recycled



Transport into the nucleus

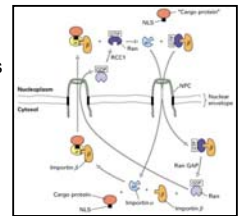
- All proteins found in the nucleus are synthesized in the cytoplasm
- Examples:
 - Histones
 - Ribosomal proteins
 - DNA and RNA polymerases
 - Transcription factors

Transport into the nucleus

- Transport requires nuclear localization sequences (NLS)
- Transport occurs through the nuclear pores
 - Nuclear import receptor (Importin α and β)
 - Energy from GTP
 - GTPase Ran
- Fully folded proteins are transported

Transport into the nucleus

- Importin α and β bind to the protein to be transported
 - Nuclear localization signal binds to importin α
- The complex is translocated through the nuclear membrane



Transport into the nucleus

- Activated Ran (GTP) causes the complex to dissociate
- Ran transports importin β are back to cytosol
- Importin α becomes a part of export receptor

