Week III Lecture I
'Protein Sorting Mechanisms'

Proteins
- Linear polymer of amino acids
- Major macromolecular constituent of cells
- Functional machinery encoded by DNA blueprint
- Unique compliments of proteins determine structure and function of organelles
- Must be targeted to specific organelle/compartment
- Free (cytosolic) proteins – synthesized free in cytosol
- Bound proteins – synthesized on ribosomes attached to the rER
- Protein must be converted from a linear chain of amino acids to a specific 3D shape (conformation) to gain function

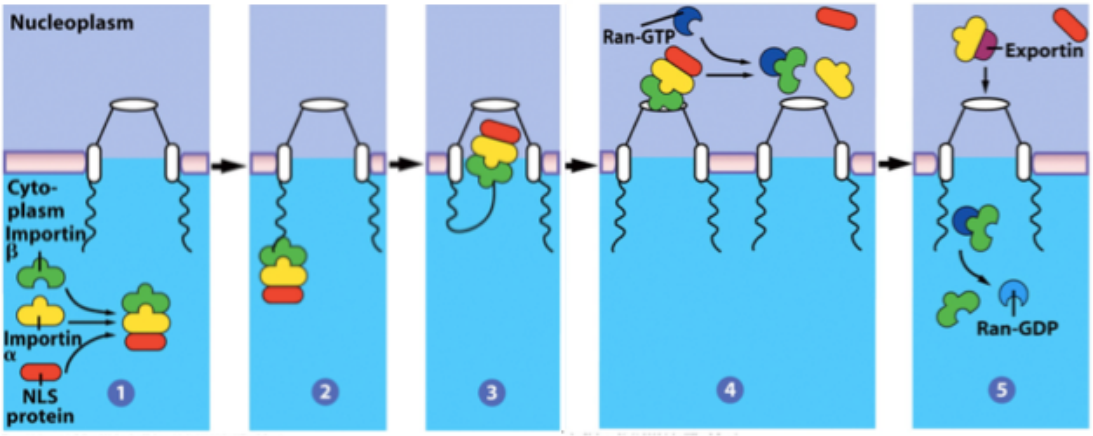
Protein Road Map
- Cytosolic protein
- Transport to nucleus, mitochondria, chloroplasts or peroxisomes
    - Post-translational importation
- Transport to ER, endomembrane system or transported out of cell
    - Co-translation importation

Distinct Routes/Methods of Transport of Proteins
- Transport through nuclear pores
    - Gated transport
- Transport across membranes
    - Transmembrane transport
- Transport by vesicles
    - Vesicular transport

Sorting Signals
- Signal sequences/signal peptides
- Act as 'postcodes'
- Information stored in the amino acid sequence
- Located at
    - End of protein (N terminus)
        - I.e. signal peptide for transport to ER
    - Middle of protein (separate regions contribute to form 'signal patch' when protein folds)
        - I.e. signal patch for vesicular transport (to golgi, lysosome, etc.)
- Transfection experiments
    - Prove that a signal sequence can direct protein trafficking
    - Signal sequence created and fused to GFP in plasmid
    - Plasmid transfected into cells
    - GFP present in organelle that signal sequence was targeting
- Lack of sorting signal leads to protein remaining in cytosol

Protein Trafficking to Nucleus
- Nucleus structure
    - Double layered nuclear envelope (inner and outer membrane)
    - Perinuclear space between inner and outer membrane of nuclear envelope
    - Nuclear lamina at base of nuclear envelope for support/scaffolding
    - Lumen of nuclear envelope is continuous with ER lumen
    - Nuclear pore complex
        - Made of ~50 different proteins
        - Series of concentric rings
        - Cytoplasmic filaments that protrude from the pore to the cytosol
        - Bidirectional traffic occurs through the nuclear pore complex
        - Passageway for macromolecules across nuclear envelope
        - Freely permeable to small water soluble molecules <5000Da
        - Molecules >60,000Da cannot pass
        - Proteins with nuclear localization sequence (NLS) are recognized by nuclear import receptors in the cytosol, which interact with filaments
        - RNA and new ribosomal subunits have nuclear export signals recognized by nuclear export receptors

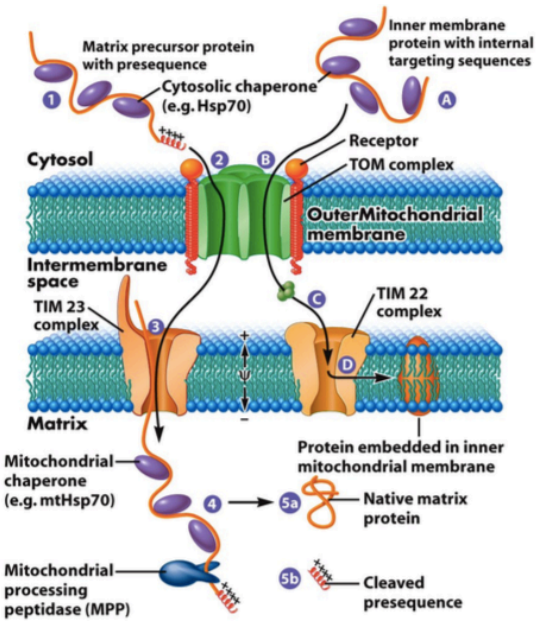
- Importation
    - Energy dependent process (GTP hydrolysis)



1. Protein with NLS binds to soluble heterodimeric receptors (importin α/β)
2. Protein-receptor complex binds to cytoplasmic filament of nuclear pore
3. Protein-receptor complex moves through nuclear pore
4. Protein-receptor complex interacts with Ran-GTP in the nucleoplasm and dissociates
5. Importin β is transported to through the nuclear pore, back to the cytoplasm by binding to Ran-GTP
6. Ran-GTP is hydrolyzed to Ran-GDP and dissociates from importin β
7. Importin α is transported through the nuclear pore, back to the cytoplasm by binding to exportin

Protein Trafficking to Mitochondria

- Membrane bound organelle
- Powerhouse of the cell (convert energy to ATP)
- Mitochondria structure
    - Outer membrane (porins – leaky)
    - Inner membrane (ATP synthase)
    - Intermembrane space (contain kinases)
    - Cristae (inner membrane folds increase surface area)
    - Matrix (enzymes of the TCA cycle)
- Importation
    - Requires the protein to be linear
    - Requires ATP, signal and electrochemical gradient
    - Mitochondria is negatively charged
    - TOM – Translocator, Outer Membrane
        - Receptor + protein channel
    - TIM – Translocator, Inner Membrane
        - Receptor + protein channel
    - Occurs in areas where the inner and outer membrane of the mitochondria are close together
        - TOM + TIM only located at this point
        - Form continuous channel

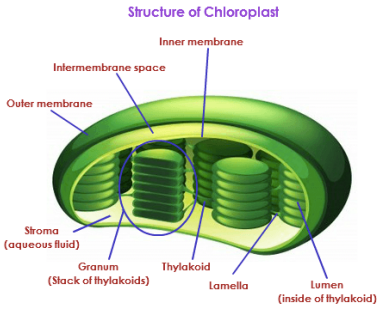


1. Cytosolic chaperone molecules (i.e. Hsp70) unfold protein (requires ATP hydrolysis to dissociate)
2. Tip of protein contains a positive charge (attracted to negative mitochondria)
3. Passes through TOM
4. Passes through TIM
5. Mitochondrial chaperone molecule (i.e. mtHsp70) facilitates folding of protein into native conformation (requires ATP hydrolysis to dissociate)
6. Mitochondria processing peptidase (MPP) cleaves positive signal sequence

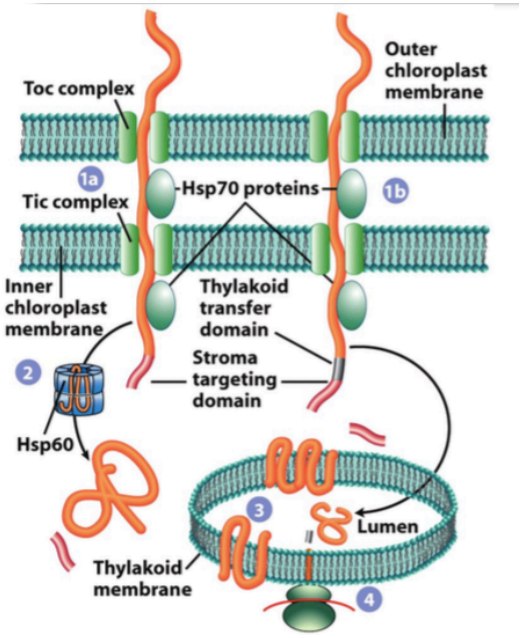
- **Note:** if directed by signal sequence, protein may also enter TOM or TIM complex and remain imbedded in the mitochondrial membrane rather than progress to matrix

Protein Trafficking to Chloroplasts

- Specialized plastid for photosynthesis
- Chloroplast structure
    - 6 compartments
        - Inner and outer membrane
        - Intermembrane space
        - Stroma
        - Thylakoid membrane
        - Lumen

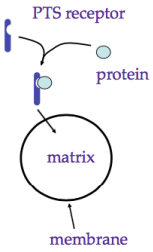


- Importation
    - TOC – Translocator, Outer-membrane Chloroplast
    - TIC – Translocator, Inner-membrane Chloroplast
    1. Cytosolic chaperon molecules (i.e. Hsp70) unfolds protein (requires ATP hydrolysis to dissociate)
    2. Passes through TOC
    3. Chloroplast chaperone molecule (i.e. Hsp70) in intermembrane space keeps protein unfolded
    4. Passes through TIC
    5. Protein passes through the stroma chaperone (i.e. Hsp60) where it is folded and stroma targeting sequence is cleaved
    6. Proteins with only a stroma targeting sequence remain in stroma
    7. Proteins with thylakoid signal sequence are transported to the thylakoid membrane or the thylakoid lumen where their signal sequence is then cleaved (requires GTP)



Protein Trafficking to Peroxisome

- Oxidizes organic molecules
- Uses O_2 to remove H_2
- Produces and degrades H_2O_2
- Breakdown of fatty acids
- Structure
    - Single membrane
    - Matrix



- Importation
    - Proteins destined for peroxisomes contain a peroxisomal targeting signal
    - PTS → matrix protein
    - mPTS → membrane protein
    - PTS receptors bind to peroxisomal proteins in the cytosol and shuttle them to the surface of the peroxisome
    - Mechanism of entry unknown
    - Chaperone involvement not confirmed
        - Proteins are believed to cross the membrane in their native, folded form