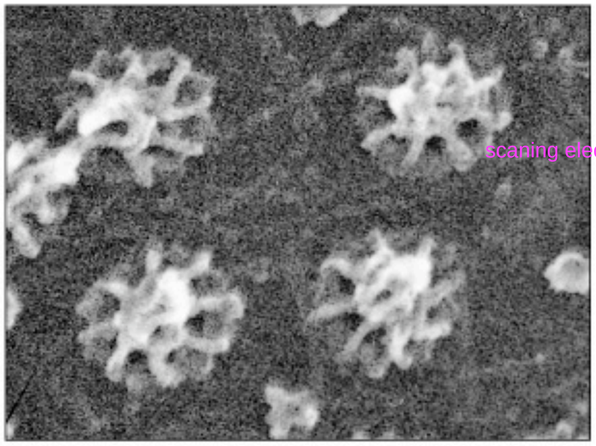
Protein Sorting: Nucleus and Mitochondrion



scaning electron micrograph

NPCs from inside of nucleus

1

Protein Sorting

- "*Sorting*": movement to appropriate destinations (compartments).
- Eukaryotic cells are compartmentalized.
- Each organelle requires specific proteins (e.g. enzymes, transporters etc.) to perform their function.
- About 10 billion proteins of 10 – 20 thousand types.
- Most of these are synthesized elsewhere in the cytosol and must be transported ("sorted") to appropriate sites.

2

Movement of Protein Between Compartments

1. Gated transport: nucleus

proteins move betwen the cytosol and the nucleus through nuclear pore complexes in the nuclear envelope.

2. Transmembrane transport: mitochondria and ER

transmembrane protein translocators directly transport specific proteins across a membrane from the cytosol into a space.
the transported protein molecule usually msut unfold to snake through the translocator.

3. Vesicular transport: secretory pathway

membrane-enclosed transport intermediates-which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments.-ferry proteins from one to another compartment. the transport vesicles and fragments become loaded with a cargo of molecules derived from the lumen of one compartment as they bud and ponch off from its membrane ; they discharge their cargo into a second compartment by fusing with the membrane enclosing that conpartment

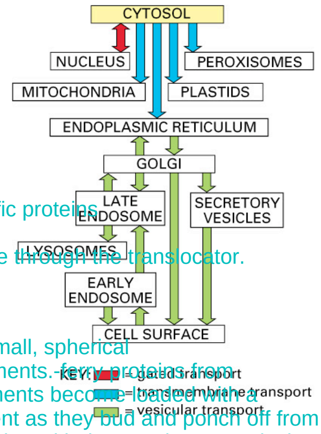


Figure 12–6. Molecular Biology of the Cell, 4th Edition.

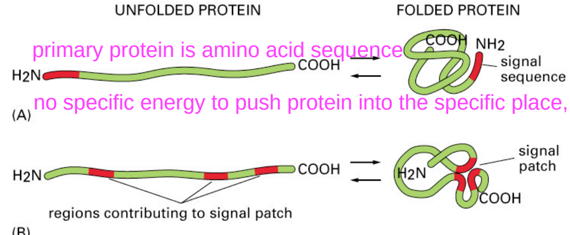
3

Signal Sequence or Patch

identifier proteins that allow them to go to the certain place in the cell

the synthsis of all proteins begins on tibosomes in the cytosol, except for the few that are synthesized on the ribosomes of mitochondria and plastids. then, their fate depends on their amino acid sequence, which can contain sorting signals that direct their deliverv to locations outside the cvtosol

- Amino acid sequence that directs protein to specific destination.
- Signal sequence: signal is composed of consecutive amino acid sequence at terminal end of protein.
- Signal patch: signal amino acids are internal to protein.



primary protein is amino acid sequence

no specific energy to push protein into the specific place, just random movement

intrisinc signal, when the protein is folded, the signal wind up very closely to form a signal patch

Figure 12–8. Molecular Biology of the Cell, 4th Edition.

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Protein Sorting Between Cytosol and Nucleus

- Important proteins, such as polymerases, gene regulatory proteins etc. are synthesized in the cytosol and imported into the nucleus.
- Others (e.g. mRNA) are synthesized in the nucleus and exported to the cytosol.
- *Bidirectional traffic*
- Nuclear envelope composed of inner and outer membrane, and nuclear pore complexes.

it sythesized in the cytosol and direct to the nucleus, that's how it regulate the transcription of the gene

many proteins that function in the nucleus(histones, DNA and RNA polymerases, gene regulatory proteins and RNA processing proteins) are selectively imported into the nuclear compartment from the cytosol, where they are made. at the same time, tRNA and mRNA are synthesized in nuclear compartment and then exported to the cytosol.

even inner and outer membrane are continuous,they maintain distinct protein compositions

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Nuclear Pore Complex

- Perforate nuclear envelope.
- Octagonal structure.
- Composed of *nucleoporins*.
- Allow passage of molecules.

this complex is composed of nucleoporins

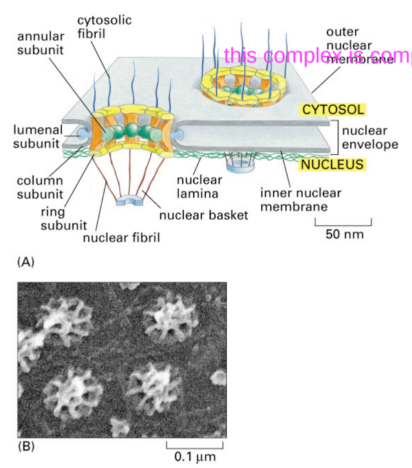

Figure 12–10 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

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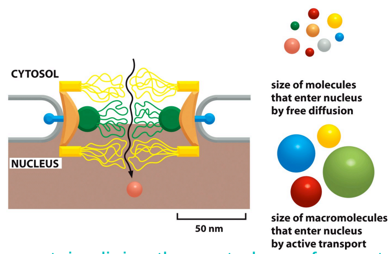
Nuclear Pore Complex

small can be passed by diffusion,

- Structure includes an aqueous pore.
- Small proteins traverse by passive diffusion.
- Recent evidence indicates a tangled meshwork lines the pore to block passive diffusion of large molecules.
- Large proteins traverse by active transport mechanism.
- Proteins are imported/exported in *folded* conformation.



unstructrued regions of the proteins lining the central pore form a tangled meshwork which blocks the passive diffusion of large macromolecules

Figure 12-10 *Molecular Biology of the Cell* (2008)

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Nuclear Import Receptors

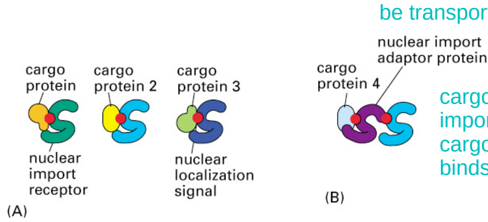
the import receptors can bind both to the nuclear localization signal on the protein to be transported and to NPC proteins

receptors themselves interact with both

- Bind to nucleoporins and nuclear localization signals (NLSs).
- NLSs are signal sequences or patches.
- NLS-receptor recognition initiates import of cargo proteins.
- NLSs are specific for receptors.
- Sometimes adaptor proteins involved.
- Export works the same way, but in reverse, and utilize export receptors.

nuclear import receptors do not always bind to nuclear proteins directlyadditional adaptor proteins sometimes form a bridge between the import receptors and the nuclear localization signals on the proteins to be transported

NLS indicated by red circles



cargo protien 4 requires an adaptor protein to bind to its nuclear import receptors and recognize nuclear localization signals on cargo proteins. they also contain a nuclear localization signal that binds them to an import receptor

Figure 12–14. Molecular Biology of the Cell, 4th Edition.

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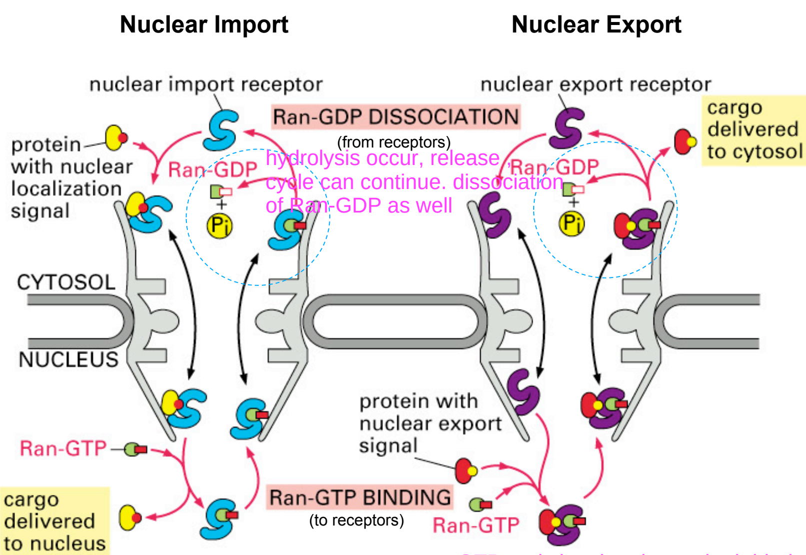
Directional Transport

- Import and export consumes energy.
- A GTPase called Ran acts as a *molecular switch*.
- *Directionality* occurs primarily because Ran-GDP is concentrated in the cytosol, while Ran-GTP exists in the nucleus.
- This gradient of two conformational forms of Ran drives nuclear transport in the appropriate direction.

initiate reaction

Ran is found in both cytosol and nucleus, and it's required for both nuclear import and export. like other CTPases, Ran is a molecular switch that can exist in two conformational states, depending on whether GTP or GDP is bound.



hydrolysis occur, release cycle can continue. dissociation of Ran-GDP as well

Figure 12–16. Molecular Biology of the Cell, 4th Edition.

nire GTP is maintain high concentration in nucles GDP in cytosol.
receptor bind to cargo and signal involve as well pass through the pore. GTP bind to receptor and leads to release cargo, deliver the cargo\ to nucleus

GTP and signal and cargoboth binds to receptor, binding to GTP initiate export and hydrolysis leads to release of cargo deliver to cytosol

GAP triggers GTP hydrolysis and thus converts Ran-GTP to Ran -GDP, and GEF promotes the exchange of GDP to GTP and thus converts Ran-GDP to Ran-GTP. because Ran-GAP is located in the cytosol and Ran-GEF is located in the nucleus, the cytosol contains mainly Ran-GDP and the nucleus contains mainly Ran-GTP. if they reach the nuclear side of the pore complex, Tan-GTP binds to them, and if they arrive loaded with cargo molucules, the Ran-GTP binding causes the import receptors to release the cargo. because the Ran-GDP in the cytosol does not bind to the cargo receptors, unloading occurs only on the nuclear side of the NPC. in this way, the nuclear localization of Ran-GTP creates the directionality.having discharged its cargo in the nucleus, the empty import receptor with Ran-GTP bound is transported back through the pore complex to the cytosol. there ran_GAP triggers Ran-GTP to hydrolyze its bound GTP, thereby converting it to Ran-GDP. the import receptor is then ready for another cycle of nuclear.

Compartmentalization of Ran-GDP and Ran-GTP

- Ran is a molecular switch that exists in two conformations: Ran-GDP in cytosol, Ran-GTP in nucleus.
- GTPase-activating protein (GAP) hydrolyses GTP, and converts Ran-GTP to Ran-GDP.
- Guanine exchange factor (GEF) promotes GDP-GTP exchange, and converts Ran-GDP to Ran-GTP.
- The activity of GAP and GEF maintain the Ran-GDP/Ran-GTP gradient that drives import and export.

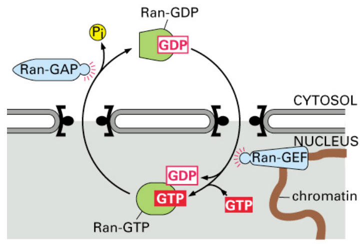


Fig. 12-15, Alberts 4th ed.

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Protein Sorting to the Mitochondrion

- Many mitochondrial proteins are necessary for electron transport, oxidative phosphorylation, and ATP synthesis.
- Most proteins must be encoded in the nucleus and imported from the cytosol. to the mitochondria
- *Posttranslational translocation*.
- Double membrane structure.
- Proteins are transported to matrix or inserted into membrane.

mitochondrial proteins are first fully synthesized as mitochondrial precursor proteins in the cytosol and then translocated into mitochondria by post-translational mechanism.

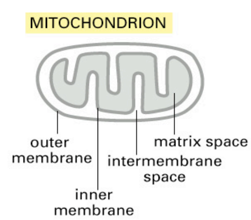

Fig. 12-22 Alberts

proteins can't be foloded
this called molecular champeron
this protein binds to foled protein and maintain their unfold state. once the protein is folded, it will never go to the mitochondria

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The Mitochondrial Signal Sequence

- Exposed amino acid sequence that directs protein to appropriate address.
- Mitochondrial signal sequence is modified as an α helix.
- (+) charged amino acids are exposed at one side, uncharged ones at the other.

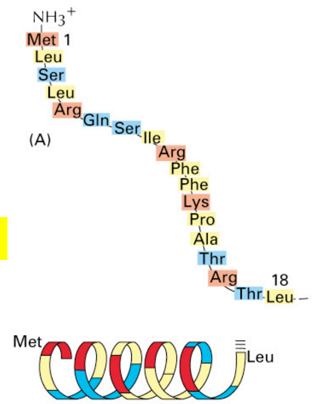

Figure 12–23. Molecular Biology of the Cell, 4th Edition.

one or more signal sequences direct all mitochondrial precursor proteins to their appropriate mitochondrial subcompartment. many proteins ertering the matrix space contain a signal sequence at their N-terminus that a signal peptidase rapidly removes after import. others, including all outer membrane and many inner membrane and intermembrane space proteins, have an internal signal sequence that is not removed. the signal sequences are both necessary and sufficient for the import and correct localization of the proteins: when genetic engineering techniques are used to link these signals to cytosolic protein, the signals direct the protein to the correct mitochondrial subcompartment.

Protein Translocator Complexes

1. *TOM*: translocase of the outer mitochondrial membrane.
2. *TIM*: translocase of the inner mitochondrial membrane.
3. *OXA complex*

these complexes contain some components that act as receptors for mitochondrial precursor proteins, and other components that form the translocation channels

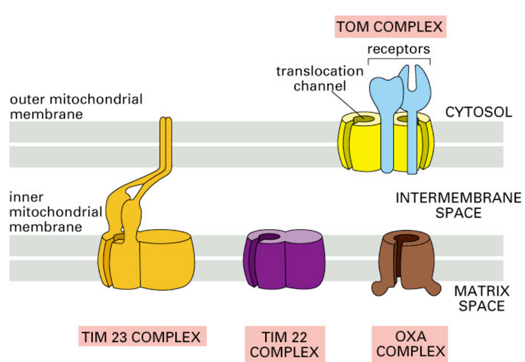

Figure 12–24. Molecular Biology of the Cell, 4th Edition.

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long unfolded protein can through two membrane at the same time

Protein Translocator Complexes

1. *TOM*: imports all mito-destined proteins to intermembrane space; membrane insertion at outer membrane.
2. *TIM*: imports proteins to matrix (TIM 23); insertion into inner membrane (TIM 23); insertion of carrier proteins (TIM 22).
3. *OXA complex*: insertion of proteins into inner membrane.

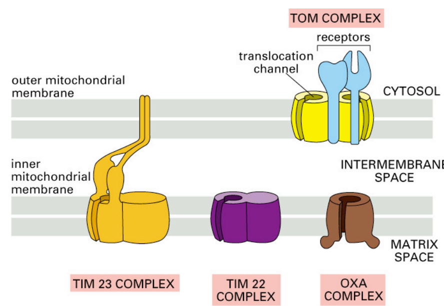

Figure 12–24. Molecular Biology of the Cell, 4th Edition.

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the TOM complex is required for the import of all nucleus-encoded mitochondrial proteins. it initally transports their signal sequences into the intermembrane space and helps to insert transmembrane proteins into the outer membrane. then passed onto an additional translocator, the SAM complex, which helps them to fold properly in the outer membrane. The TIM23 complex transports some soluble proteins into matrix space and helps to insert transmembrane proteins into the inner membrane proteins. the TIM22 conplex mediates the insertion of a subclass of inner membrane proteins, including the transporter that moves ADP,ATP, and phosphate in and out of mitochondria. the OXA complex mediate the insertion of those inner membrane proteins that are synthesized within mitochondria. it also helps to insert some imported inner membrane proteins that are initially transported into matrix space by the other complex

Translocation Across the OM

Formation of precursor protein

↓

maintain the protein unfolded

1 Interacting proteins (e.g. chaperones, *cytosolic Hsp70*) maintain unfolded structure

mitochondrial precursor proteins do not fold into their native structures after they are synthesized; instead, tehy remain unfolded in the cytosol through interactions with other proteins.

↓

2 Binding and recognition of signal sequence at TOM receptor proteins (TOM 20, TOM 5)

↓

3 Transfer of signal sequence and polypeptide to TOM channel (TOM 40)

↓

4 Interacting proteins (e.g. Hsp70) stripped away by ATP hydrolysis

↓

5 Unfolded polypeptide fed through TOM channel

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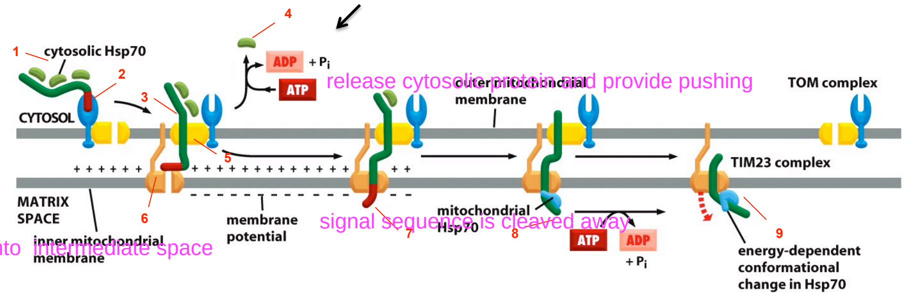
Energy released by ATP hydrolysis pushes polypeptide chain through TOM channel.



separation charge, push proton into intermediate space

Note: The TOM complex is composed of multiple proteins, such as smaller receptor proteins (TOM 20 and TOM 5) and a pore-forming region (TOM 40).

bound cytodolic Hsp 70 is released from the protein in a step that depends on ATP hydrolysis. after initial insertion of the signal sequence and of adjacent portions of the polypeptide chain into the TOM complex, the signal sequence interacts with a TIM complex.
the signal sequence is then translocated into the matrix space in a process that requires a membrane potential across the inner membrane
Mitochondrial Hsp70, which is part of an import ATPase complex, binds to regions of the polypeptide chain as they become exposed in the matrix space, pulling the protein through the translocation channel

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Figure 12-26 *Molecular Biology of the Cell* (© Garland Science 2008)

Translocation Across the IM

6 Polypeptide bound to TIM complex

↓

7 Electrochemical H^+ gradient pulls (+) charged signal sequence through TIM

↓

8 Signal sequence cleaved away by *matrix processing peptidase* (MPP), and mitochondrial Hsp70 binds

↓

9 ATP hydrolysis induces conformational change of mitochondrial Hsp70, polypeptide is pulled through

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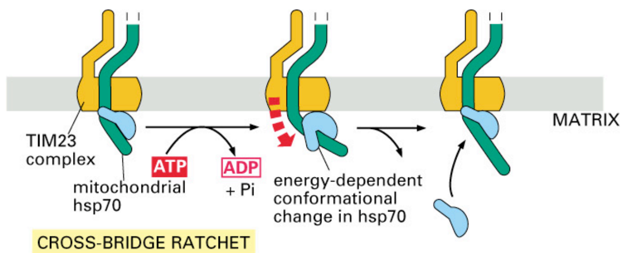
Cross-Bridge Ratchet Model

- Mitochondrial Hsp70 associated with TIM 23.
- ATP hydrolysis drives conformational change in Hsp70 that actively pulls chain through.



Insertion Into the Inner Membrane

delivers the protein completely into the matrix space. cleavage of the signal sequence used for the inital translocation expose the hydrophobic signal sequence. this signal directs the protein to OXA in the inner membrane

SS reaches matrix

↙ ↘

Chain pulled into matrix

↓

1st **SS** cleaved

↓

2nd **SS** guides chain to <u>OXA</u>

direct protein back to membrane

↓

Insertion in IM by OXA

2nd **SS** binds to <u>TIM 23</u> and stops translocation

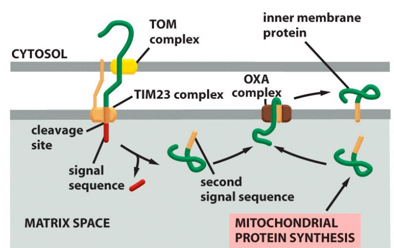
↓

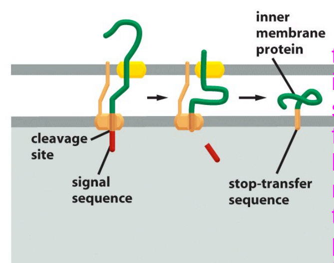
1st **SS** cleaved

↓

Insertion in IM by TIM 23

Also for proteins produced by mitochondrion





the N-terminal signal sequence intitiates import into the matrix space. a hydrophobic sequence that fllows the matrix-targeting signal binds to the TIM in the inner membrane and stops translocation. the remainer of the protein is then pulled into intermembrane space through the TOM in the outer membrane, the hydrophobic sequence is released into the inner membrane.

Figure 12-28 *Molecular Biology of the Cell* (© Garland Science 2008)

Things to Consider...

1. Think about the utilization of energy (i.e. ATP or GTP hydrolysis) in nuclear and mitochondrial translocation.

2. How do nuclear pore complexes and mitochondrial translocator complexes differ?

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