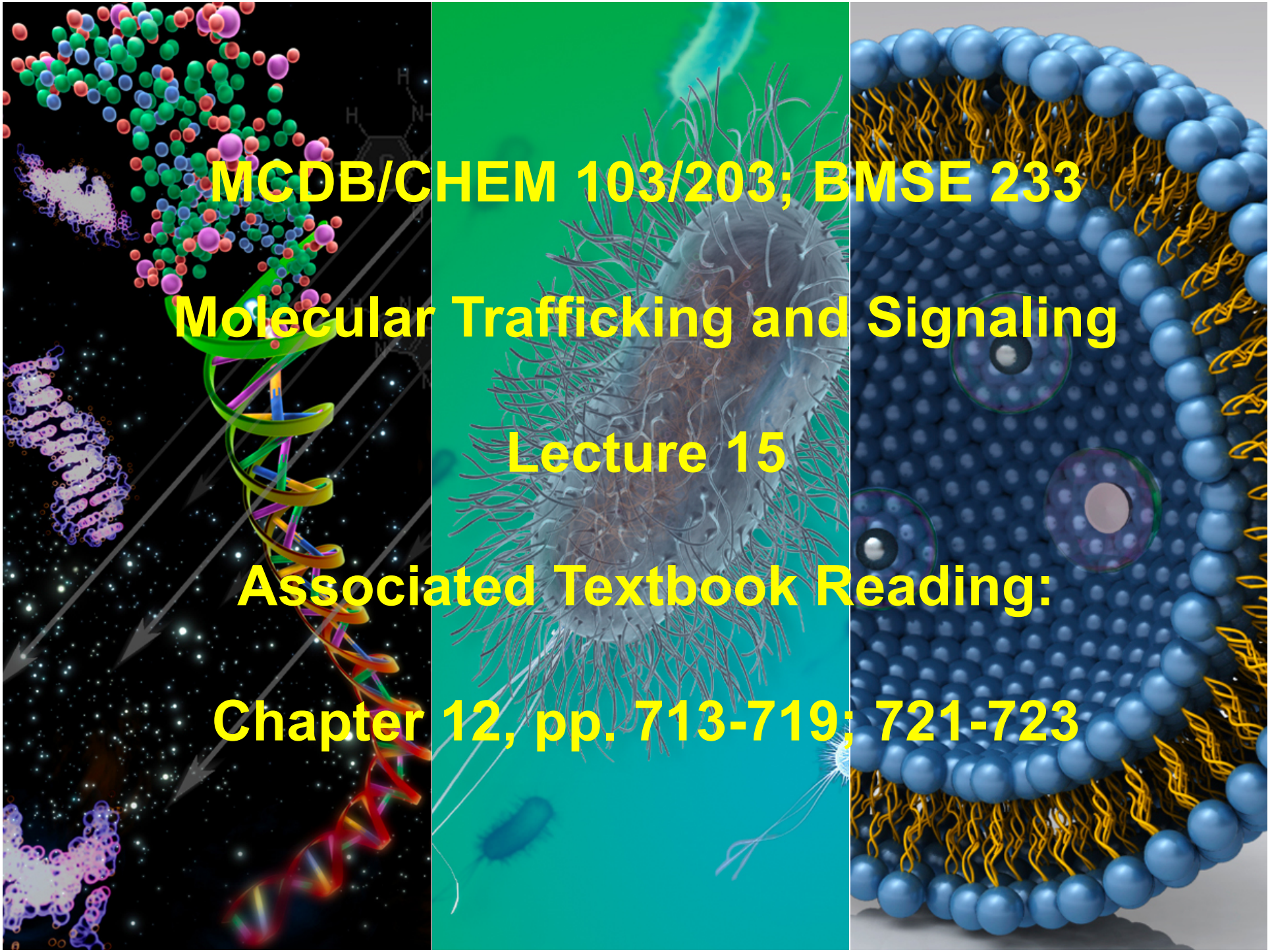


MCDB/CHEM 103/203; BMSE 233

Molecular Trafficking and Signaling

Lecture 15

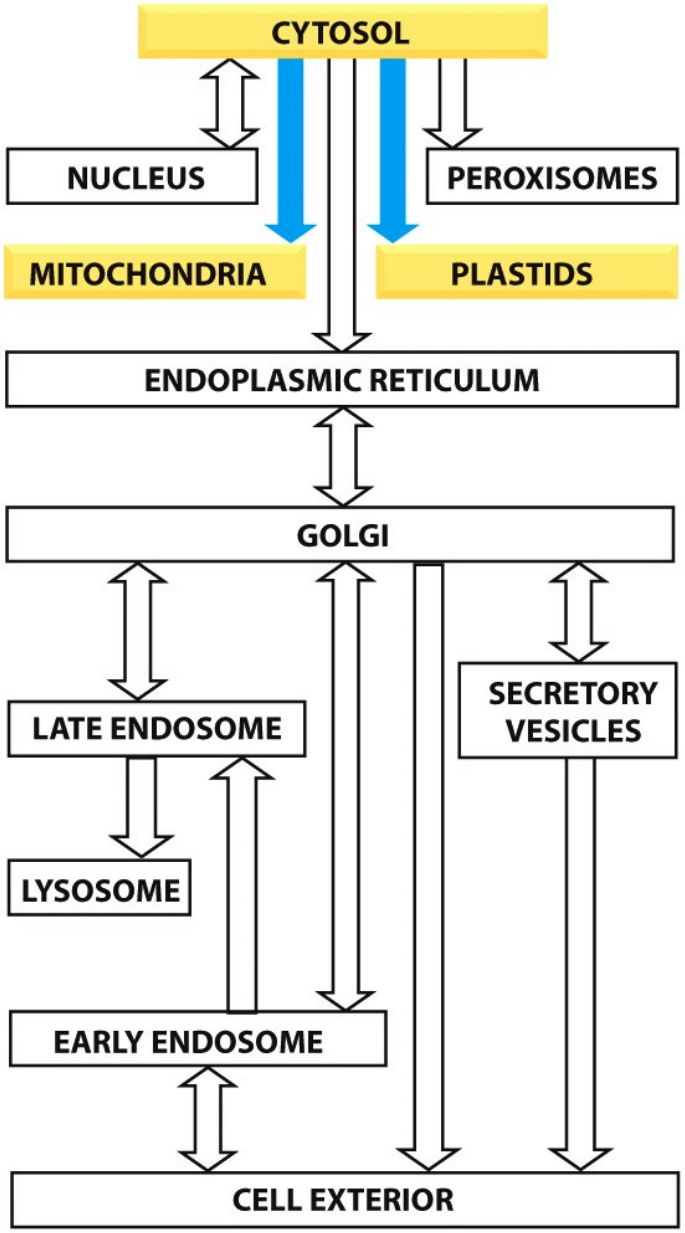
Associated Textbook Reading:

Chapter 12, pp. 713-719; 721-723

Cytosol-to-Mitochondria Transport

Cytosol-to-Peroxisome Transport

Protein transport into mitochondria



General Features:

mediated by TM translocators

mostly uni-directional transport

import signal sequence required at the N-terminus of an imported protein

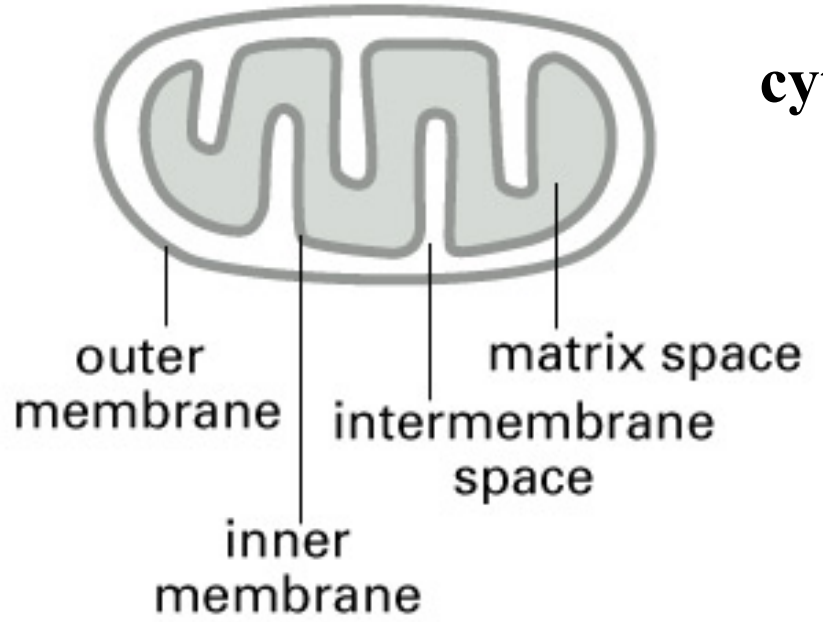
signal sequence cleaved once it is imported into the matrix

post-translational import mechanism

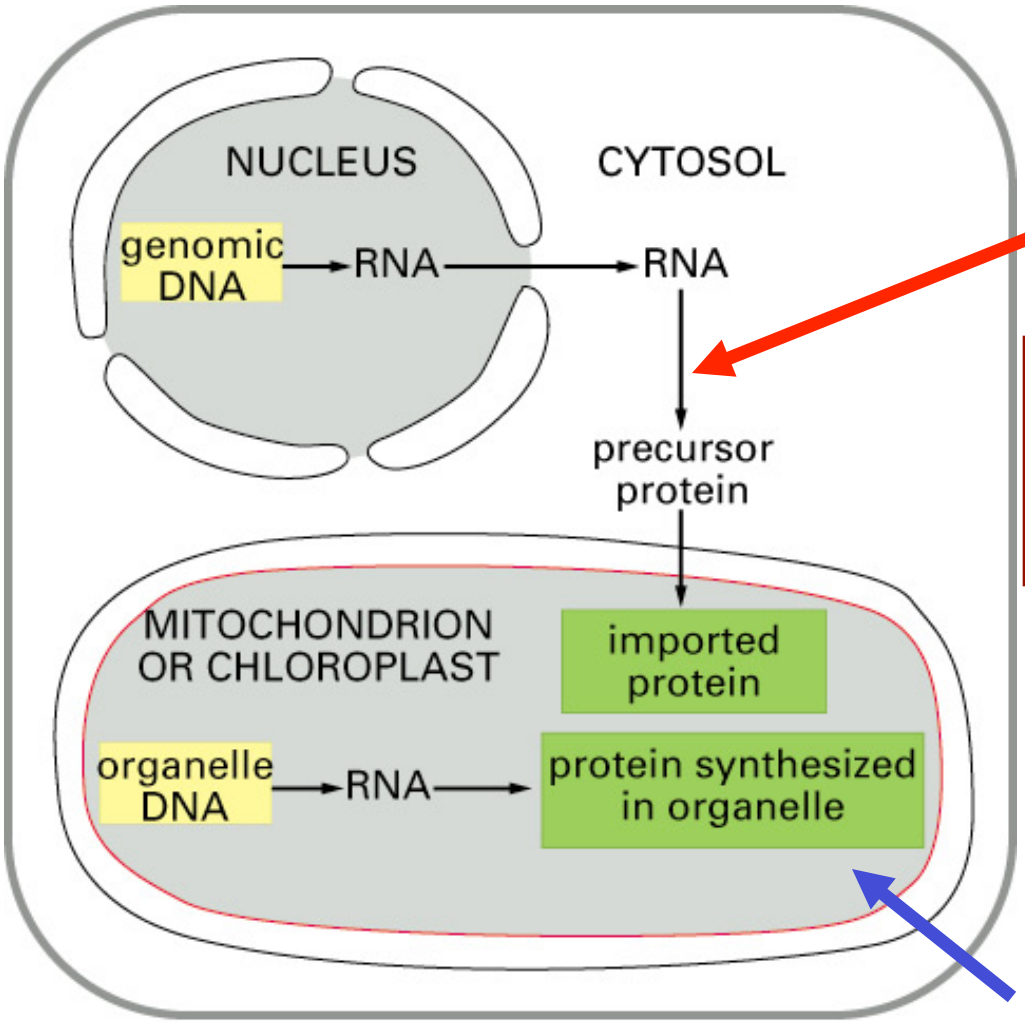
imported as unfolded protein

Molecular chaperones involved (chaperones are proteins assisting the folding or unfolding of other proteins)

Basic Structure and Topology of Mitochondria



Two Separate Systems to Generate Mitochondrial Proteins



the major pathway (99%)

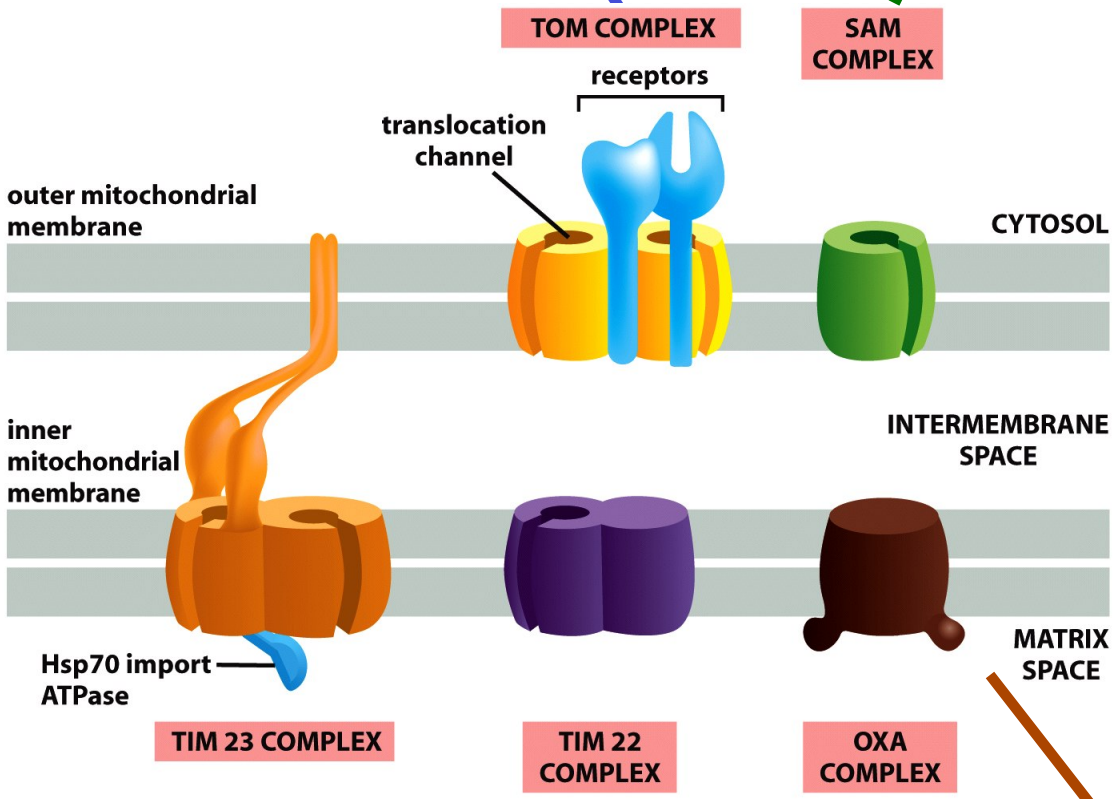
How are proteins synthesized in the cytosol transported into mitochondria?

the minor pathway (1%, 37 genes in human); 30/37 genes are linked to diseases

Transport Machinery for Cytosol-to-Mitochondria Transport

The entry port for all imported proteins, including matrix, inner membrane, intermembrane and outer membrane proteins

By working together w/ TOM, it helps the folding of outer membrane proteins

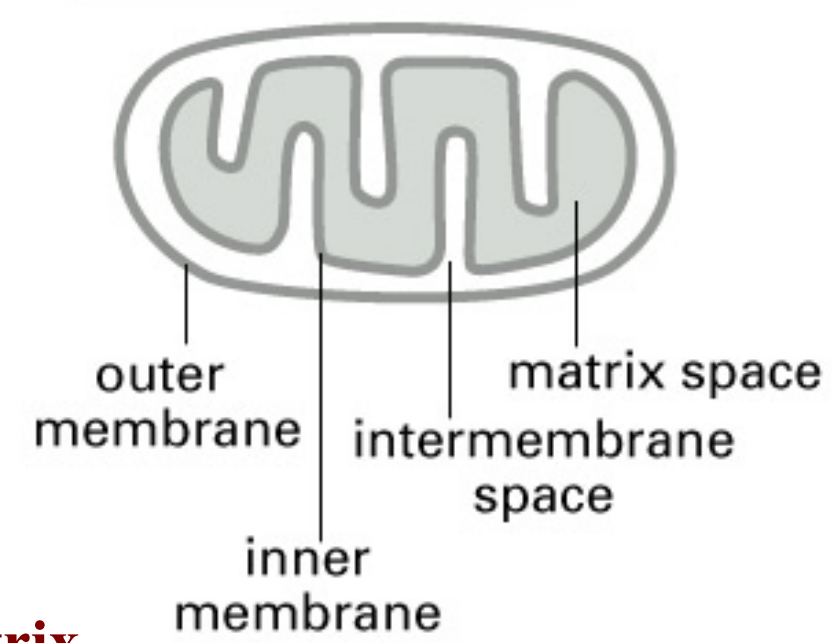


For some imported matrix proteins & a subset of imported inner membrane proteins

For a subset of imported inner membrane proteins

For a subset of imported inner membrane proteins and mitochondria-encoded inner membrane proteins

While multiple pathways exist to transport mitochondrial proteins into different subcompartments, most (but not all) mitochondrial proteins first enter the matrix from the cytosol before being targeted to their final destinations



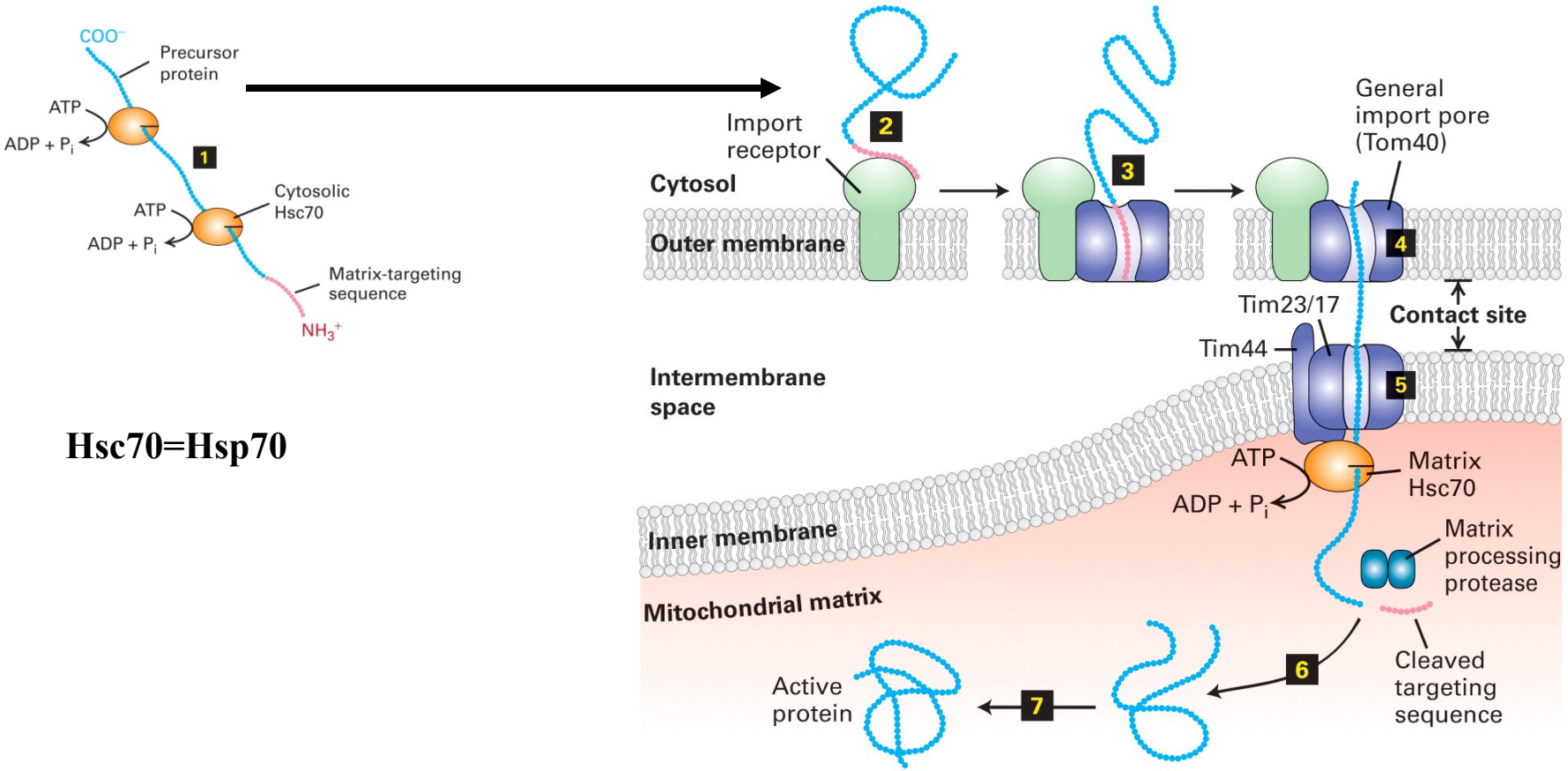
cytosol → matrix

cytosol → matrix → other destinations

(inner & outer membranes, intermembrane space)

We will focus on the cytosol-to-matrix transport

Overview of Cytosol-to-Mitochondrial Matrix Transport



Hsc70=Hsp70

The matrix targeting sequence is recognized by an import receptor of the TOM complex, then followed by another receptor of the TIM complex

Proteins are imported in a post-translational but unfolded state.

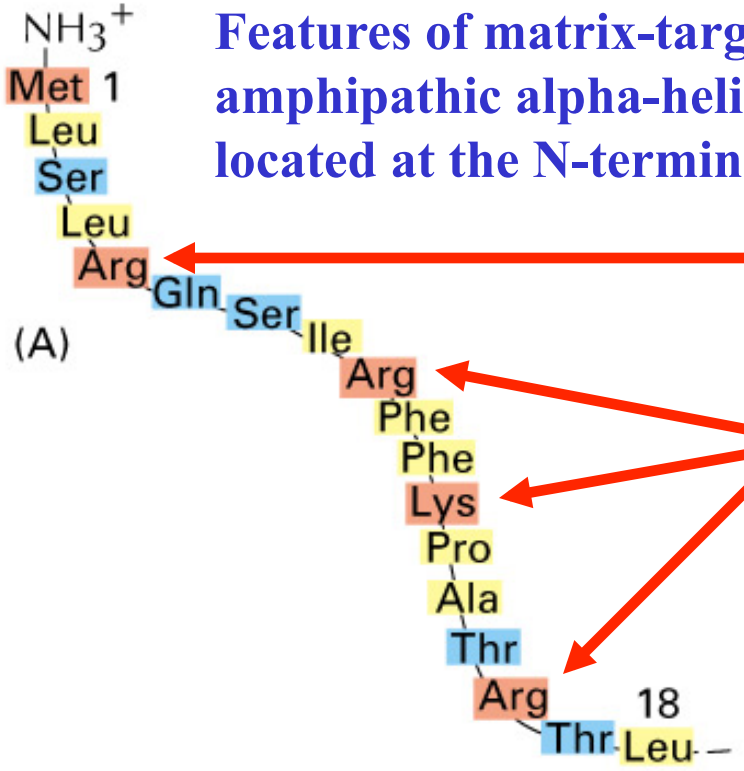
Transport occurs via the contact sites between outer and inner membranes.

Chaperones are required on both cytosolic and matrix sides.

ATP is required on both cytosolic and matrix sides.

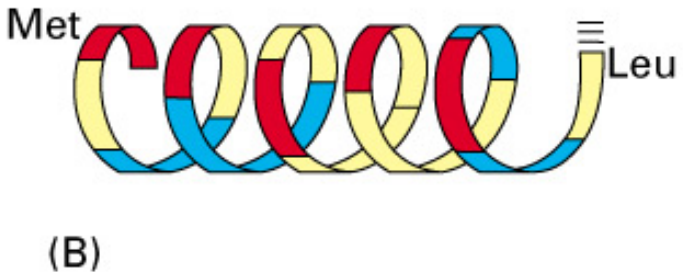
H^+ gradient across the inner membrane is also required.

Cytosol-to-matrix transport is mediated by matrix-targeting sequences found within the N-termini of proteins



Features of matrix-targeting sequence:
amphipathic alpha-helix (20-50 a.a. long) but no specific a.a. sequence.
located at the N-termini of imported proteins

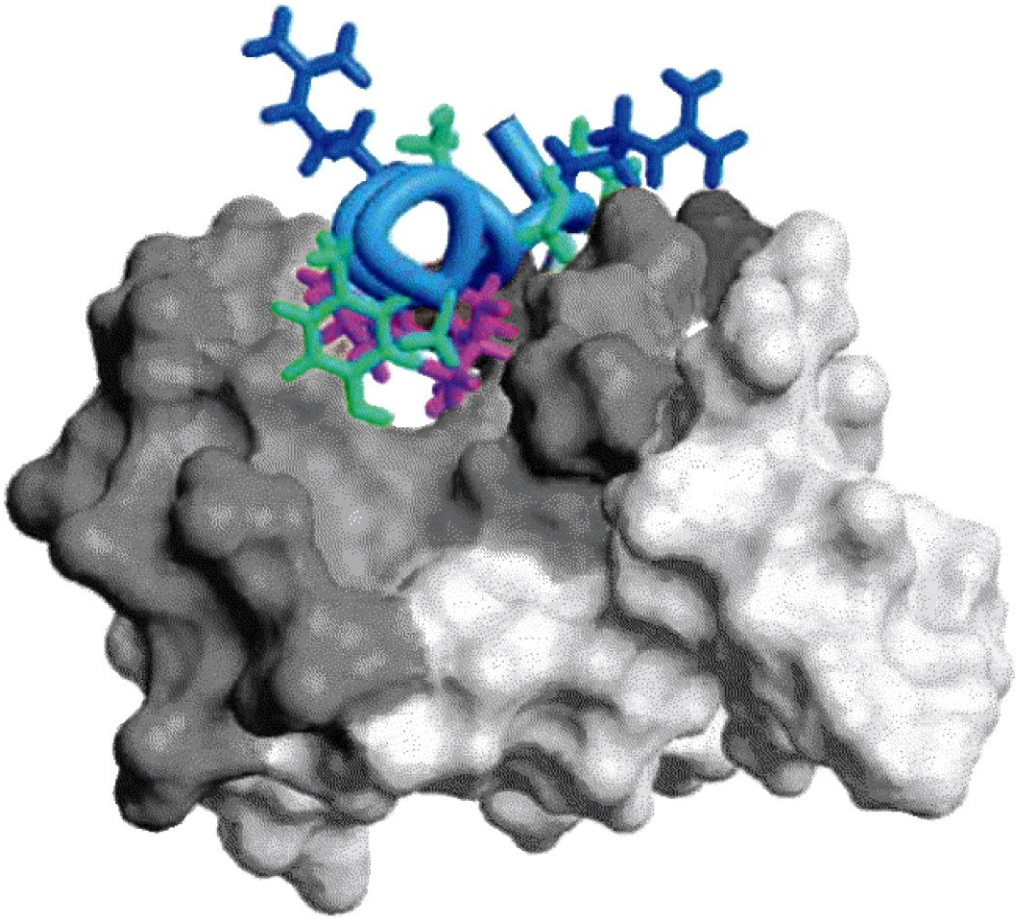
a.a. composition of one targeting sequence:
positive residues every 3 or 4 a.a.
hydrophobic residues about every 3 or 4 a.a.



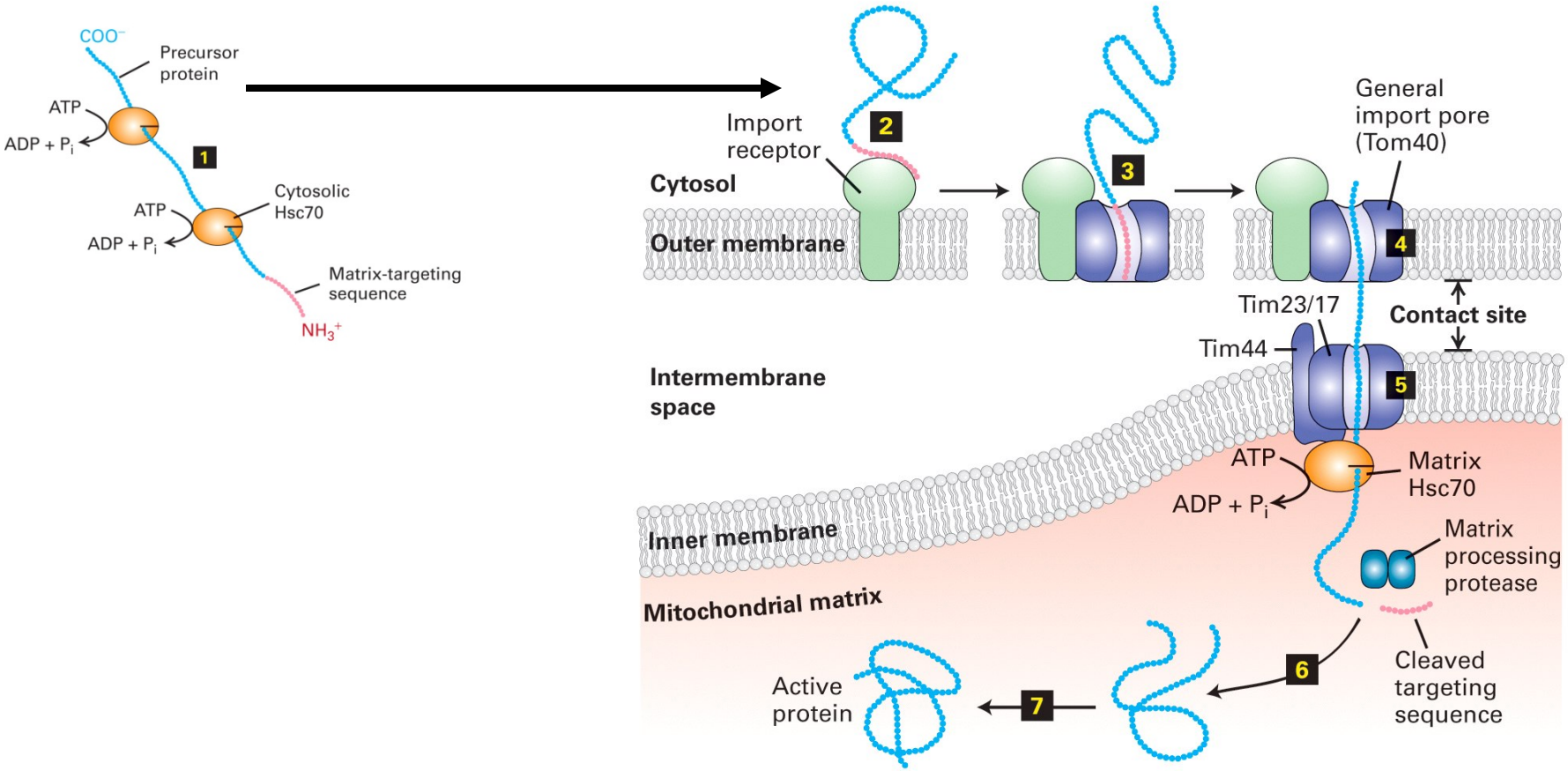
the secondary structure of targeting sequences
positive a.a. residues (in red) on one side
hydrophobic residues (in yellow) on the other side

Structure of the Complex between a Matrix-Targeting Sequence of Alcohol Dehydrogenase and the Import Receptor

The hydrophobic side of the amphiphilic alpha helix binds to a hydrophobic groove of the import receptor (another example of the general principle: hydrophobic likes hydrophobic)



Overview of Cytosol-to-Mitochondrial Matrix Transport



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Transport occurs via the contact sites between outer and inner membranes.

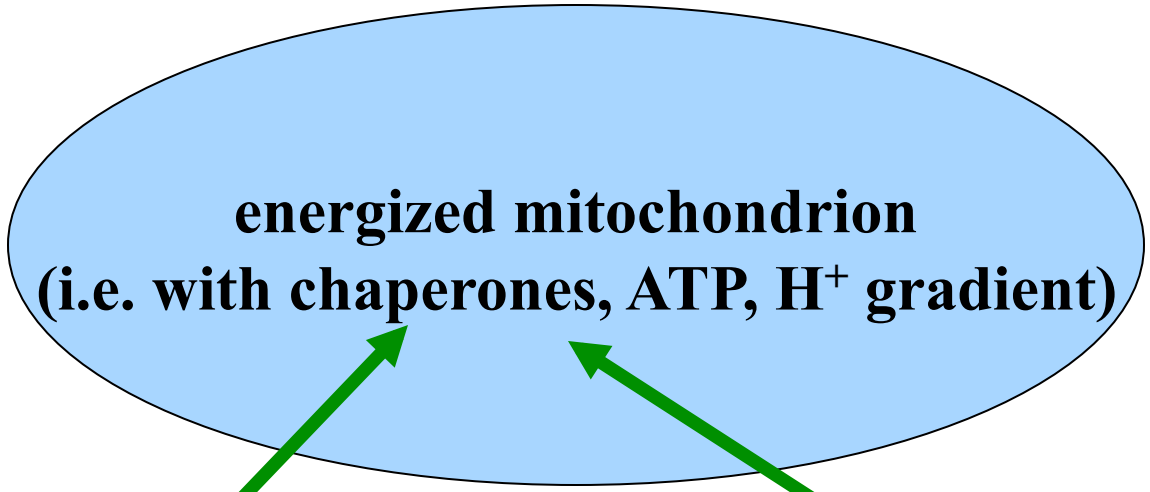
Chaperones are required on both cytosolic and matrix sides.

ATP is required on both cytosolic and matrix sides.

H^+ gradient across the inner membrane is also required.

How do we know that import into the mitochondria can occur post-translationally in an unfolded state?

Utilize energized mitochondria to conduct an *in vitro* reconstituted mitochondrial transport assay



no import

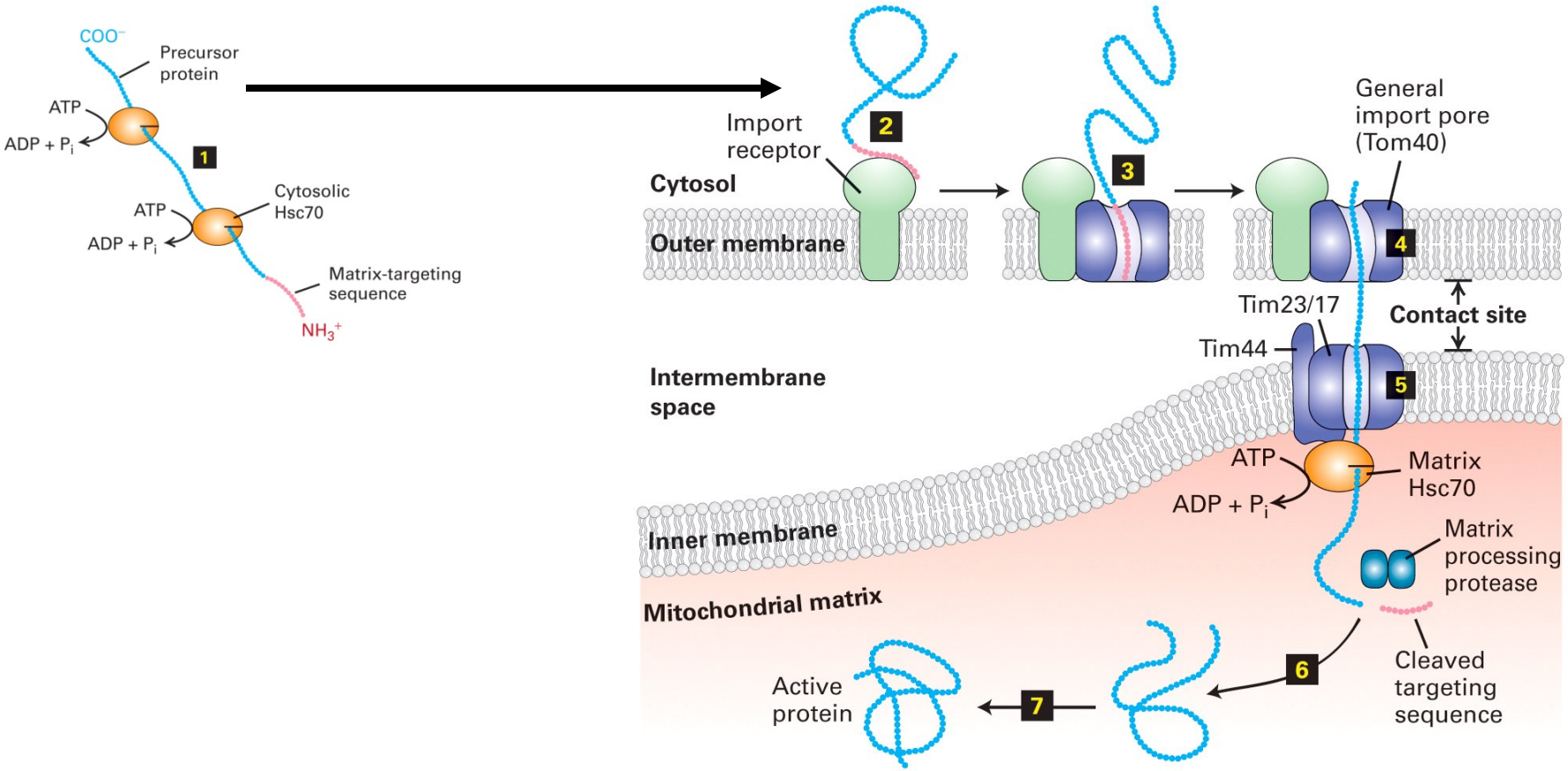
import occurs

cytosol extract and folded precursor protein

cytosol extract and urea-treated precursor protein (urea denatures a protein)

The above results suggest that a protein can be imported into a mitochondrion post-translationally but only when it is unfolded (by its binding to chaperones and mitochondrial targeting sequence-binding proteins).

Overview of Cytosol-to-Mitochondrial Matrix Transport



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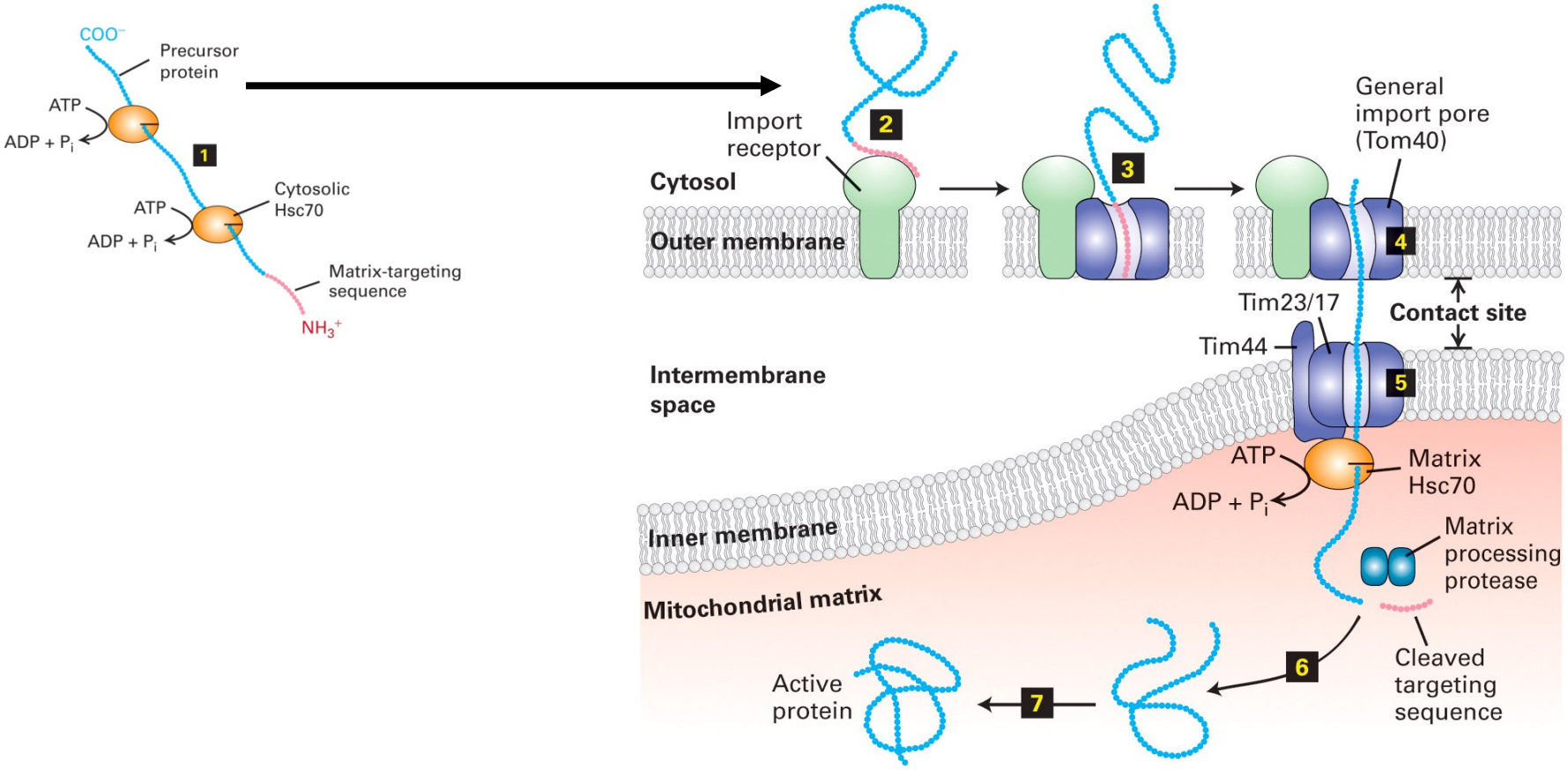
H^+ gradient across the inner membrane is also required.

How do we know that import into the mitochondria occurs at the contact sites between the outer and inner membranes?

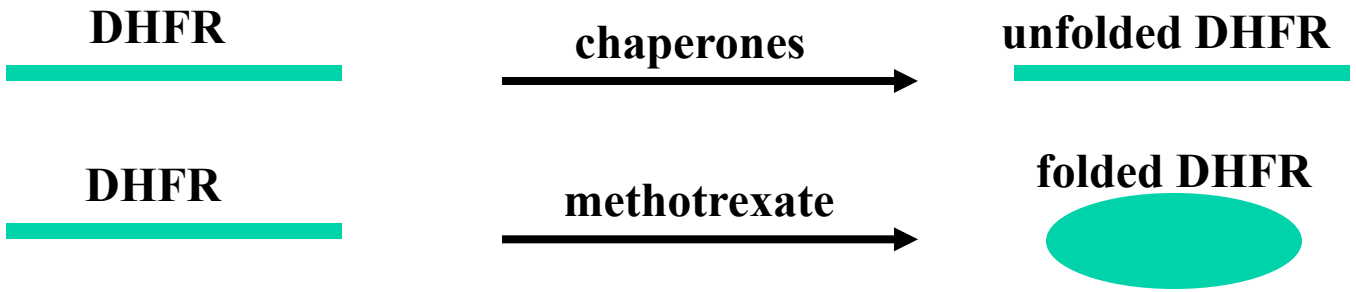
Method 1

In vitro reconstituted mitochondrial translocation assay using a DHFR-derived reporter protein

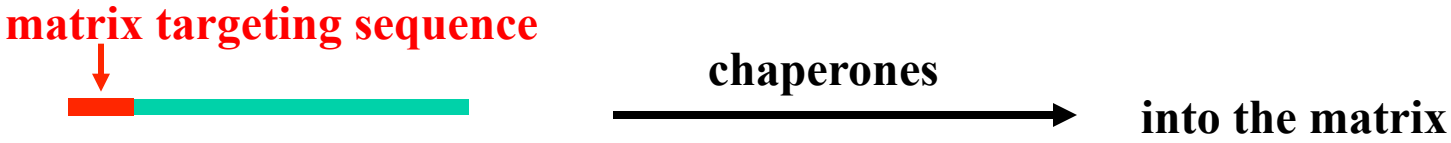
In order to form a stable transport intermediate within the translocator, the precursor protein needs to span both outer and inner membranes to be anchored.



DHFR (dihydrofolate reductase) is a cytosolic protein. When synthesized in vitro, its folding status can be differentially controlled by chaperones or methotrexate.

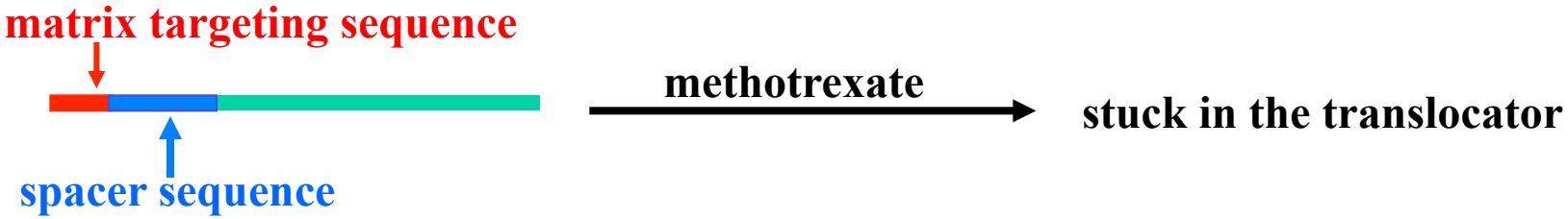


Fusion of a mitochondrial targeting sequence to DHFR allows the fusion protein to be transported into the matrix only in the presence of chaperones.



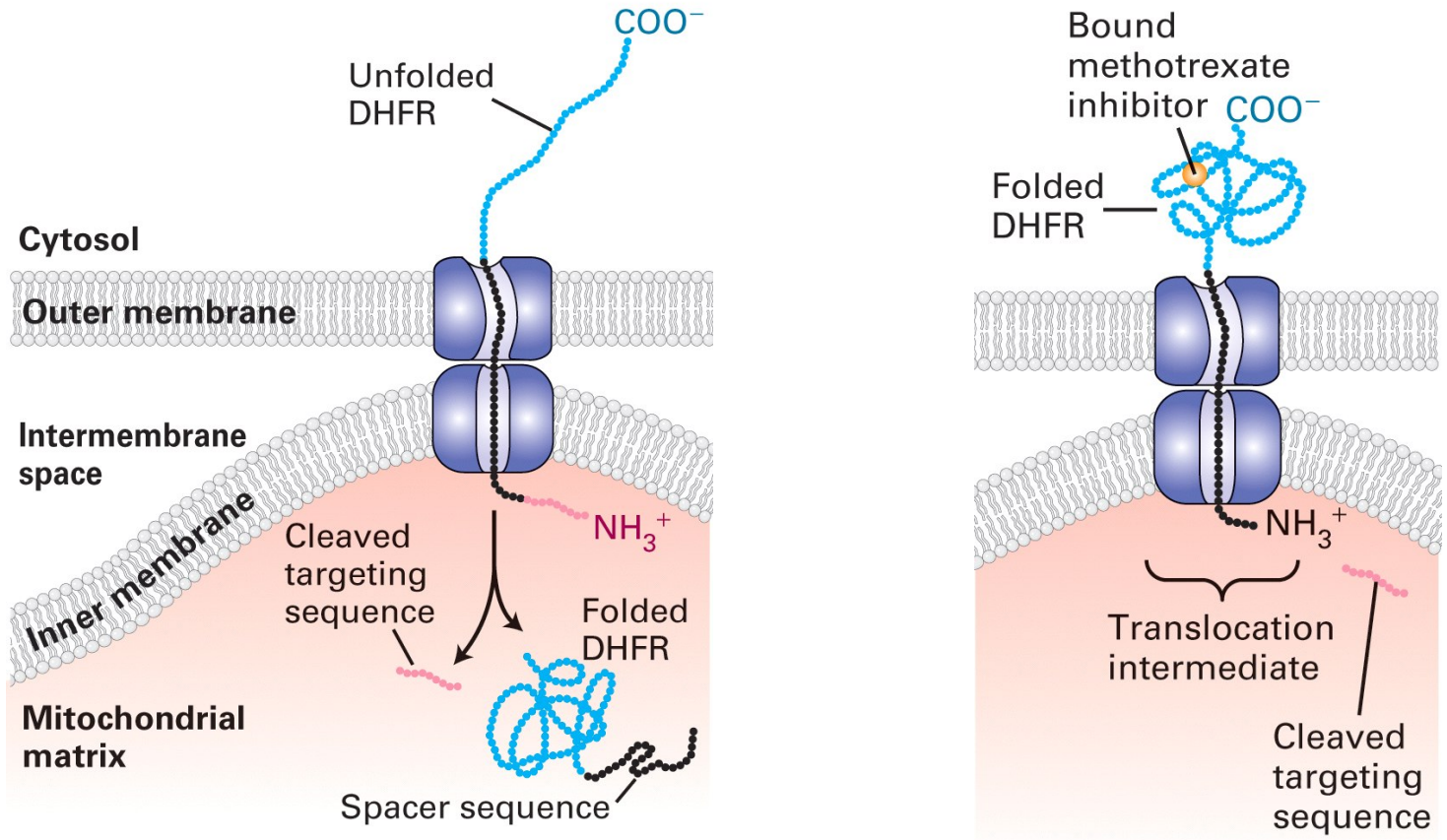
In the presence of methotrexate, the fusion protein stays in the cytosol since the matrix targeting sequence cannot form a stable interaction with the translocator (too short).

However, the insertion of a long enough spacer sequence to the fusion protein allows it to form a stable interaction with the translocator in the presence of methotrexate.



How do we know that import into the mitochondria occurs at the contact sites between two membranes?

In the presence of methotrexate, if the spacer sequence is long enough, the DHFR fusion protein would get stuck within the translocator with its N-terminal matrix targeting sequence cleaved and its C-terminal DHFR part sensitive to the protease treatment.



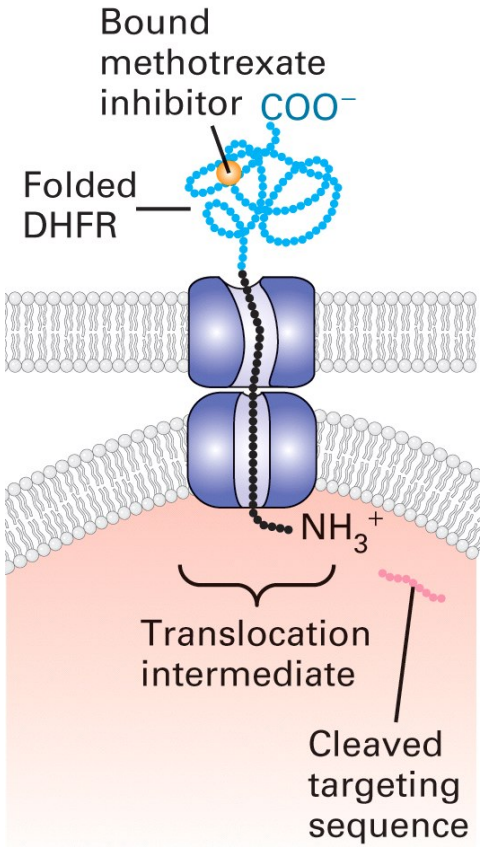
How do we know that import into the mitochondria occurs at the contact sites?

Since signal cleavage takes place in the matrix, the N-terminal part of the fusion protein must be in the matrix

Since the C-terminal part of the fusion protein is sensitive to proteases in the absence of detergents, it must be in the cytosol.

When combined together, these observations suggest that the stuck transport intermediate of the fusion protein spans both outer and inner membranes.

Since it requires a spacer sequence of about 50 a.a long in an alpha-helix to get the above stable transport intermediate, the transport must happen at the contact site of the outer and inner membranes -Why?

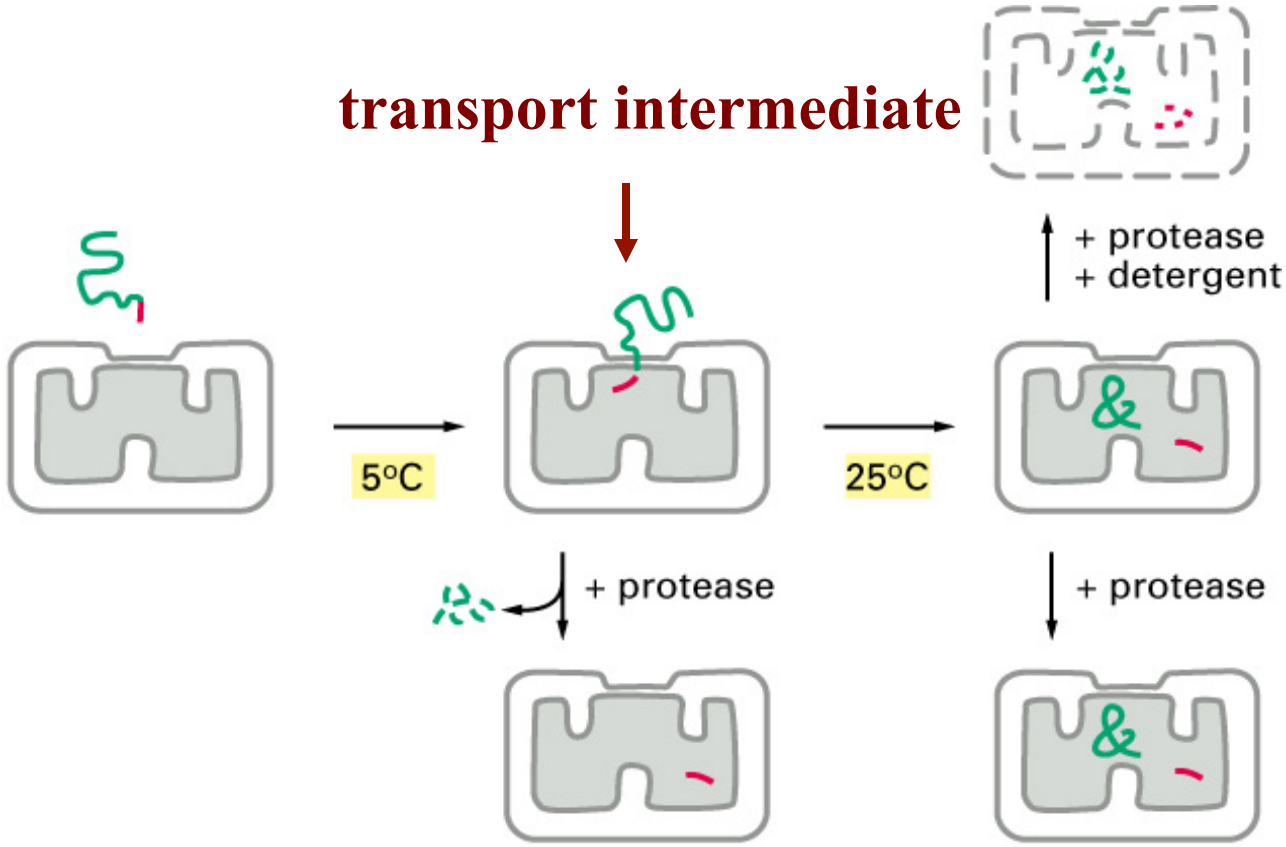


25-30 a.a.

25-30 a.a.

Method 2
In Vitro Reconstituted Mitochondrial Translocation Assay

The initial translocation of signal sequence can proceed at a low temp, but the subsequent translocation of the bulk portion of the protein cannot. By performing the translocation at the low temp, one can "freeze" the transport intermediate.



Method 3
Imaging Approach:
Gold conjugate particles & Electron microscopy

Gold labeling is one of the best methods in visualization via electron microscope. It provides the following advantages:

high sensitivity

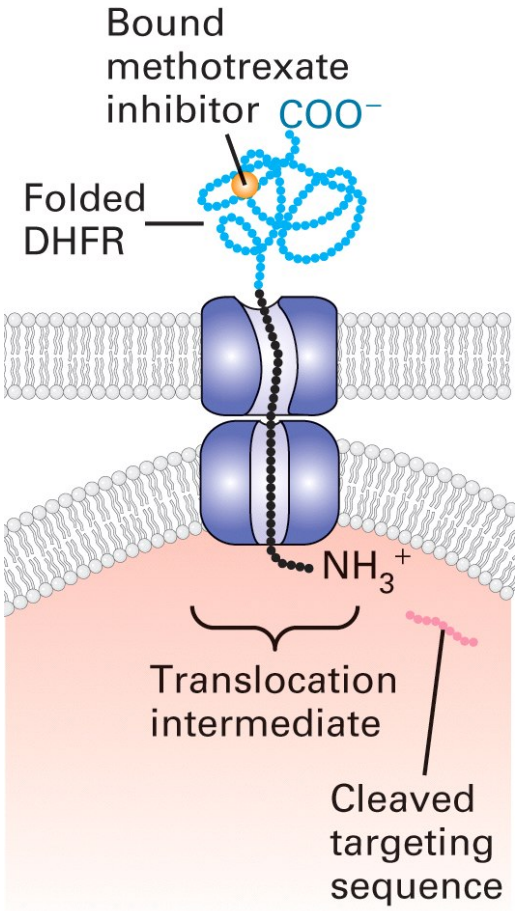
superior resolution

high contrast

stable signals

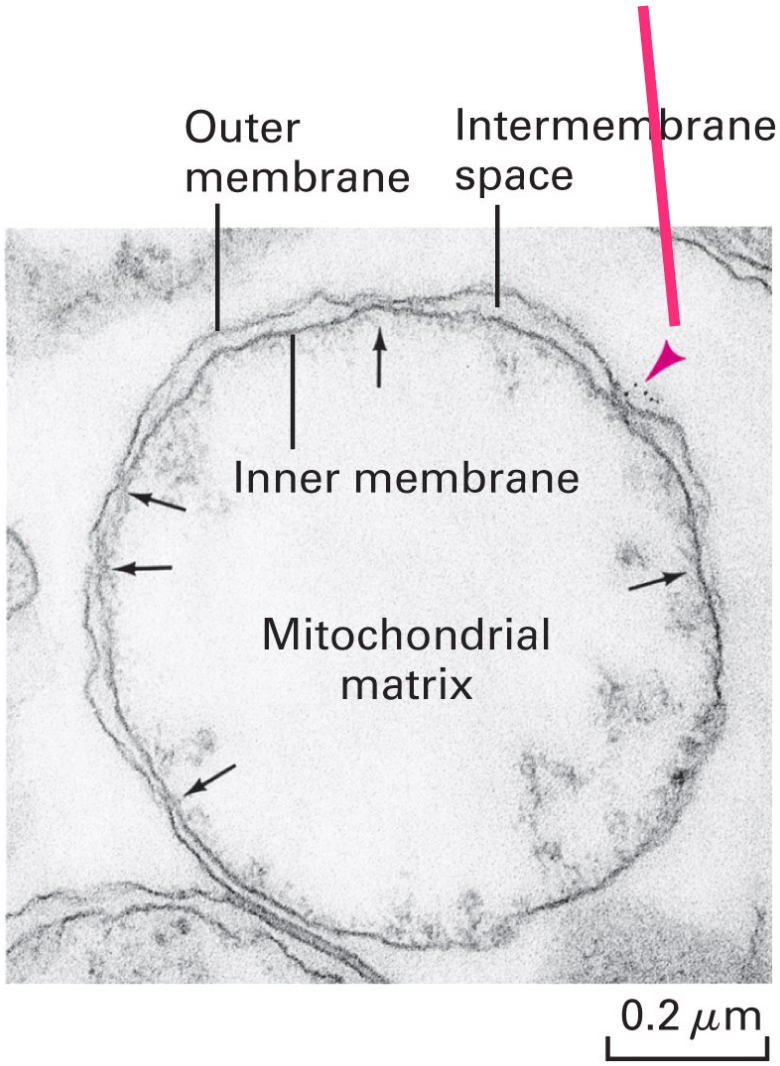
different sized particles are available

Method 3
Imaging Approach



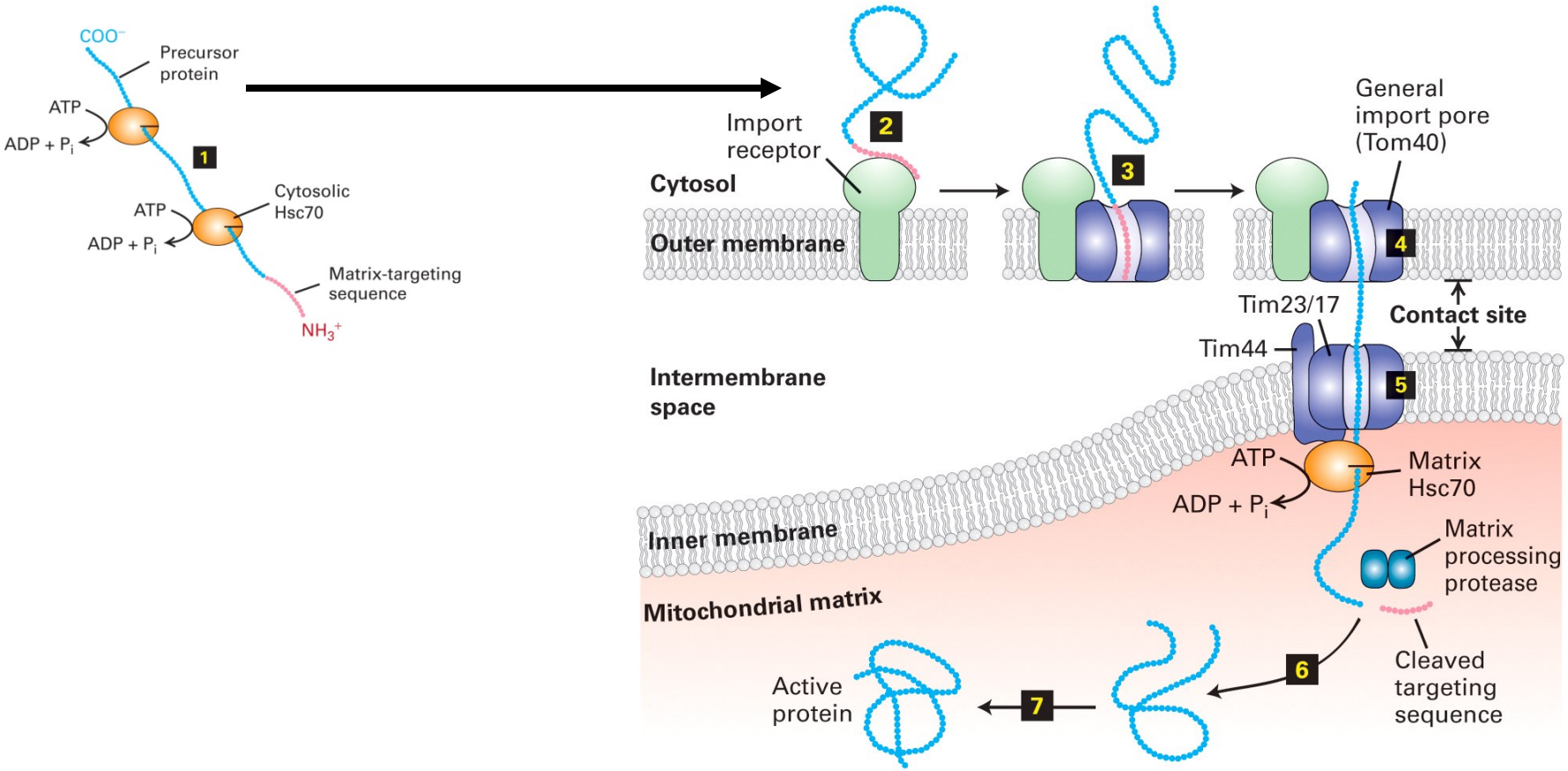
anti-DHFR antibody
+
gold particles coated with protein A

proteins to be imported



Protein A is a bacterial protein which has a high affinity to the antibodies

Overview of Cytosol-to-Mitochondrial Matrix Transport



The matrix targeting sequence is recognized by an import receptor of the TOM complex, then followed by another receptor of the TIM complex

Proteins are imported in a post-translational but unfolded state.

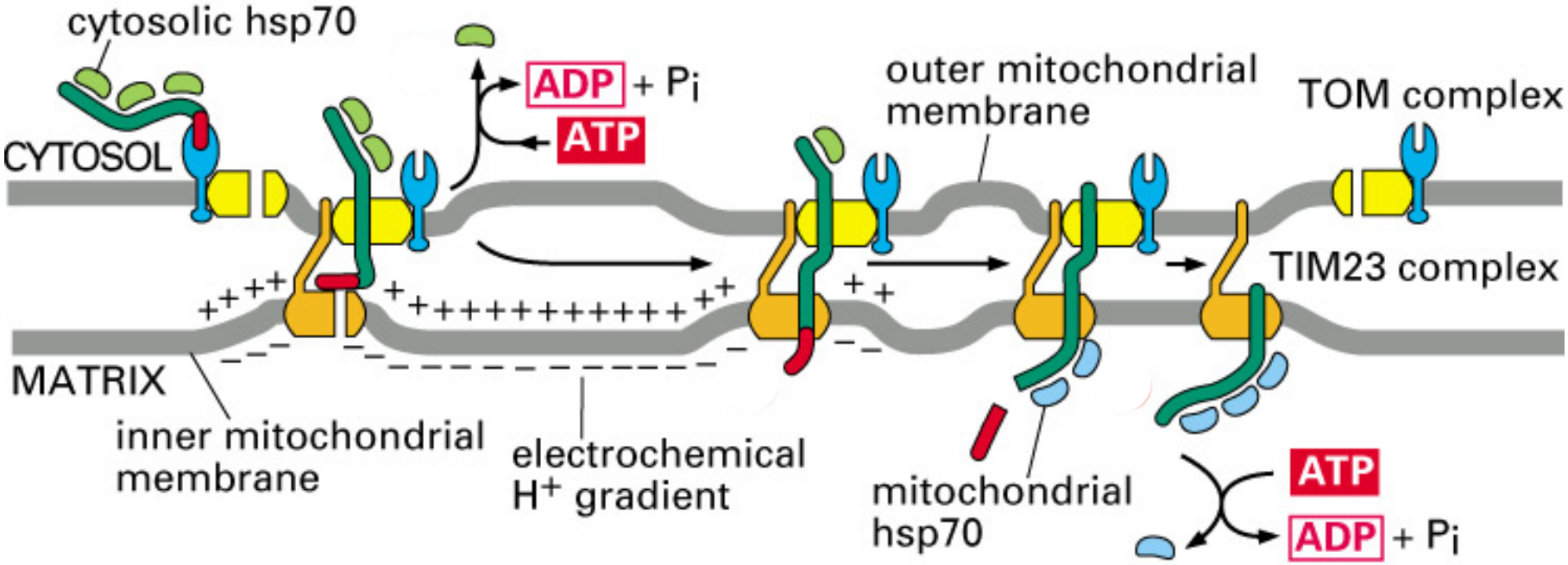
Transport occurs via the contact sites between outer and inner membranes.

Chaperones are required on both cytosolic and matrix sides.

ATP is required on both cytosolic and matrix sides.

H^+ gradient across the inner membrane is also required.

Energy and Chaperone Requirement During Cytosol-to-Matrix Transport



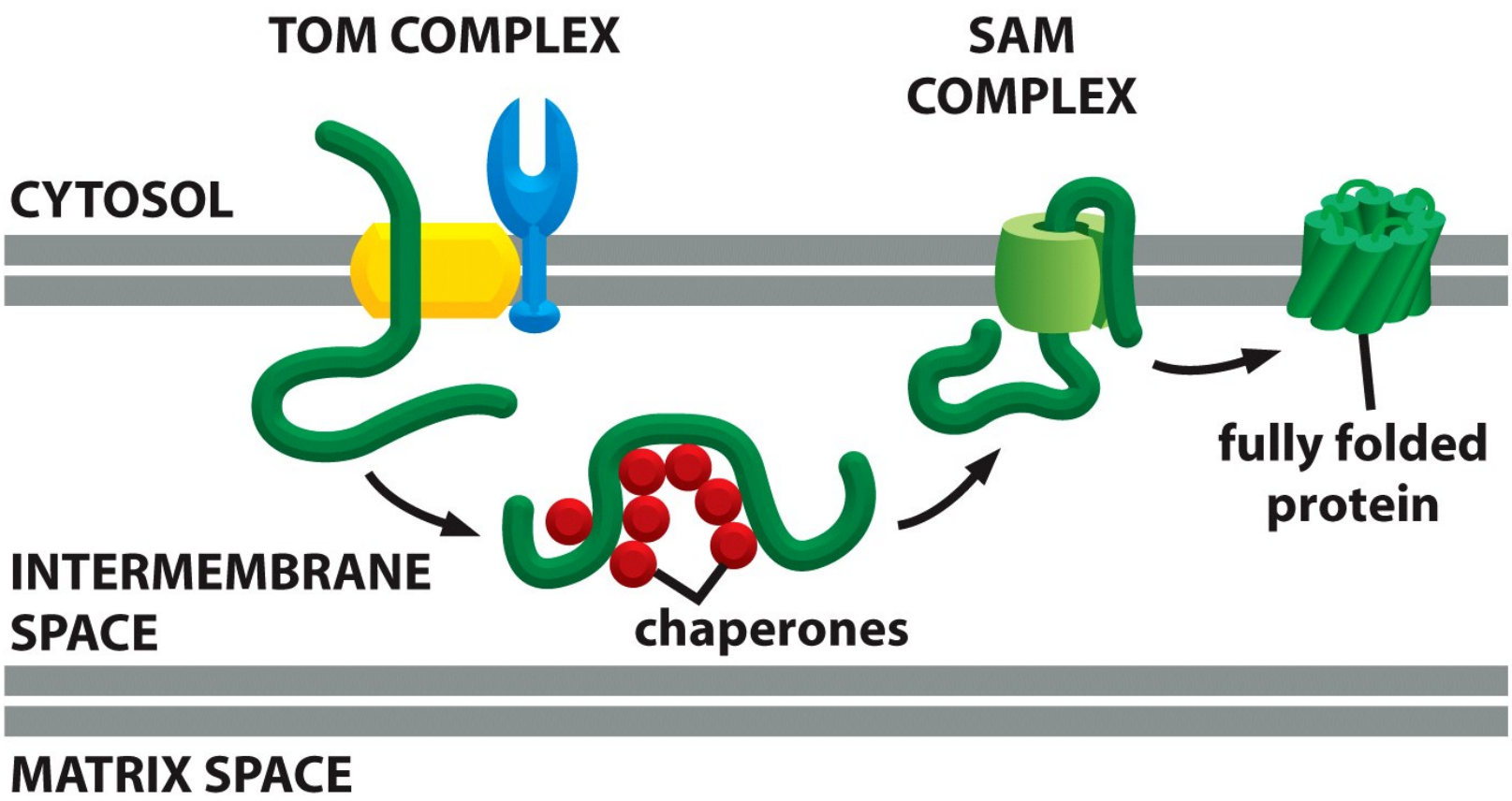
cytosolic hsp70: a chaperone required to keep the polypeptide chain unfolded in the cytosol until translocation

matrix hsp70: a chaperone required to pull the emerging polypeptide chain into the matrix (the pulling assures the directional transport)

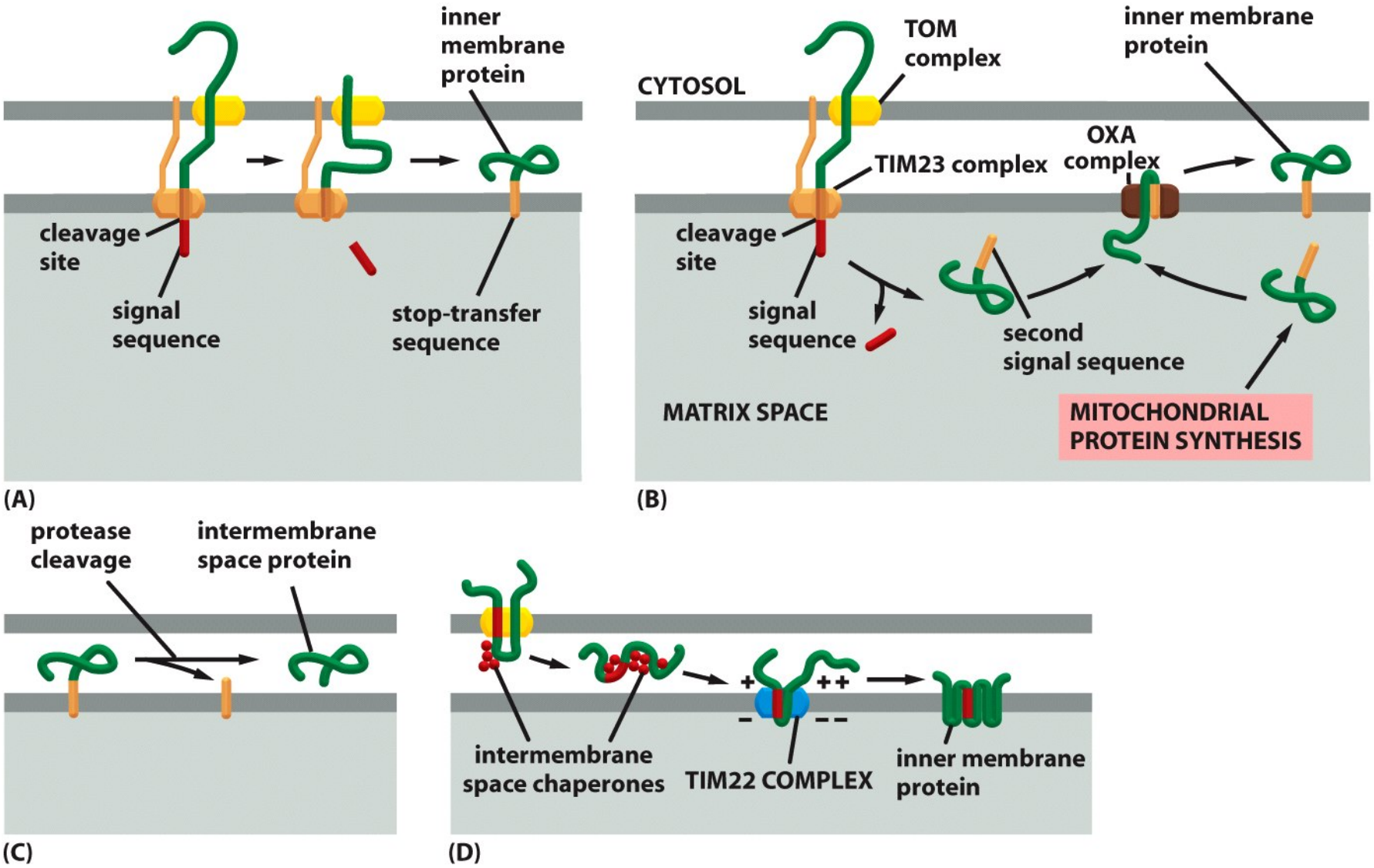
ATP is required for the functions of both types of chaperones

H^+ gradient is required at an intermediate step, probably the translocation of the matrix targeting sequence across the inner membrane.

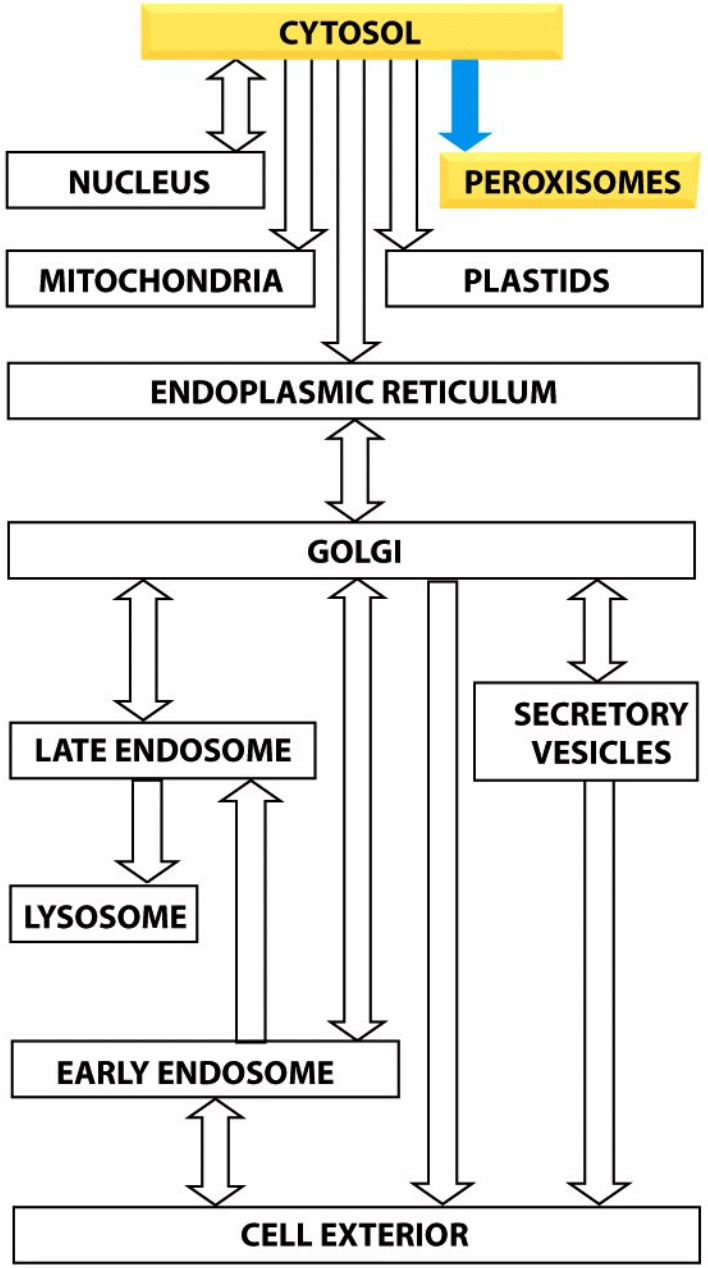
Cytosol to Outer Membrane Protein Transport



Cytosol to Inner Membrane Protein Transport



Protein transport into peroxisomes



General Features:

translocator-mediated

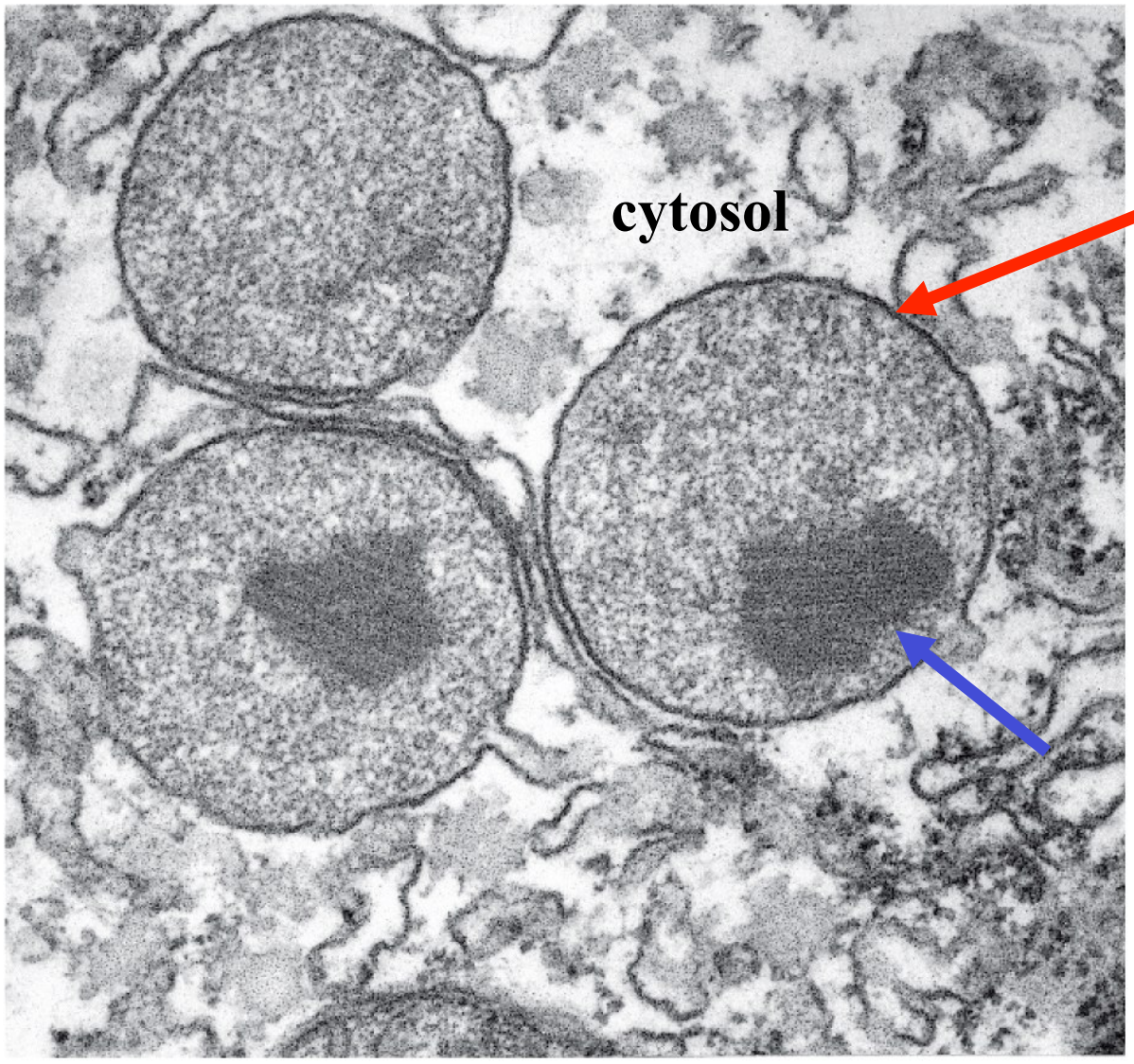
signal sequence required (found in the C-terminus)

mostly uni-directional transport

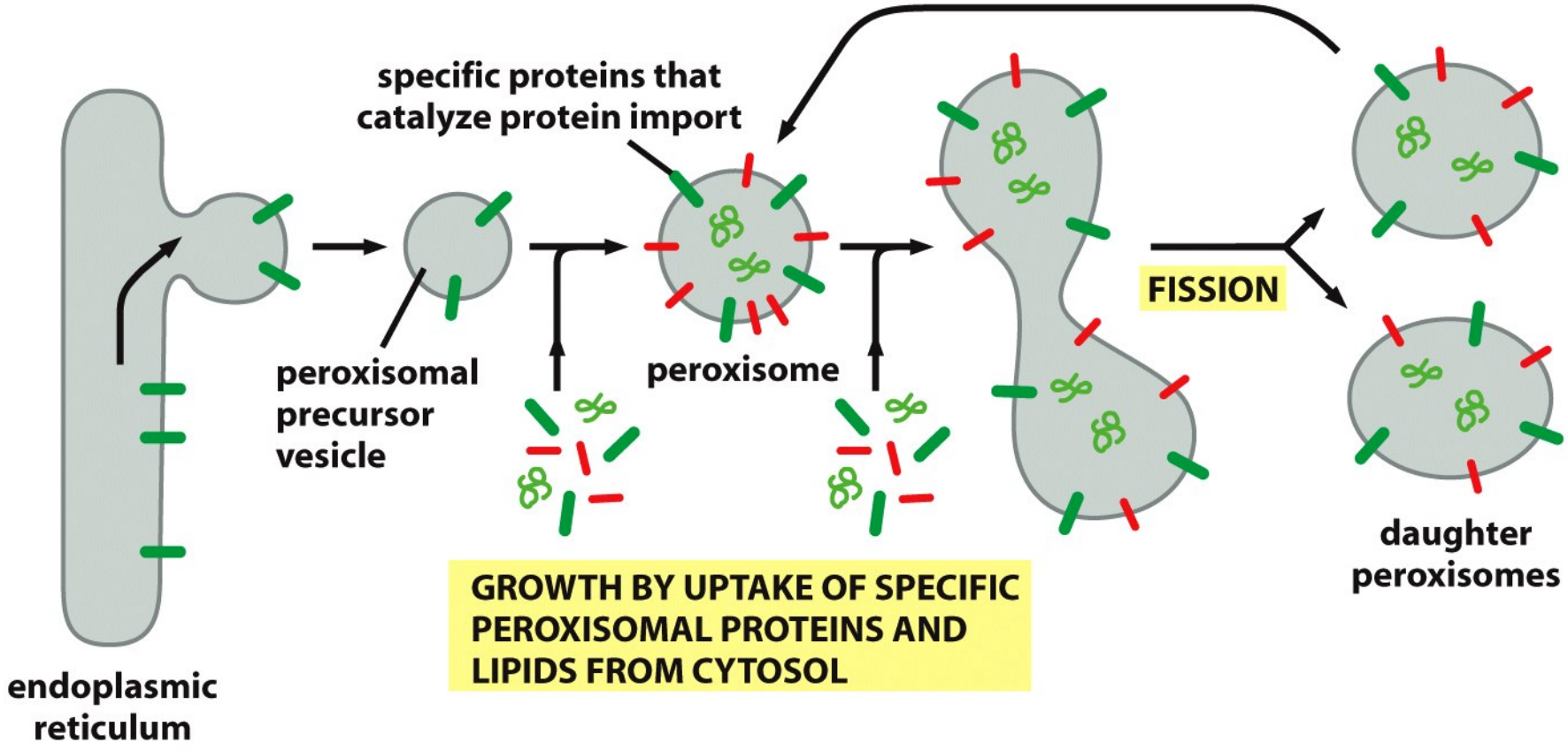
post-translational mechanism

imported as folded polypeptide chains

Basic Structure and Topology of Peroxisomes



Peroxisomes: Sources of Peroxisomal Proteins



Signal Sequences for Peroxisome Import

For most imported proteins:
(S/A/C)(K/R/H)(L/M)-COOH

For some imported proteins:
(R/K)(L/V/I)X_5(H/Q)(L/A)
(near the N-terminus)

(no need to remember these sequences)

A group of more than 20 proteins (peroxins) constitute the transport machinery.

Less is known regarding this transport mechanism

In vitro transport assays suggest that the cargo can be transported in its folded state. If so, how a cell maintains peroxisome integrity during protein import is a challenging research question.

The prevention of component mixing between two compartments during intracellular protein transport is an important consideration.