LECTURE 11

PROTEIN SORTING 1

- change of gears - we look at the path of the protein from the emergence of the ribosome (nascent protein) all the way to the expression in the plasma membrane
  - protein can be destined towards many parts of the cell
    - it is not as chaotic as you would think even though there are sooooo many proteins
  - on the first slide, he shows us an image of nuclear pore complexes - perforations in the nucleus that regulate the movement of proteins between the cytosol and nucleus

Protein sorting

- **"sorting"** movement to appropriate destinations (movement of proteins b/w compartments)
  - there will be a signal that carries them to be recognized at the target sites; which is why we' ll find some proteins in the peroxisome or vesicle, etc...
- eukaryotic cells are **compartmentalized**
- **each organelle** requires a specific protein - enzymes transporters etc.. to perform their function
- about 10 billion proteins- 10-20 thousand types
- most of these are synthesized elsewhere in the cytosol and must be transported "sorted" to appropriate sites
  - sometimes there are enzymes and transporters that originate in the ribosome , and are now found in the plasma membrane

Movement of proteins between compartments

In red, there is the GATE TRANSPORT

In blue, there is TRANSMEMBRANE TRANSPORT

Mitochondria and the ER

In green, there is VESICULAR TRANSPORT

Secretory pathway

For example, nuclear pore complexes are gated: you can think of them as being opened or closed, a signal allows them to function

Ion channels that transport ions can be via a different mechansim such as G proteins (seen in later lecture)

For vesicular pathway , we will see how some

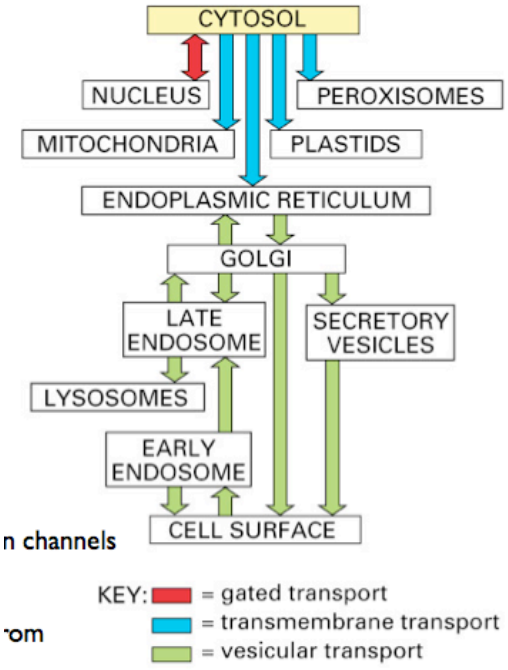
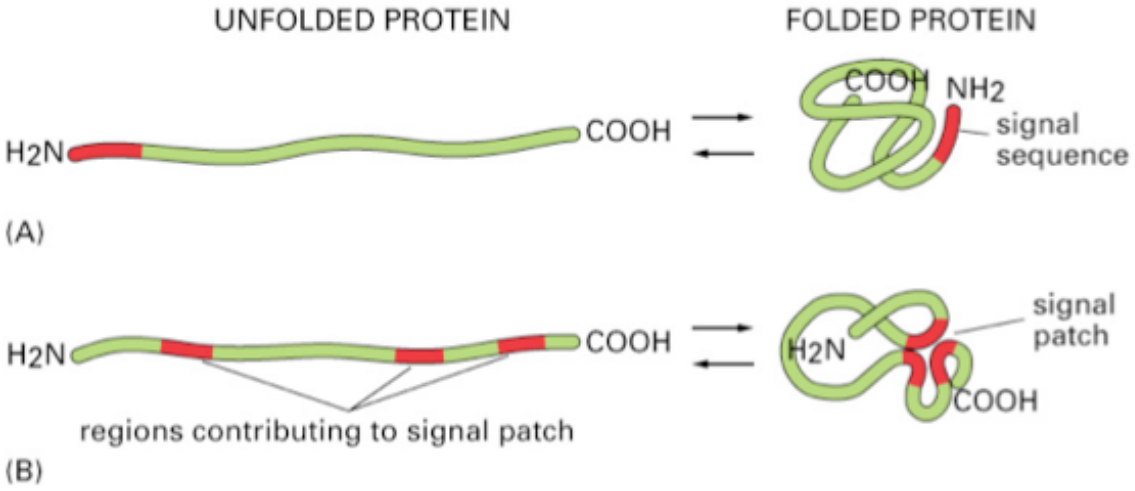


Figure 12–6. Molecular Biology of the Cell, 4th Edition.

of the proteins will go from golgi to cell surface of the plasma membrane
-(bi directionality via the cytosol and nucleus)

Signal sequence or patch

- **amino acid sequence that directs protein to specific destination**
    - they are identifiers of proteins to allow them to reach their destination
- **a signal sequence (A) is composed of consecutive amino acid sequence at a terminal end of the protein**
    - can be seen at the primary structure of the protein and at the end
    - typical to be the N terminus



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N terminus has a + charge- there is a specific seq (SIGNAL SEQ) of aa residues that identify that protein and help mark that protein for sorting to a specific place.

- **a a signal patch (B) is a signal as well, but the amino acids are internal to the protein and they do not need to be consecutive**
    - unfolded protein it doesn't look like anything but then it does fold so that a signal does become a whole - the residues will be grouped within this the same **space. - different sets of sequences and it becomes a whole as that signal**
    - collectively form a signal patch. so it is intrinsic to the primary structure of the protein.

*so whats pushing the protein in that direction?? just random motion! it will jossell around, and there are TONS that move in all sorts of directions

but these specific sequences allow the protein to be translocated to a specific organelle once it interacts with it (**but there is no specific energy that pushes it in that direction)**

so this is how proteins are marked for translocation: signal seq and signal patch

Protein sorting between the cytosol and nucleus

- **important proteins, such as polymerases, GRPs are synthesized in the cytosol and imported into the nucleus**
  - GRPs are essential in upregulation and downregulation of proteins
  - myc pathway for example! - we saw this in the first half of the lecture
- **others (eg mRNA) are synthesized in the nucleus and exported to the cytosol**
- Bi directional traffic (key word)
- nuclear envelope composed of inner and outer membrane and NPC
  - nuclear pore omplexes perforate the envolope - they are channels AKA GATES that allow the passage of the proteins

Nuclear pore complex

- **perforates nuclear envelope**
- **octagonal structure - 8 subunits**
- **composed of nucleoporins**
  - type of protein - 8 of them
  - this is their general name
- **allow passage of molecules**
  - small molecules can fit easily through via free diffusion
  - but larger molecules, c' est tough! via active transport
- **structure includes an aqeuous pore**
  - the pore works depending on the size of the molecule (some proteins are quite large) small ions move freely by diffusion
    - **small proteins transverse by passive diffusion**
- **recent evidence indicates a tangled meshwork lines the pore to block passive diffusion of large molecules**
- **large proteins transverse by active transport mechanism**
  - large molecules can only be transported by active mechanism- transported in folded conformation
  - translocation via gated channel= active transport ENERGY NEEDED
- **proteins are imported/exported in folded conformation**

・<u>at mitochondria and ER</u>, only **unfolded proteins can be translocated. the pores there are not large enough to allow folded proteins to go,** but what happens b/w **cytosol and nucleus is ALREADY folded proteins**

・so these are proteins that express a signal patch OR signal seq- they are folded and fully functional

・ex: gene regulatory protein: already folded and functional and moves through nuclear pore complex

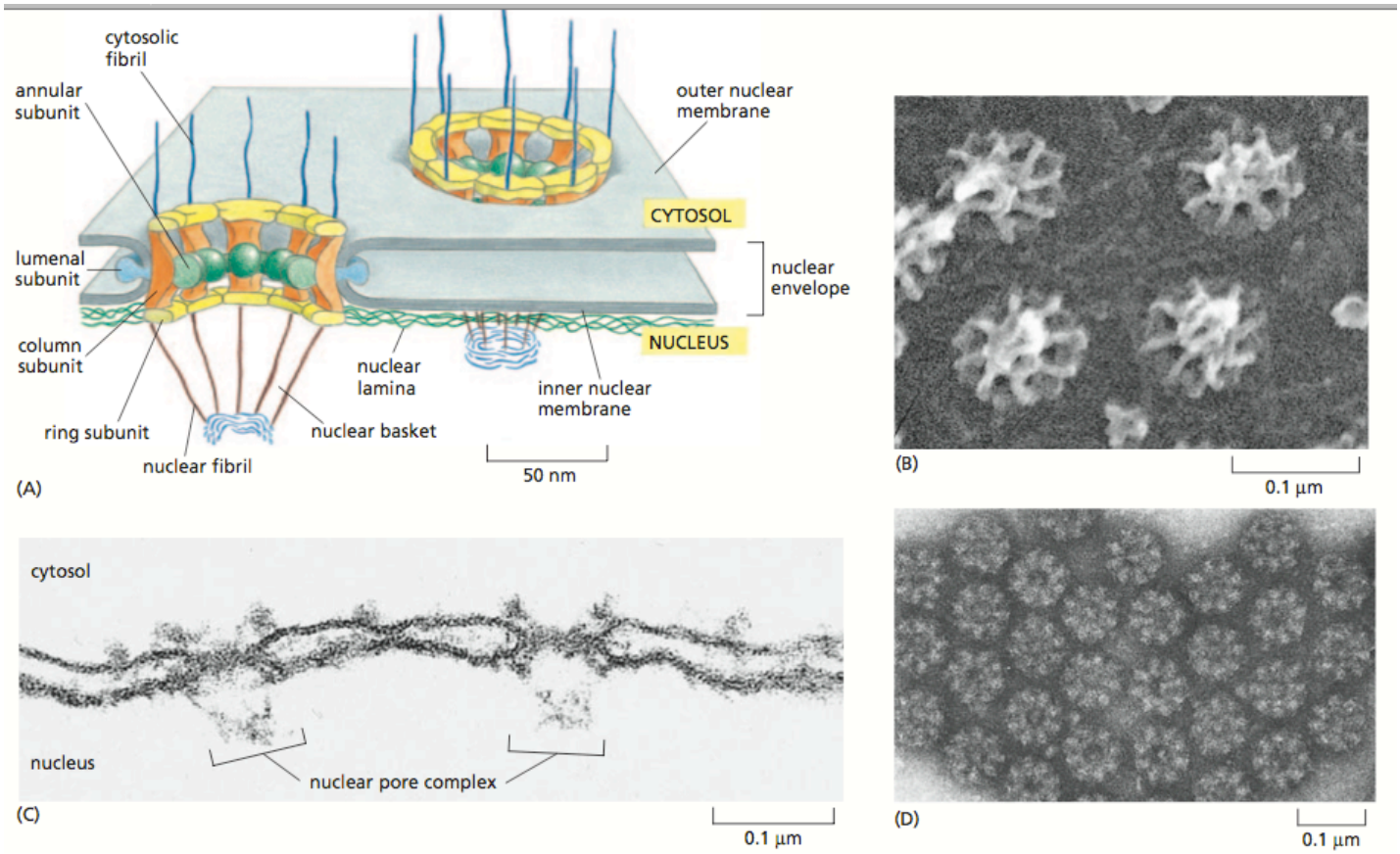


Figure 12–9 The arrangement of NPCs in the nuclear envelope. (A) A small region of the nuclear envelope. In cross section, an NPC seems to have four structural building blocks: column subunits, which form the bulk of the pore wall; annular subunits, which are centrally located; lumenal subunits, which contain transmembrane proteins that anchor the complex to the nuclear membrane; and ring subunits, which form the cytosolic and nuclear faces of the complex. In addition, fibrils protrude from both the cytosolic and the nuclear sides of the NPC. On the nuclear side, the fibrils converge to form basketlike structures. Immunoelectron microscopic studies show that the proteins that make up the core of the NPC are oriented symmetrically across the nuclear envelope, so that the nuclear and cytosolic sides of the core look identical. In contrast, the proteins that make up the fibrils are different on the cytosolic and nuclear sides of the NPC. The eight-fold rotational and two-fold transverse symmetry of the core NPC explains how such a huge structure can be formed from only about 30 different proteins: many of these proteins are present in 16 copies (or multiples of 16). Disordered domains of the core proteins (not shown) are thought to extend toward the center of the NPC, blocking the passive diffusion of large macromolecules. (B) A scanning electron micrograph of the nuclear side of the nuclear envelope of an oocyte (see also Figure 9–55). (C) An electron micrograph showing a side view of two NPCs *(brackets);* note that the inner and outer nuclear membranes are continuous at the edges of the pore. (D) An electron micrograph showing face-on views of negatively stained NPCs. The membrane has been removed by detergent extraction. Note that some of the NPCs contain material in their center, which is thought to be macromolecules in transit through these NPCs. (B, from M.W. Goldberg and T.D. Allen, *J. Cell Biol.* 119:1429–1440, 1992. With permission from The Rockefeller University Press; C, courtesy of Werner Franke and Ulrich Scheer; D, courtesy of Ron Milligan.)

This is an example post transloational translocation - refers to the movement or the translocation of proteins after its translated soon where proteins are translocated at the same tim

**we will see that in the next lecture there is also cotranslational translocation

・we know very little about these pore structures: there is a meshwork that lines the pore that doesn't allow large molecules to pass by diffusion, and only small ones by diffusion

Nuclear localization signals can be located almost anywhere in the amino acid sequence and are thought to form loops or patches on the protein surface. Many function even when linked as short peptides to lysine side chains on the surface of a cytosolic protein, suggesting that the precise location of the signal within the amino acid sequence of a nuclear protein is not important. Moreover, as long as one of the protein subunits of a multicomponent complex displays a nuclear localization signal, the complex can be imported into the nucleus.

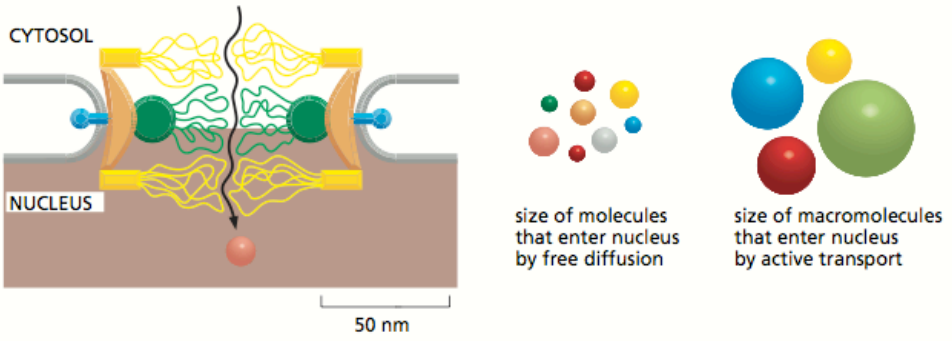


Figure 12–10 A model for the gated diffusion barrier of the NPC. The drawing shows a side view of an NPC. Unstructured regions of the proteins lining the central pore form a tangled meshwork, which blocks the passive diffusion of large macromolecules. During active transport, however, even particles up to 39 nm in diameter can pass through NPCs.

Nuclear import receptors

- **bind to nucleoporins and nuclear localization signals (NLS)**
- **NLSs are signal sequences or patches**
- **NLSs are specfic for receptors**

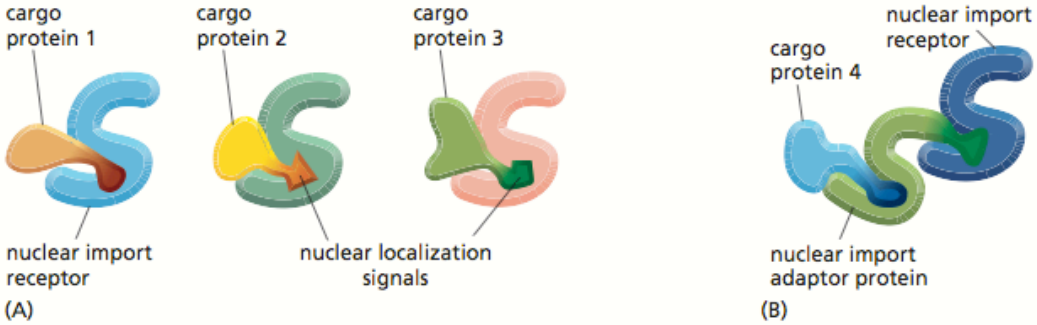


Figure 12–13 Nuclear import receptors. (A) Many nuclear import receptors bind both to NPC proteins and to a nuclear localization signal on the cargo proteins they transport. Cargo proteins 1, 2, and 3 in this example contain different nuclear localization signals, and therefore each binds to different nuclear import receptors. (B) Cargo protein 4 requires an adaptor protein to bind to its nuclear import receptor. The adaptors are structurally related to 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.

import receptors and adaptors, cells are able to recognize the broad repertoire of nuclear localization signals that are displayed on nuclear proteins.

Nuclear Export Works Like Nuclear Import, But in Reverse

- **sometimes there's an an adaptor protein involved**
- **export works the same way, but in reverse and utilizes export receptors**

this is where story begins: **nuclear import receptors and nuclear export receptors** are the same thing just are involved in opposite directions

*export works in same way but we have nuclear export receptors **(NER not NIR)**

- so nuclear import receptor has a cargo protein, which has nuclear localization signal
  - **(NLS is like a signal seq or path, but specific to translocation INTO nucleus, this is NLS)**
- so lets say this is a GRP that is destined for import into the nucleus.,
- --> the binding of NLS to the nucleus import receptor **INITIATES the transport of cargo protein-** the receptors themselves will then bind to the nucleoporins (remember - they are still bound to NLS (SS sequence)
  - the nucleopore (protein complex that lines the nucleopore complex) and that will interact with **BOTH NLS and receptor**
- So when the nuclear localization signal and the cargo protein are bound via the receptor - we initiate sorting
  - there is a different type of nulcear important receptor for each cargo protein
    - high level of specifity between NIR and NLS in order to bring specific cargo proteins into the nucleus

Directional transport

- **import and export consumes energy**
- **A GTPase called Ran acts as a** molecular switch (allows off and on like behaviour)
  - **G protein**
  - depending on which phosphorylate nucleoside it is bound, it will allow to dertemines its directionality
  - (it initiates a rxn upon binding of the hydrolysis of GTP to GDP)
    - import and export consumes energy via hydrolysis
- **Directionality occurs primarily because Ran-GDP is concentrated in the cytosol while Ran-GTP exists in the nucleus**
- **this GRADIENT for the two conformational forms of Ran drives the nuclear transport in the appropriate direction**
  - the gradient maintains the flow of imported and exported proteins
- since there is a bidirectional traffic, we need to remember there are import receptors carrying cargo in, and export receptors carrying cargo out.

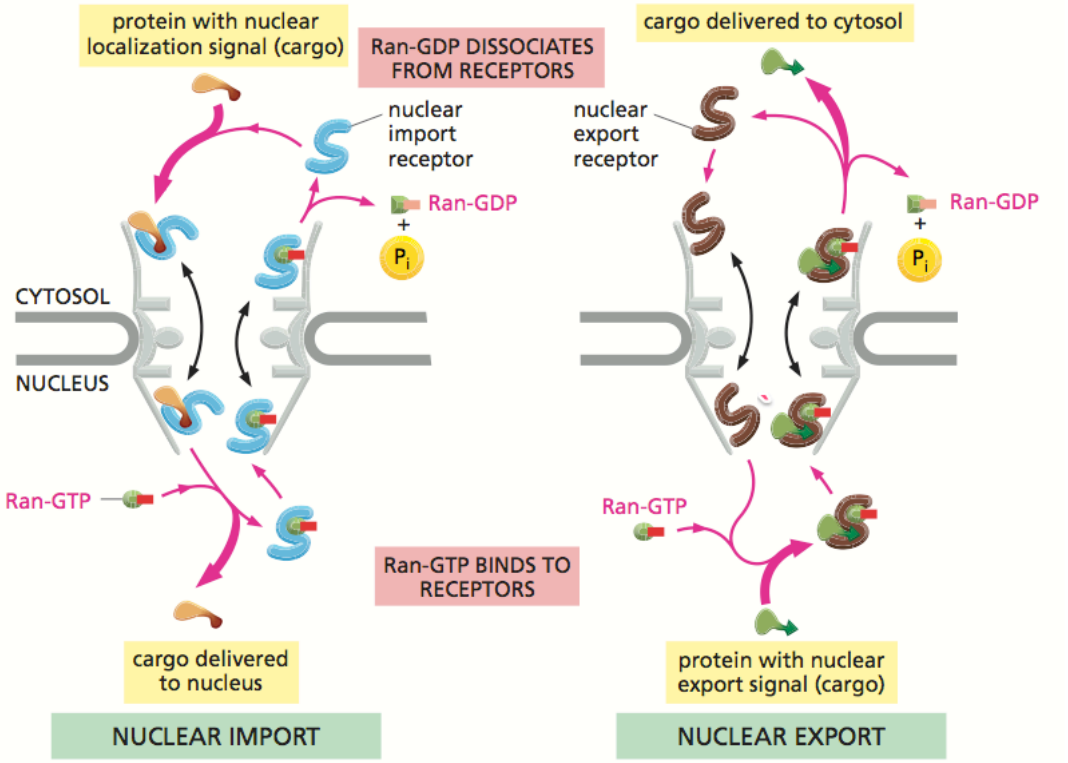


Figure 12–15 A model explaining how GTP hydrolysis by Ran in the cytosol provides directionality to nuclear transport. Movement through the NPC of loaded nuclear transport receptors occurs along the FG-repeats displayed by certain NPC proteins. The differential localization of Ran-GTP in the nucleus and Ran-GDP in the cytosol provides directionality *(red arrows)* to both nuclear import *(left)* and nuclear export *(right)*. Ran-GAP stimulates the hydrolysis of GTP to produce Ran-GDP on the cytosolic side of the NPC (see Figure 12–14).

Ran-GDP is imported into the nucleus by its own import receptor, which is specific for the GDP-bound conformation of Ran. The Ran-GDP receptor is structurally unrelated to the main family of nuclear transport receptors. However, it also binds to FG-repeats in NPC proteins and hops across the NPC.

・so there is a **mechanism that maintains each form of Ran** (the molecular switch in their appropriate compartments)

・GDP in cytosol, GTP in nucleus!!

・maintenance of these forms of Ran is what maintains the gradient to allow bidirectional transport

・in both import and export there is a **more Ran-GDP in cytosol and more Ran-GTP in nucleus --> will never be extinguished, will always be replenished to keep system moving.**

・MECHANISM!

・nuclear import receptor (NIR) binds with a protein with a NLS

・this initiates the process of import.

・it increases the likelihood of binding: once the NIR binds to protein with NLS, the affinity of that complex to nuclear porin in the nuclear pore complex is increased **BY A CONFORMATIONAL CHANGE**

・this initiates transport from cytosol to nucleus.

・here, **Ran-GTP's role is to deliver the cargo to the nucleus- release the cargo from the NIR**

・Ran-GTP will bind to the NIR, it displaces the cargo protein.

・so now cargo (ex: GRP) has entered the nucleus

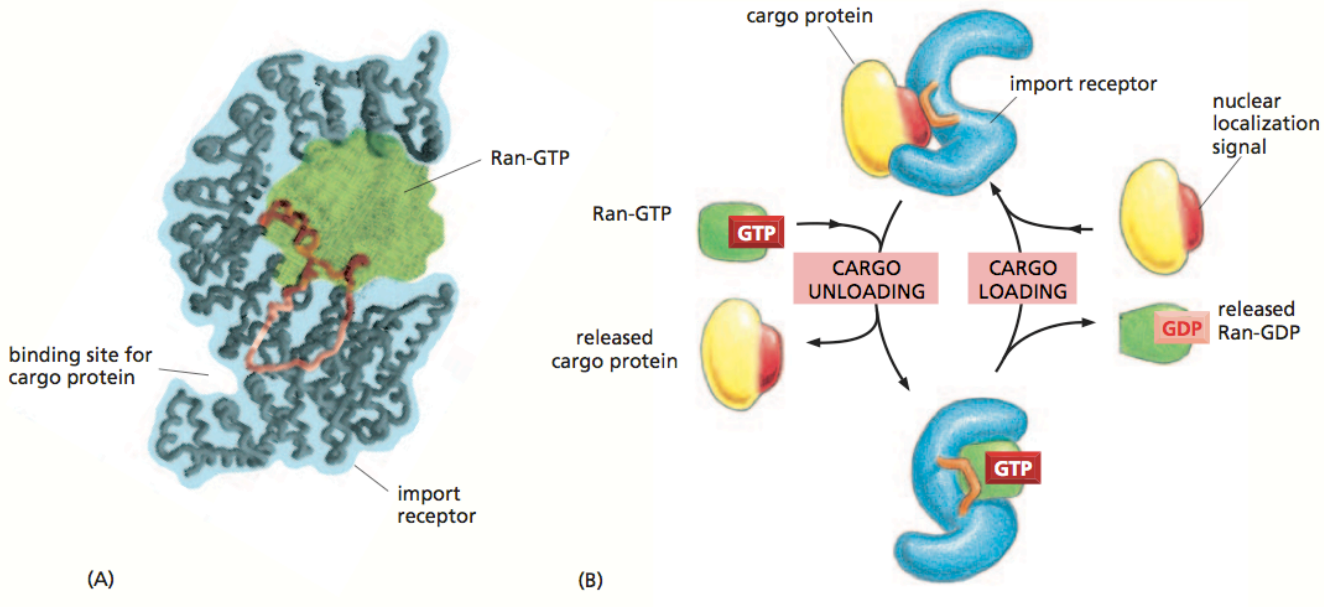
・import receptor proteins will be sent all the way back out to the cytosol. once Ran is associated with NIR, **it binds back to the nucleopore complex and interacts with nucleoporins all the way out.**

・once it gets out to the nucleus, <u>this is where hydrolysis occurs.</u>

・GTP hydrolysis releases the NIR back into the cytosol and sucks it up for the next cycle. and you get back to a NIR not bound to anything.

・so hydrolysis of ran GTP releases the NIR back into the cytosol.

・so binding of ran gtp that displaces the cargo protein, and it is the hydrolysis of gtp that <u>frees the receptor again so cycle can continue (and GDP dissociates)</u>



・so in **nuclear export:**

・start at nucleus: there is a protein with a NES (nuclear export signal)

・in order to get protein from nucleus to cytosol, we need ran-gtp and cargo protein, **both will bind with NER (nuclear export receptor)- binding of the 3 partners that initiates the export of the complex.**

<u>DIFFERENCES???</u>

・in import it was only binding of receptor and cargo, in this case it was binding of receptor, cargo AND ran-gtp

・the role here of gtp hydrolysis and dissociation of gdp **is TO deliver a cargo to the cytosol.**

・binding of ran gtp delivers cargo in nuclear import, and binding of ran gtp initiates export in nuclear export

・once that occurs, the export receptor associates with the nucleoporins and makes its way up.

・once delivered, NER is going to head back by interacting with nucleoporins

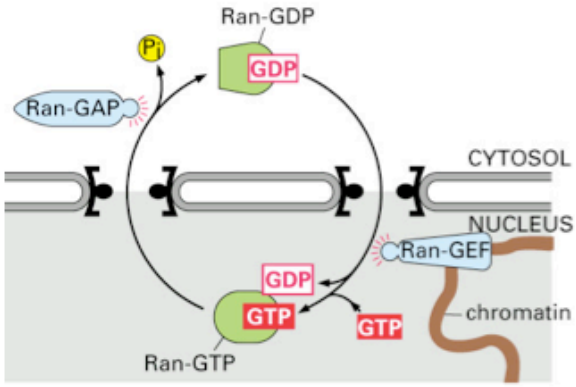
KEY IDEAS FOR COMPARTMENTILIZATION OF RAN!!!!!!!!!! AND HOW GRADIENTS ARE MAINTAINED!!!!

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.



2-15, Alberts 4th ed.

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*so GAP hydrolyses GTP, so whatever form of Ran exists in cytosol, if there is GAP around, it will hydrolyse the GTP form into Ran-GDP. so lets say ran-gtp comes out of the nucleus, GAP will remove a phosphate, and will convert to GDP.

(so this is how Ran-GDP is maintained in the cytosol)

Protein sorting to the mitochondrion

・many mitochondrial proteins are necessary for electron transport, OP, ATP synthesis

・most proteins must be encoded in the nucleus and imported from the cytosol

• **post translational translocation**

・the protein that is destined for mitochondria is first **translated by ribosome in the cytosol,** and then brought

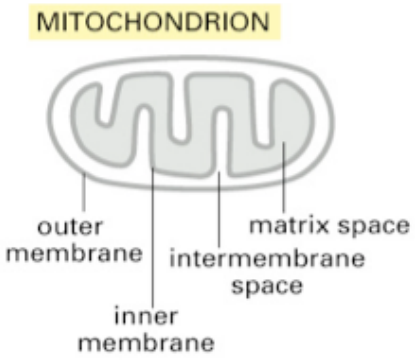


Fig. 12-22 Alberts

to the mitochondria, then translocated)

- **double membrane structure -** inner membrane and outer membrane allow intermembrane space + matrix
- proteins are transported to matrix or inserted into the membrane
  - these proteins are found via signal sequences (translocated into the mitochondria) -
- so from cytosol to mitochondrion: **this is a one way mechanism**
  - most proteins need to be encoded in the nucleus and imported from the cytosol into the mitochondria
- any protein that is going to translocated into the mitochondira CANNOT be folded and is therefore NOT functional before entering the mitochondria
  - a peptide chain from ribosome will get **CHAPERONED** to mitochondria
  - will quickly fold once reaching its destination
- molecular chaperones: molecules like heat shock protein 70 (HSP70), bind to formal protein coming out of ribosome in order to maintains it in its unfolded state

Mitochondria signal sequence

- exposed amino acid sequence directs protein to appropriate address
- mitochondrial signal sequence is a modified as an alpha helix
  - the number of amino acids involved in alpha helix are important in terms of stability
- mitochondria signal sequence is modified as an alpha helix
  - as well as signal patch - it conveys the information after folding has been completed - there are number of residues in the image that have a positive charge happening on all one side and uncharged ones at the other
- so just like nuclear localization, there is a SIGNAL SEQ that directs proteins to the mitochondria
  - BUT instead of a specific aa seq that is recognized by a patch or terminus, rather it is the **CHARGES that is recognized by the mito.**
    - primary structure is aa, secondary is alpha helix (folding), and at the terminal end of a protein that is destined for the mito, we find a specialized alpha helical seq of aa that **have + charged aa on ONE side of the structure**
      - it is similar to the lsignal patch because they are not sequential residues (aas) that may occur every few aas.
      - but when the protein is folded as an alpha helix, they end up being close together so as to direct the protein to mito.
- **SO: + charged aas are exposed in one region (one side) of that alpha helix, and that signal directs proteins that are destined for the mito to the center**

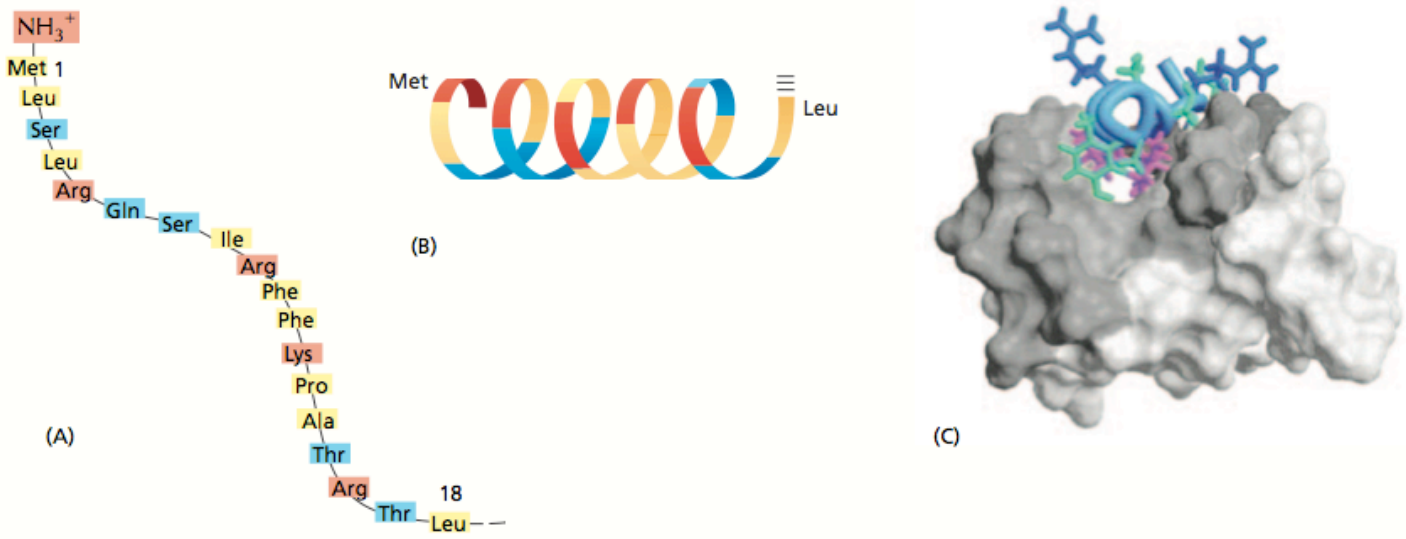


Figure 12–22 A signal sequence for mitochondrial protein import. Cytochrome oxidase is a large multiprotein complex located in the inner mitochondrial membrane, where it functions as the terminal enzyme in the electron-transport chain (discussed in Chapter 14). (A) The first 18 amino acids of the precursor to subunit IV of this enzyme serve as a signal sequence for import of the subunit into the mitochondrion. (B) When the signal sequence is folded as an α helix, the positively charged residues *(red)* are clustered on one face of the helix, while the nonpolar residues *(yellow)* are clustered primarily on the opposite face. Amino acids with uncharged polar side chains are shaded *blue*. Signal sequences that direct proteins into the matrix space always have the potential to form such an amphiphilic α helix, which is recognized by specific receptor proteins on the mitochondrial surface. (C) The structure of a signal sequence of alcohol dehydrogenase, another mitochondrial matrix enzyme, bound to an import receptor was determined by NMR. The amphiphilic α helix binds with its hydrophobic face to a hydrophobic groove in the receptor. (C, adapted from Y. Abe et al., *Cell* 100:551–560, 2000. With permission from Elsevier.)

The signal sequences that direct precursor proteins into the mitochondrial matrix space are best understood. They all form an amphiphilic α helix, in which positively charged residues cluster on one side of the helix, while uncharged hydrophobic residues cluster on the opposite side. Specific receptor proteins that initiate protein translocation recognize this configuration rather than the precise amino acid sequence of the signal sequence (**Figure 12–22**).

PROTEIN TRANSLOCATOR COMPLEXES (TRANSLOCONS)

they allow unfolded proteins to cross the membrane of the mitochondria

1) TOM: TRANSLOCASE OF THE OUR MITOCHONDRIA MEMBRANE

imports all mito-destined proteins to the intermembrane space, membrane insertion on the outer membrane

so if its destined for **matrix, and handles intermembrane space, or insertion in one of the membranes.**

2) TIM: TRANSLOCASE OF THE INNER MITOCHONDRIA MEMBRANE

***TIM23 complex extends an arm across the inter membrane space - it is thought that it helps align the outer membrane with the inner membrane so it may speed up the translocation across both membranes**

TIM23 imports proteins to matrix

allows passage all the way through the matrix

TIM23 also **inserts into the inner membrane**

TIM22 inserts **carrier proteins**

3) OXA COMPLEX: insertion of proteins in the **inner membrane that usually come from the matrix**

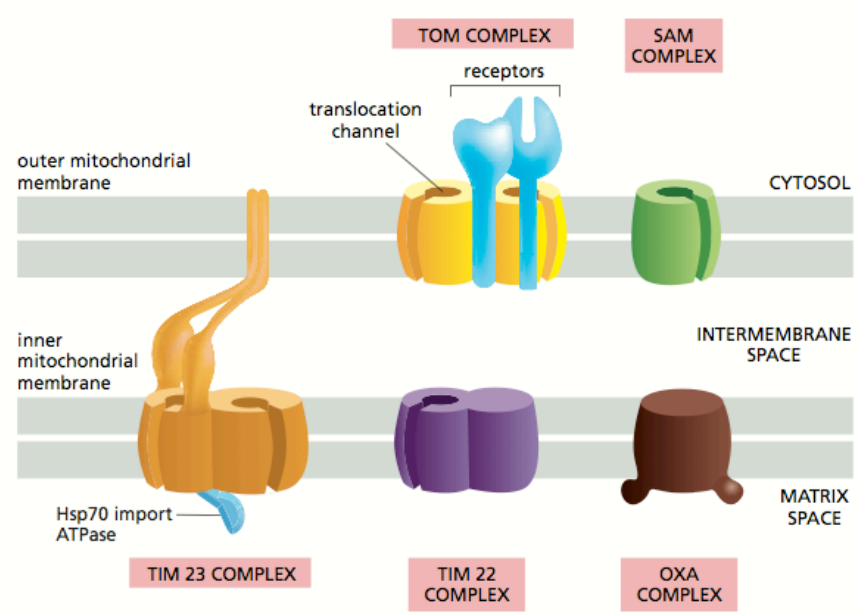
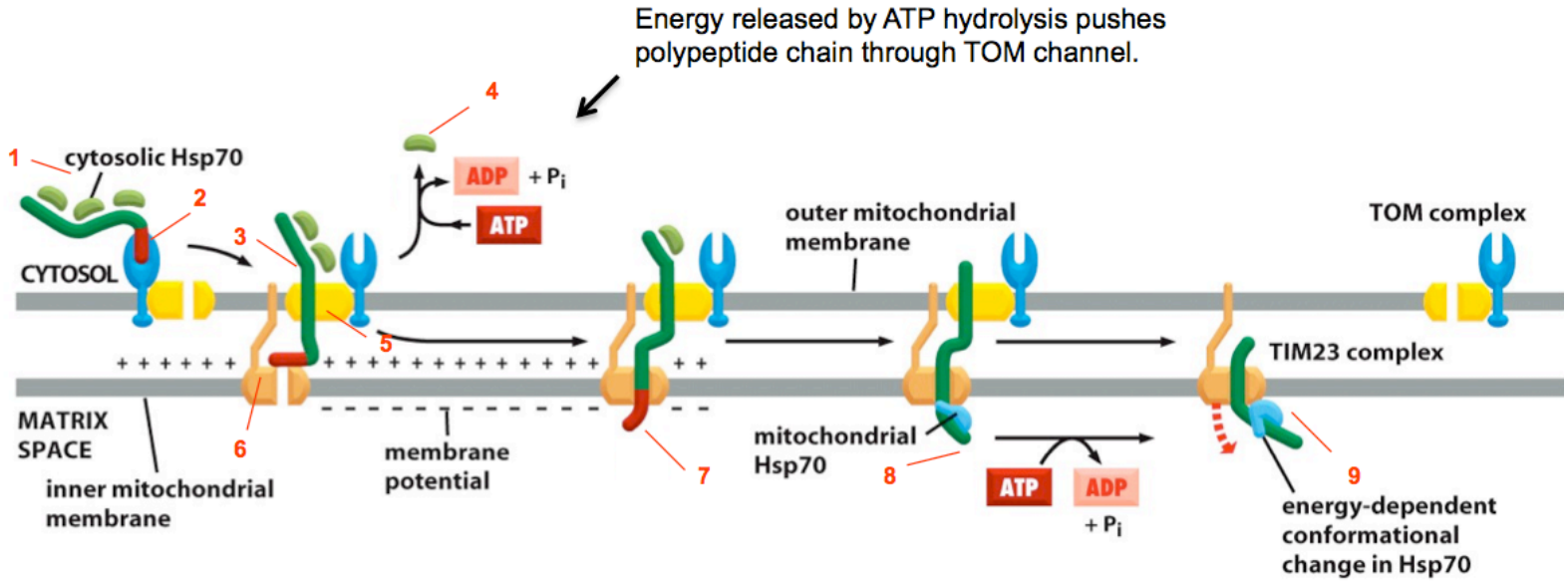


Figure 12–23 The protein translocators in the mitochondrial membranes. The TOM, TIM, SAM, and OXA complexes are multimeric membrane protein assemblies that catalyze protein transport across mitochondrial membranes. The protein components of the TIM22 and TIM23 complexes that line the import channel are structurally related, suggesting a common evolutionary origin of both TIM complexes. As indicated, one of the core components of the TIM23 complex contains a hydrophobic α-helical extension that is inserted into the outer mitochondrial membrane; the complex is therefore unusual in that it simultaneously spans two membranes.

On the matrix side, the TIM23 complex is bound to a protein complex containing mitochondrial Hsp70, which acts as an import ATPase using ATP hydrolysis to pull proteins through the pore.



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).

Translocation Across the OM

Formation of precursor protein

↓

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

↓

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

↓

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

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

translocation across the OM: – liz notes!

start with something that has **emerged from the ribosome, and it will associate with molecular chaperones (HSP70),** and they will keep them unfolded, so that they can be threaded through the translocator channels in the mitochondria

there are billions proteins in the cytosol, and they all have signals that will direct them to their destination (they are not driven to destination, **but they bounce around randomnly, and those that have specific signal will associate in with their appropriate destinations)**

when this happens, we have binding and recognition of a signal seq (so mito's signal seq is modified as an alpha helix & there is a more + charged region in this alpha helix- like a signal patch when it is folded into alpha helix, these seq express only on 1 side, and creates a + charge)

and so the protein interacts with translocator channel

so we start off at TOM complex (multiple subunits with a receptor) that identifies the protein and passes it off to a translocator channel
what happens here is that the proteins need to enter the channel threaded (NOT folded) **so HSP70 needs to be stripped away,** the protein will not be able to go through the narrow pore of the channel.

energy is released by the ATP hydrolysis that acts to: 1- get rid of HSP70, and 2- begin pushing the polypeptide through the TOM channel

tim complex is a **single complex- no separate receptor**, it is a single complex that will recognize the signal seq of the protein that will be passed through and provide the pore for translocation.

there are more + charges in the intermembrane space than in the matrix so there is a seperation of charge: membrane potential: this pushes the relatively + charged signal seq into the more – charged matrix (attracts) (proton pump **will create the membrane potential – potential energy)**

once protein is at its destination (ex: matrix, insertion in membrane, intermembrane space) the signal seq will be cleaved away **by MPP, and another mech that will act to further pull in the protein that is threading through is the use of mitochondrial HSP70**

as soon as terminus enters the mito, mitoHSP70 will bind (because the signal sequence is stripped away). it is a protein that has a high conc in the matrix, and as soon as the polypeptide is threaded through, there will be hydrolysis of ATP to induce a conformational change in mitoHSP70- this change in conformation in the matrix, pulls slowly seq by seq, like a rachet into the mitochondria

as more of the polypeptide is threaded through, **more mitoHSP70 bind to end of the protein. so lots of them bind to the terminal and act as a rachet to pull the polypeptide through.**

Directional transport requires energy, which in most biological systems is supplied by ATP hydrolysis. ATP hydrolysis fuels mitochondrial protein import at two discrete sites, one outside the mitochondria and one in the matrix space (**Figure 12–26**). In addition, protein import requires another energy source, which is the membrane potential across the inner mitochondrial membrane.

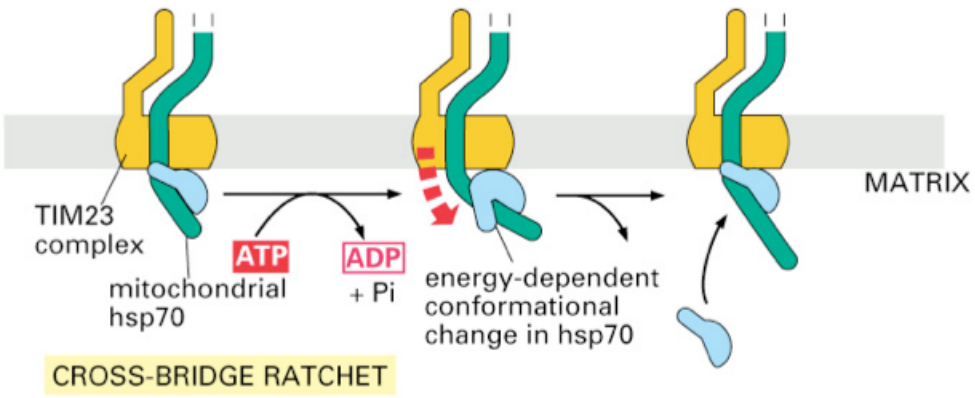
The first requirement for energy occurs at the initial stage of the translocation process, when the unfolded precursor protein, associated with chaperone proteins, interacts with the import receptors of the TOM complex. As discussed in Chapter 6, the binding and release of newly synthesized polypeptides from the Hsp70 family of chaperone proteins requires ATP hydrolysis. The requirement for Hsp70 and ATP in the cytosol can be bypassed if the precursor protein is artificially unfolded prior to adding it to purified mitochondria.

Once the signal sequence has passed through the TOM complex and is bound to a TIM complex, further translocation through the TIM translocation channel requires the membrane potential, which is the electrical component of the electrochemical H^+ gradient across the inner membrane (see Figure 11–4). Pumping of H^+ from the matrix space to the intermembrane space, driven by electron transport processes in the inner membrane (discussed in Chapter 14), maintains the electrochemical gradient. The energy in the electrochemical H^+ gradient across the inner membrane not only helps drive most of the cell's ATP synthesis, but it also drives the translocation of the positively charged signal sequences through the TIM complexes by electrophoresis.

Mitochondrial Hsp70 also plays a crucial part in the import process. Mitochondria containing mutant forms of the protein fail to import precursor proteins. The Hsp70 is part of a multisubunit protein assembly that is bound to the matrix side of the TIM23 complex and acts as a motor to pull the precursor protein into the matrix space. Like its cytosolic cousin, mitochondrial Hsp70 has a high affinity for unfolded polypeptide chains, and it binds tightly to an imported protein as soon as the protein emerges from the TIM translocator in the matrix space. The Hsp70 then releases the protein in an ATP-dependent step. This energy-driven cycle of binding and subsequent release is thought to provide the final driving force needed to complete protein import after a protein has initially inserted into the TIM23 complex (see Figure 12–26).

After the initial interaction with mitochondrial Hsp70, many imported matrix proteins are passed on to another chaperone protein, *mitochondrial Hsp60*. As discussed in Chapter 6, Hsp60 helps the unfolded polypeptide chain to fold by binding and releasing it through cycles of ATP hydrolysis.

Figure 12–26 The role of energy in protein import into the mitochondrial matrix space. (1) Bound *cytosolic Hsp70* 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. (2) The signal sequence is then translocated into the matrix space in a process that requires a membrane potential across the inner membrane. (3) *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.



Cross bridge ratchet model

- mitochondrial HSP70 proteins associated with **TIM23 complex**

- **ATP hyrdolysis drives conformational change in HSP70 that actively pulls it through**

the purpose is this to manitain the _____ important to differnetite cytosolic heat shock proteins they are important in physiology can respond to shocks of heat for certain periods of time you mostly the primary the sequence that is modified that bind to the primary structure and htis binding

unfolded structure keep in mind that the pores of these translocators of proteins to the mitochondrion very small and cannot trasnport fully proteins would not be able binding of heat shock proteins tom complex the rest of the polypeptide transfer the sequence to a tom channel

yellow thing all one multi protein complex .
one part receptor and one part trasnlcoator channel, threaded through these proteins are stripped away by atp hydrolysis get rid of heat hock protein 70 polypeptide can be threaded through the channel the unfolded protein is fed to the TOM channel and it goes on to the bind to the tim channel,
step 60 tim complex with an arm part of it extended to the innter membrane, aligning the tom with the tim and spedding up the process of trasnlcoation

7) the first thing that drew the polypeptide through the membrane the loss of heat shock protein that allowed the first push what will pul lit now an electrochemical gradient and sets up a separation of charge or membrane

potential push or pull to imagine the polypetide more protosn in the inner membrane space, and this is what dont talk about the electron trasnport chain EG changing the pH to help drive the synthesisof ATP but it also drives to intermembrane space to the mitochondrion
bound to the tim complex this seperation of charge like poles pullig away each other, taking away the polypeptide and drives it through the intermembrane space, signal sequence pulled away peptidase, an enzyme that cleaves PEPTIDE BONDS red BIT THATs been cleaved away (NOT in the image) HSP70 works with the n terminal minus the alpha terminal begins to graduall pull the popetide through the matrix

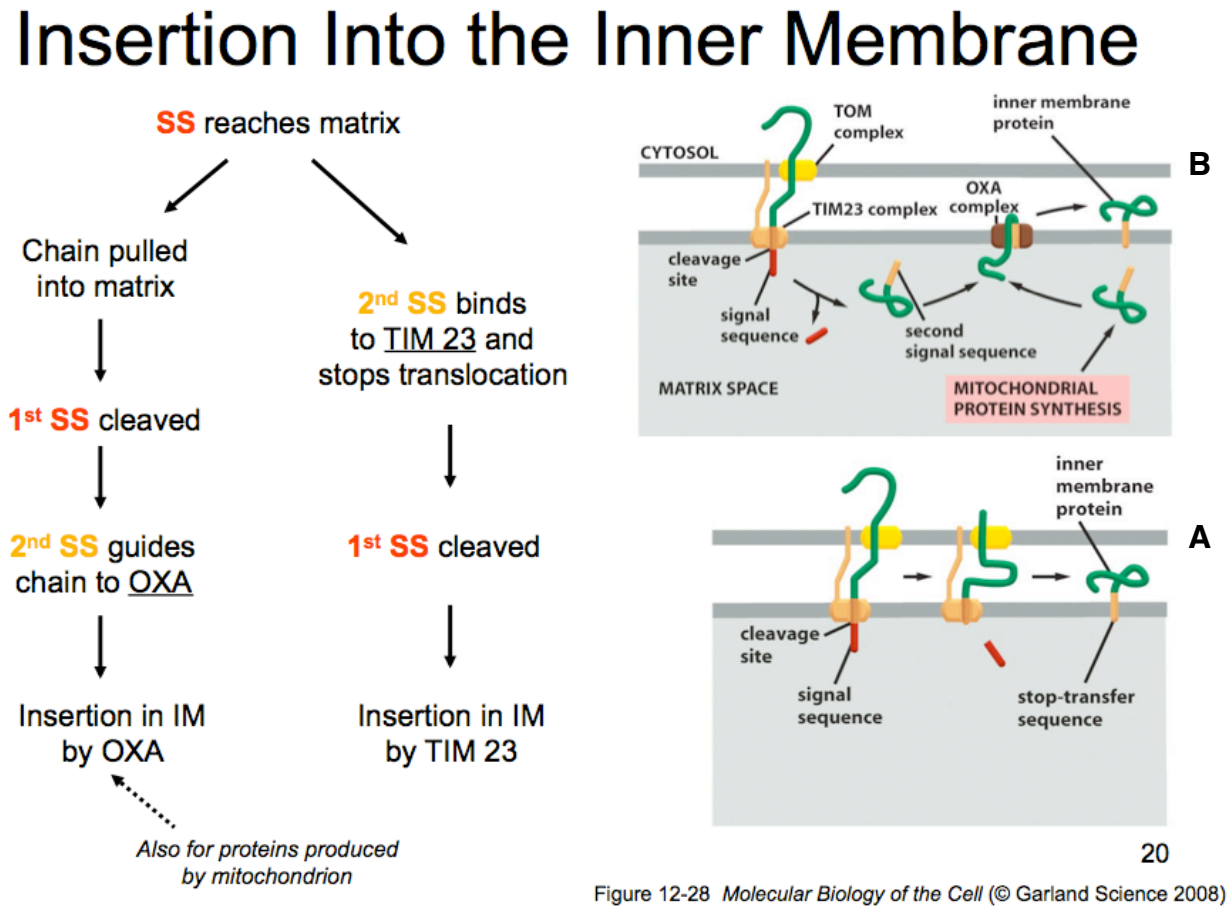


Figure 12-28 *Molecular Biology of the Cell* (© Garland Science 2008)

Figure 12–28 Protein import from the cytosol into the inner mitochondrial membrane and intermembrane space. (A) The N-terminal signal sequence (*red*) initiates import into the matrix space (see Figure 12–25). A hydrophobic sequence (*orange*) that follows the matrix-targeting signal binds to the TIM23 translocator in the inner membrane and stops translocation. The remainder of the protein is then pulled into the intermembrane space through the TOM translocator in the outer membrane, and the hydrophobic sequence is released into the inner membrane. (B) A second route for protein integration into the inner membrane first delivers the protein completely into the matrix space. Cleavage of the signal sequence *(red)* used for the initial translocation unmasks an adjacent hydrophobic signal sequence *(orange)* at the new N-terminus. This signal then directs the protein into the inner membrane, using the same OXA-dependent pathway that inserts proteins that are encoded by the mitochondrial genome and translated in the matrix space. (C) Some soluble proteins of the intermembrane space also use the pathways shown in (A) and (B) before they are released into the intermembrane space by a second signal peptidase, which has its active site in the intermembrane space and removes the hydrophobic signal sequence. (D) Metabolite transporters contain internal signal sequences and snake through the TOM complex as a loop. They then bind to the chaperones in the intermembrane space, which guide the proteins to the TIM22 complex. The TIM22 complex is specialized for the insertion of multipass inner membrane proteins.

- **so insertion of proteins into the inner membrane....**
  - what was just described was translocation all the way through from cytosol and both membranes, to matrix. this is in the case of a soluble protein: protein that is NOT integral to the membrane of organelle, but is soluble in either cytosol or lumen.
- an integral membrane protein: one that is held within the membrane- there is a diff mech here, <u>because these proteins have to be inserted into the membrane rather than just passed through.</u>
- for integral membranes, there are 2 pathways (but in both cases, there are 2 signal sequences)
- **1st pathway:**
  - so 1st signal seq (ss) reaches the matrix (by method we discussed), so ss goes all the way through **TIM and pulled into matrix, the first ss will get cleaved away,** which creates a partially folded protein still expressing the 2nd signal sequence, which directs it back to the membrane so it can be inserted. this involves OXA complex, so 2nd ss gets threaded into it, so it tells intermembrane space that 2nd ss is left, and that is a also modified. so protein is stable by the 2nd ss (cuz proteins dont like the membrane- hot and cold- like hydrophobic/ philic)
- **2nd pathway:**
- so 1st ss reaches the matrix, the 2nd ss binds to TIM 23, and stops translocation. the 1st ss is cleaved away, and simply, protein is left inserted in the inner membrane and C **terminus is left in the inner membrane space.**

****So both pathways give you the same thing - your protein will be inserted in the inner membrane - just 2 different ways ->** either TIM or you need OXA