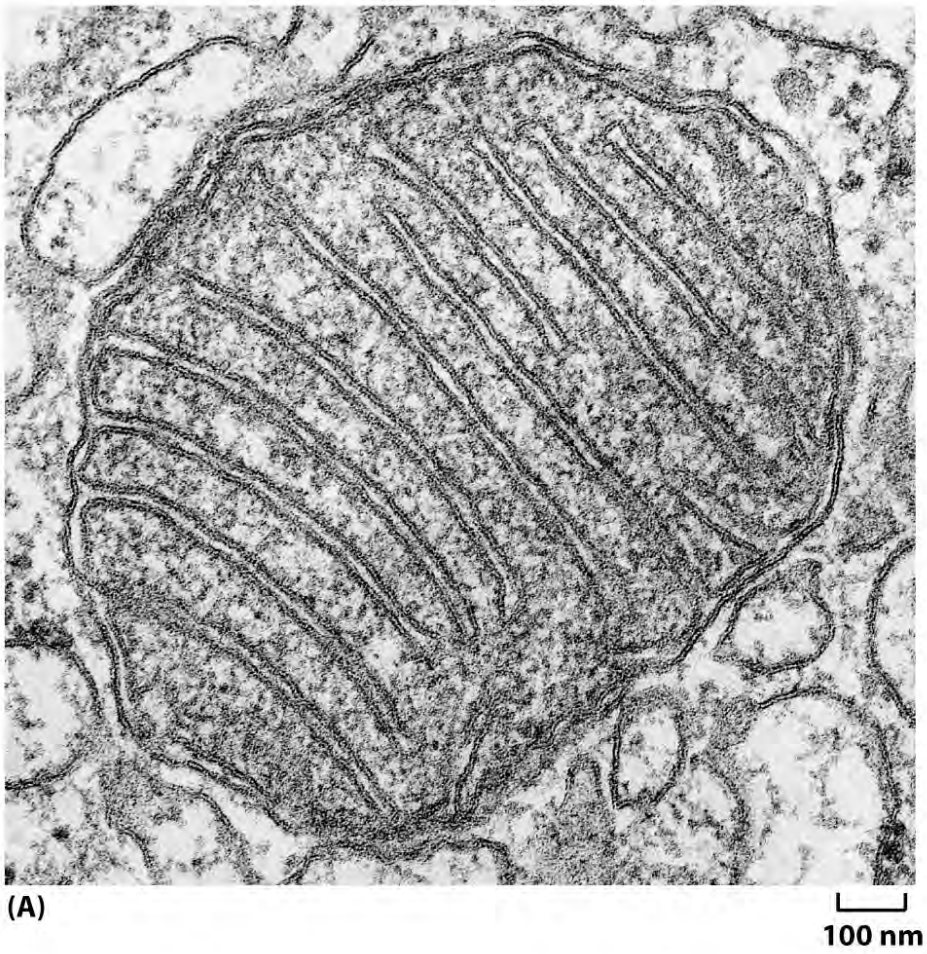
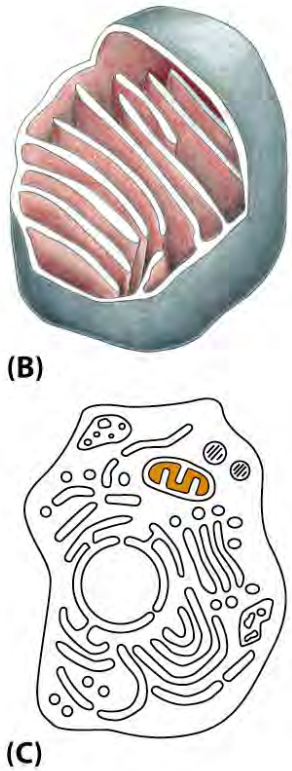
Structure of mitochondria





Subcompartments of mitochondria

Outer membrane (OM)
Inner membrane (IM)
Matrix
Intermembrane space (IMS)

Cristae membrane
Inner boundary membrane
Cristae compartment

Protein targeting in mitochondria: at least four compartments

OM: permeable to 5 kDa, contains proteins involved in import and dynamics

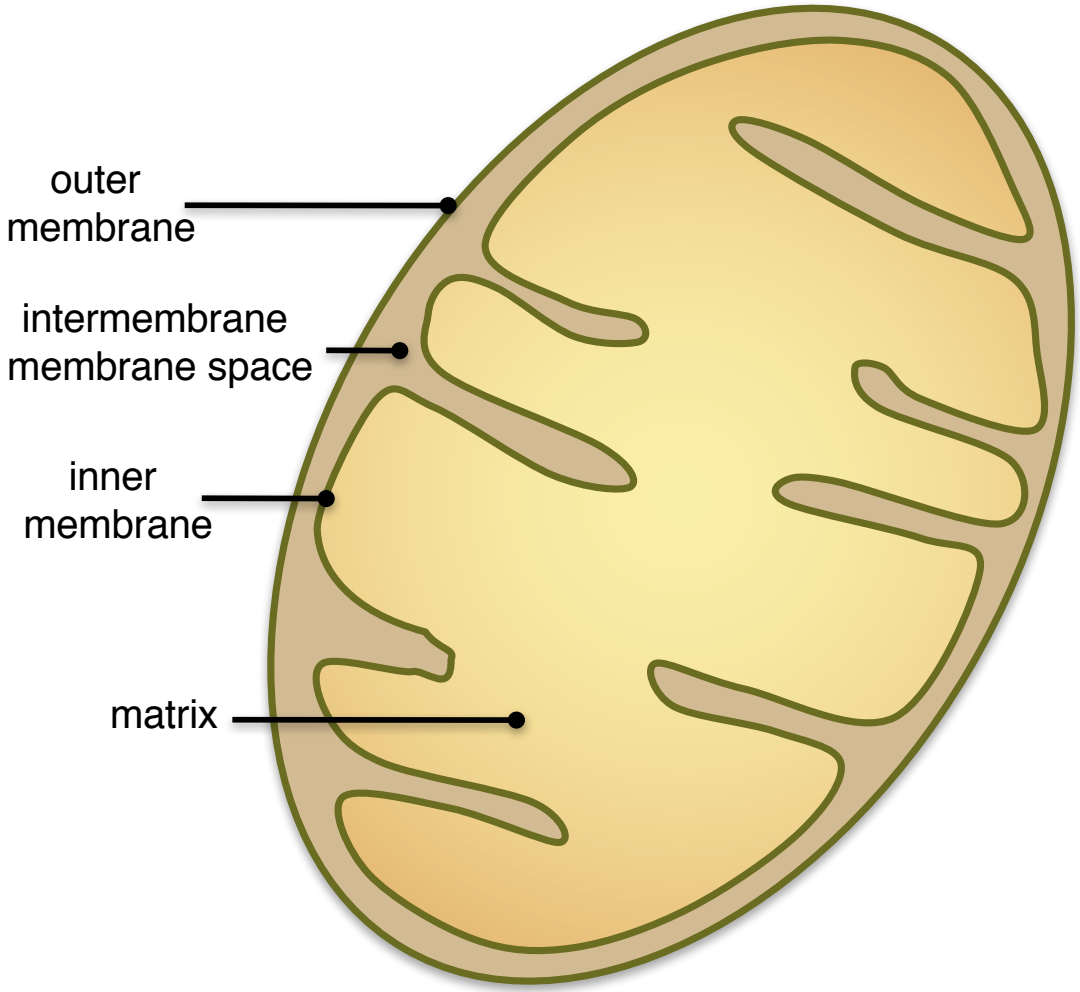
IMS: cytochrome *c*

IM: has membrane potential, proteins involved in import, respiratory chain, infolded into cristae membranes

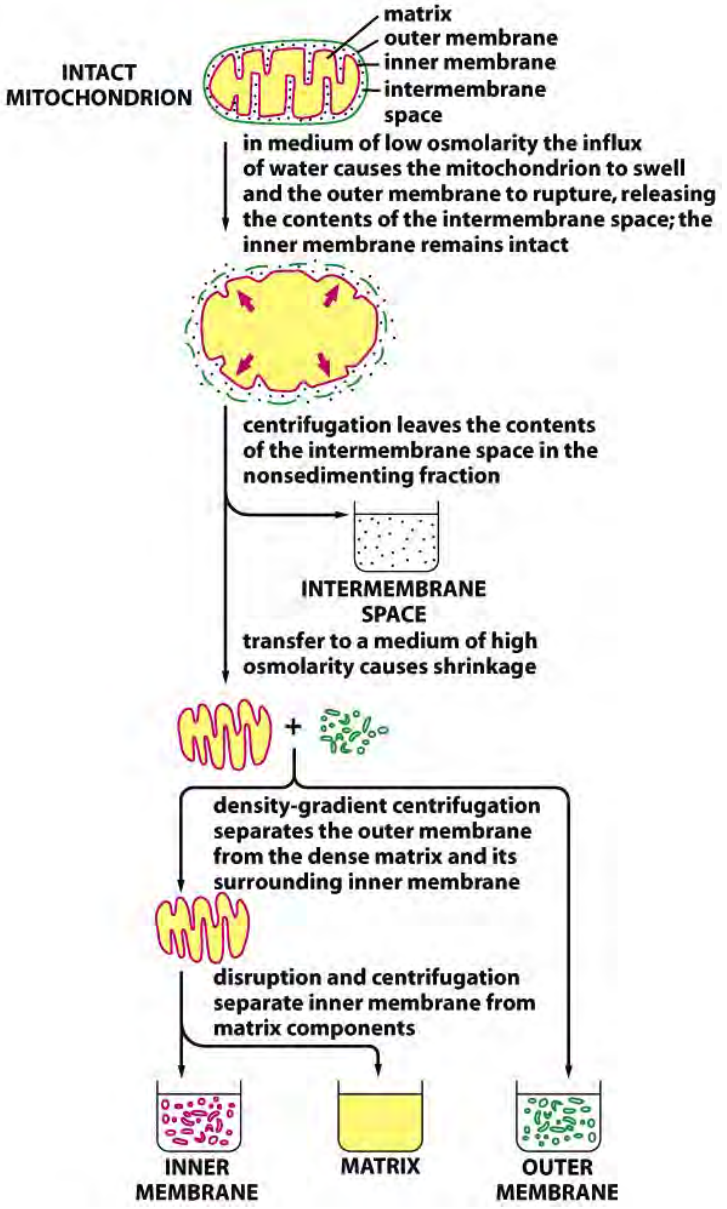
Matrix: biochemical reactions, mtDNA

Methods to determine suborganellar localization of mitochondrial proteins:

- Immunostaining (OM versus matrix)
- Immuno-EM
- Subcellular fractionation



Localization of mitochondrial proteins by subcellular fractionation



OM proteins: Regions projecting into cytosol are susceptible to proteolysis

IMS proteins: Released by hypo-osmotic shock

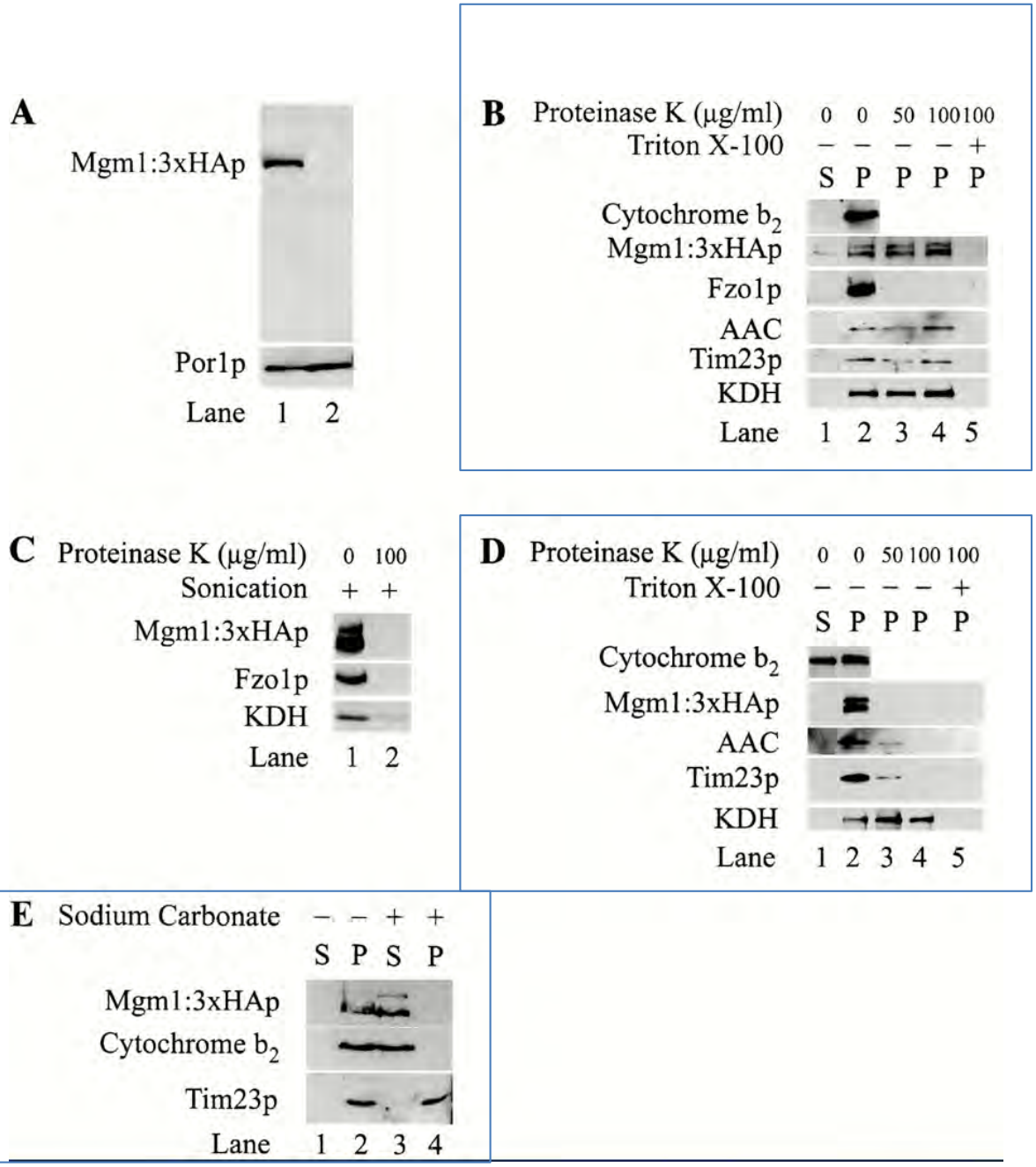
IM proteins: Regions projecting into IMS are susceptible to proteolysis after hypo-osmotic shock

Matrix proteins: Protected from proteolysis, unless mitochondria lysed with detergent or sonication.

Integral membrane proteins (OM and IM) are resistant to extraction by carbonate (converts membranes to sheets and extracts peripheral membrane proteins).

Figure 14-7 *Molecular Biology of the Cell* (© Garland Science 2008)

Sub-organellar fractionation of mitochondria



B. Enriched mitochondrial fraction
- OM proteins cleaved by protease

C. After sonication

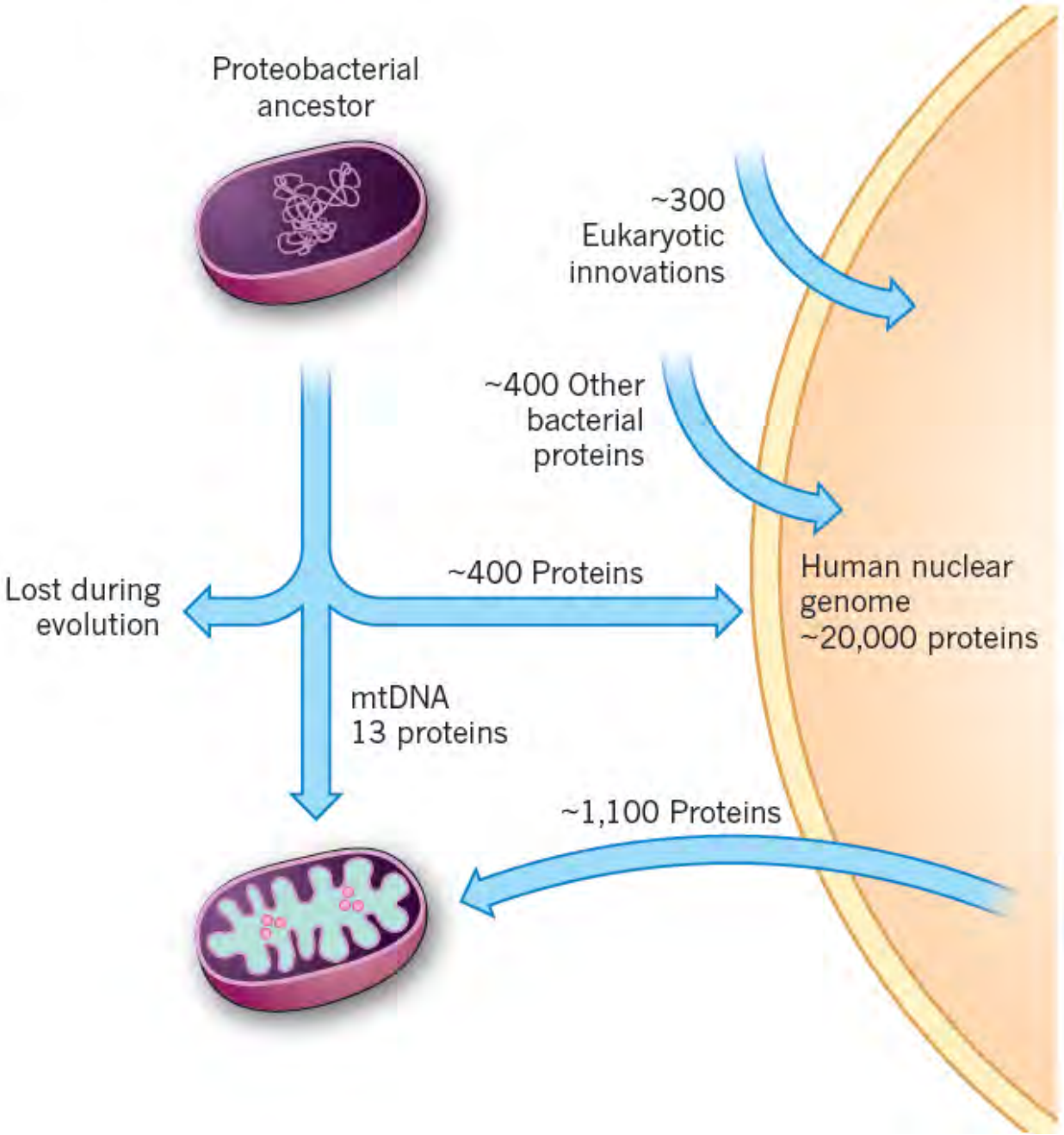
D. Hypo-osmotic shock
- Proteins exposed to IMS cleaved; matrix proteins still protected

E. 0.1 sodium carbonate, pH 10.5
- carbonate a test for integral membrane proteins

Markers:
Fzo1: OM
Tim23: IM
cytochrome b2: IMS
KDH: matrix (TCA cycle)
AAC (ADP/ATP carrier): IM

Wong et al. (2000) JCB

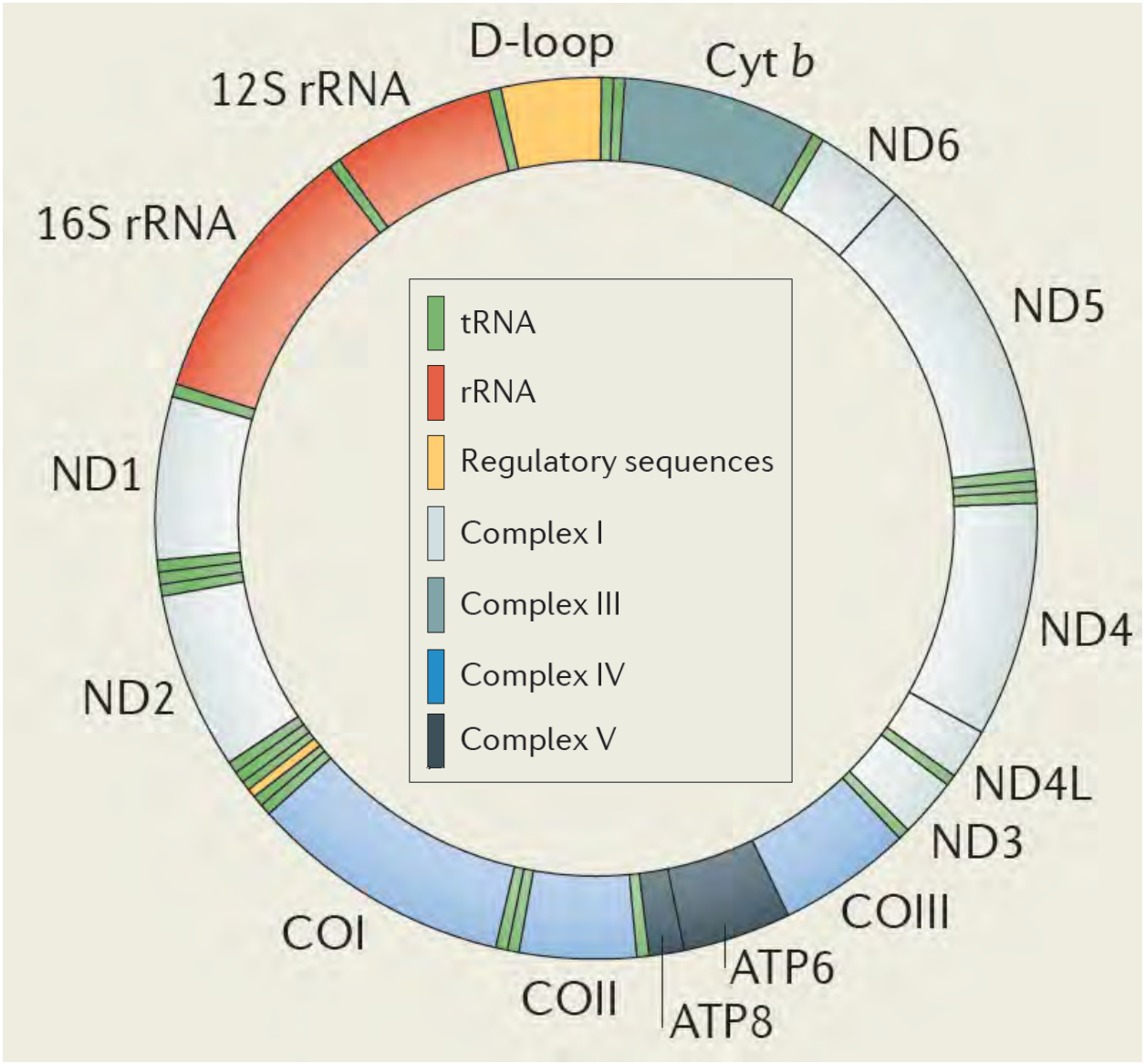
The vast majority of the mitochondrial proteome is encoded by the nucleus



- Mitochondria contain >1000 proteins; 13 are encoded by mtDNA.
- Therefore, the majority of proteins in mitochondria are synthesized in the cytosol.
- During evolution, many mtDNA-encoded genes transferred to nuclear genome. The remaining 13 proteins encoded by mtDNA are extremely hydrophobic.

Vafai & Mootha (2012) Nature

The mitochondrial genome (mtDNA)



Mammalian mtDNA is a circular 16.6 kb genome.

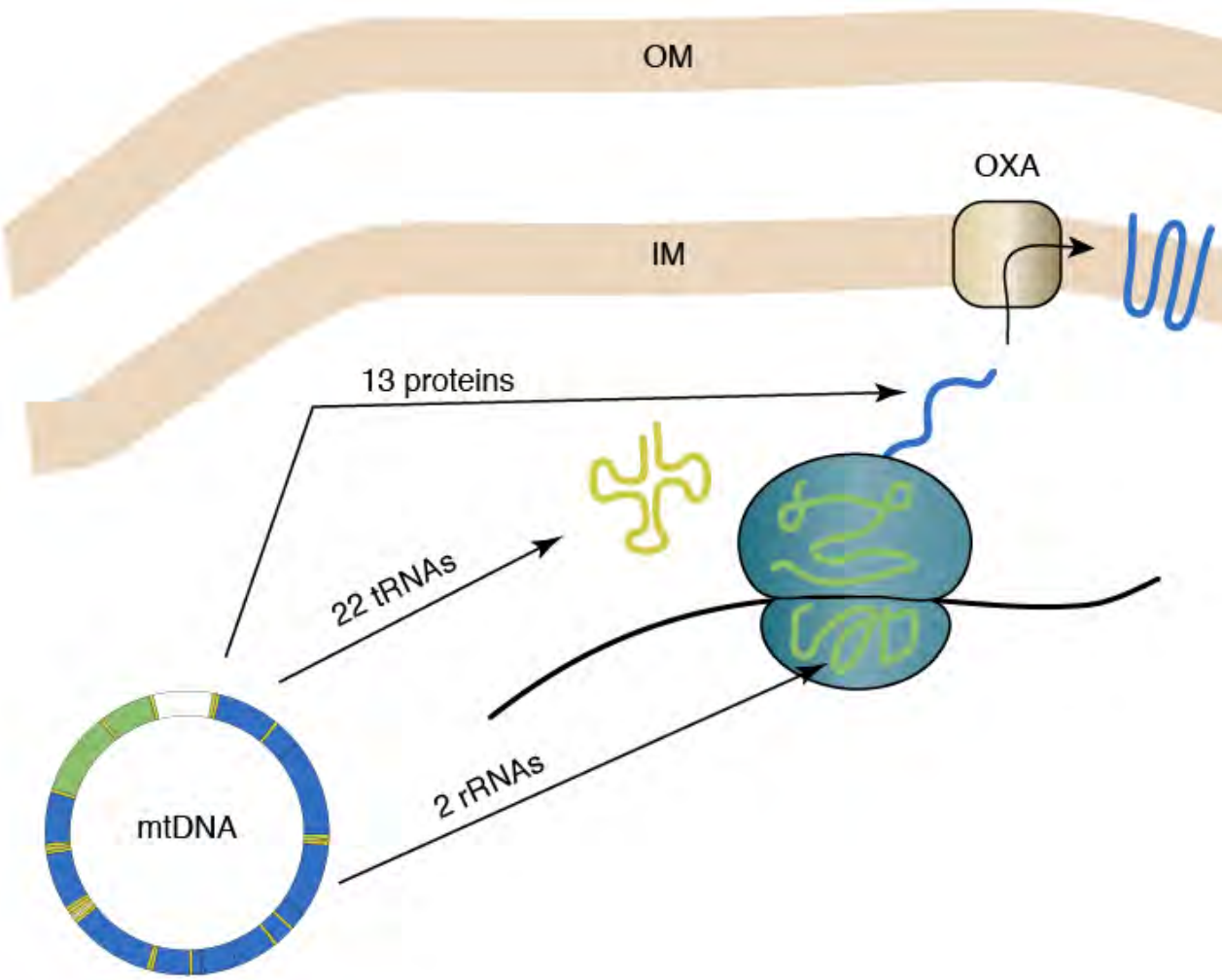
Encodes 37 genes:

13 polypeptides, 2 rRNA, 22 tRNA

Each gene essential for respiratory function.

Mishra & Chan (2014) Nat Rev Mol Cell Biol

The mtDNA gene products



To make 13 polypeptides, mtDNA encodes 2 rRNAs and 22 tRNAs.

The mitochondrial ribosomes are formed by rRNAs encoded by mtDNA and proteins subunits encoded by nuclear DNA.

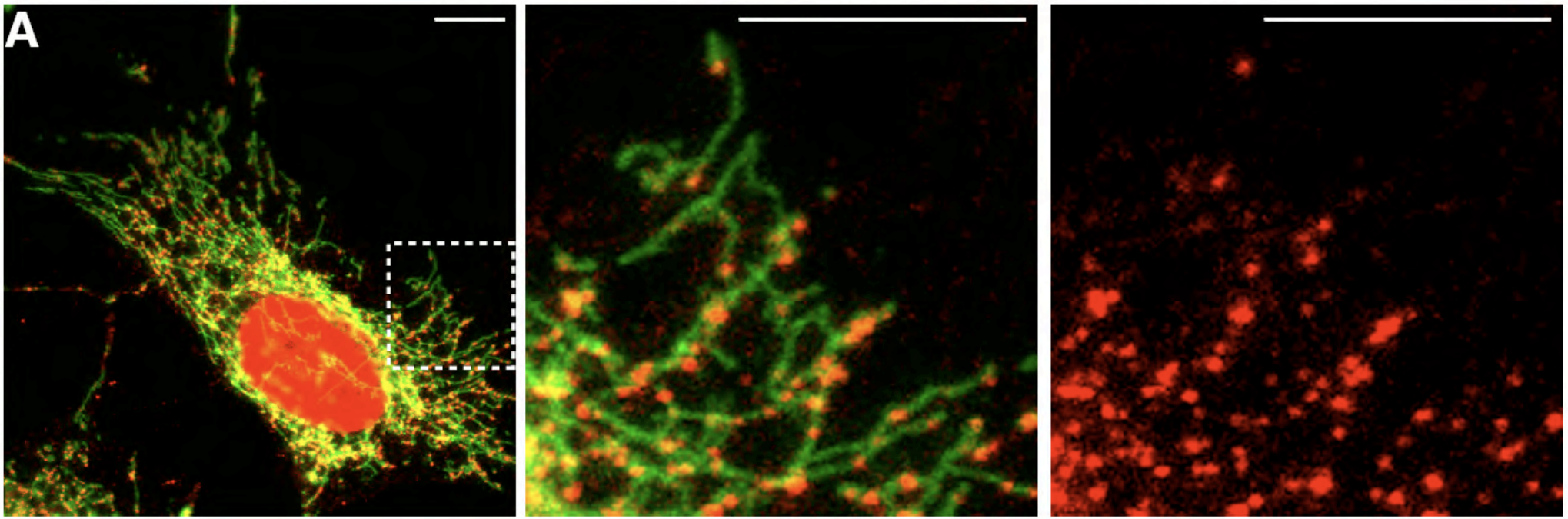
The tRNAs are charged by aminoacyl tRNA synthetases encoded by nuclear DNA*.

Humans have complete set of mito-tRNAs. But many organism do not, and then some tRNAs are imported from cytosol.

*Therefore, there must be compatibility between mitochondrial and nuclear genomes.

Carelli & Chan (2014) Neuron

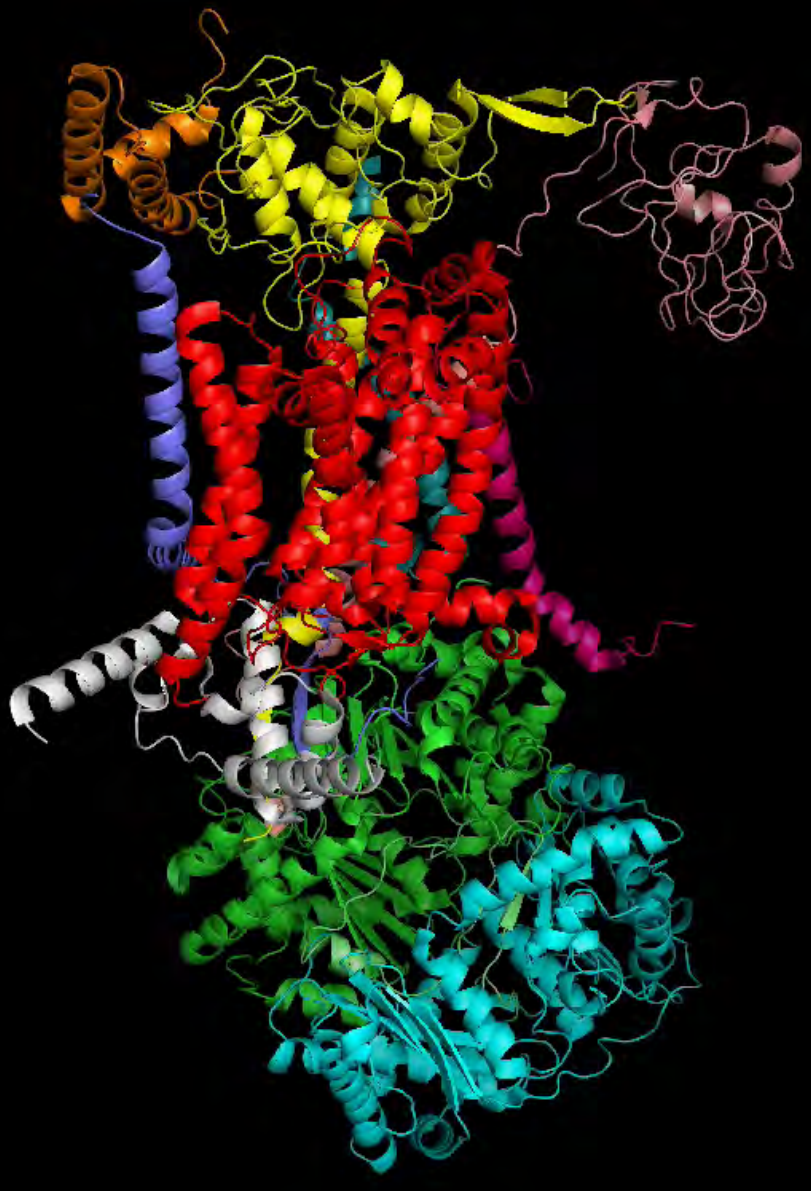
Organization of mtDNA genomes into nucleoids



- Nucleoids are mtDNA/protein complexes within matrix
- mtDNA is compacted
- Each nucleoid contains one to several mtDNA genomes
- Nucleoids tend to be inherited by both daughter mitochondria after fission

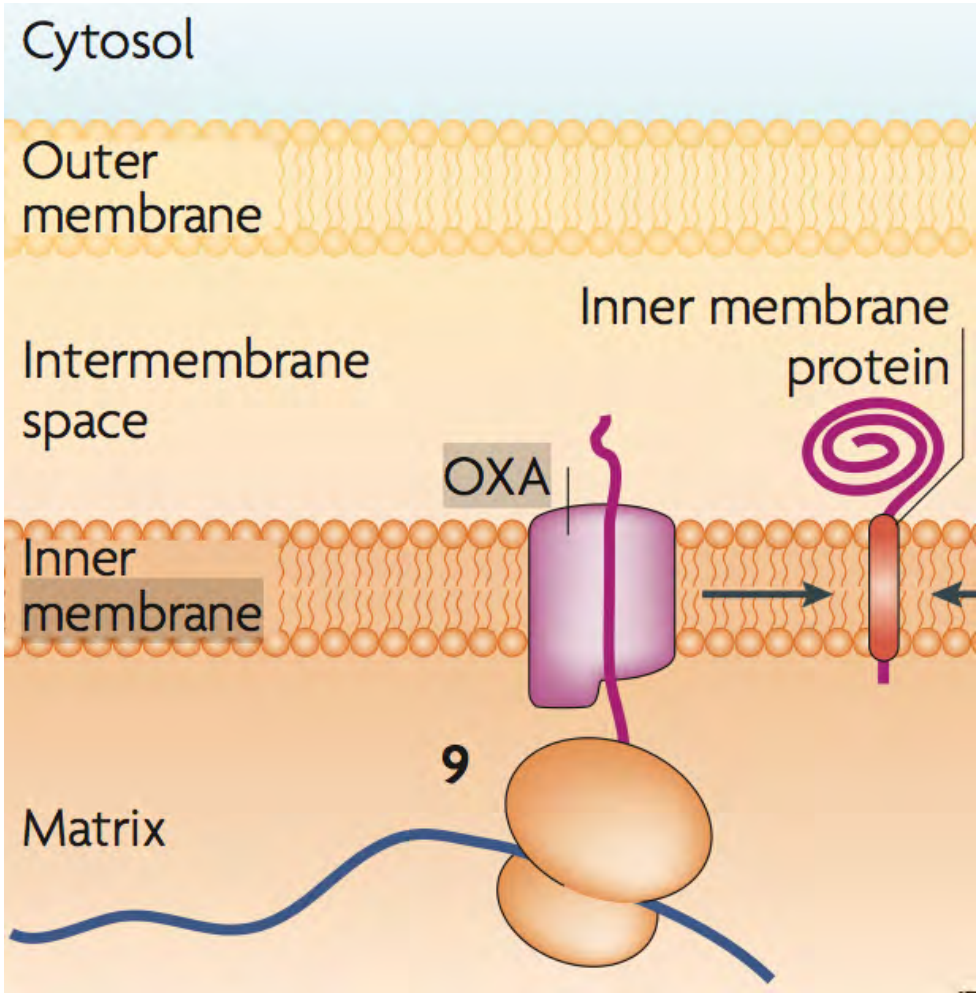
Chen et al. (2007) Cell

Example of mtDNA encoded protein: cytochrome *bc1* in complex III



- Cytochrome *bc1* is complex III
- It transfers electrons from reduced CoQ (generated from complex I) to cytochrome c and pumps protons into the IMS.
- Has 11 subunits, 1 encoded by mtDNA: cytochrome *b*
- Cytochrome *b* has multiple TMs and is the major constituent of the membrane spanning region

Insertion of mtDNA-encoded proteins



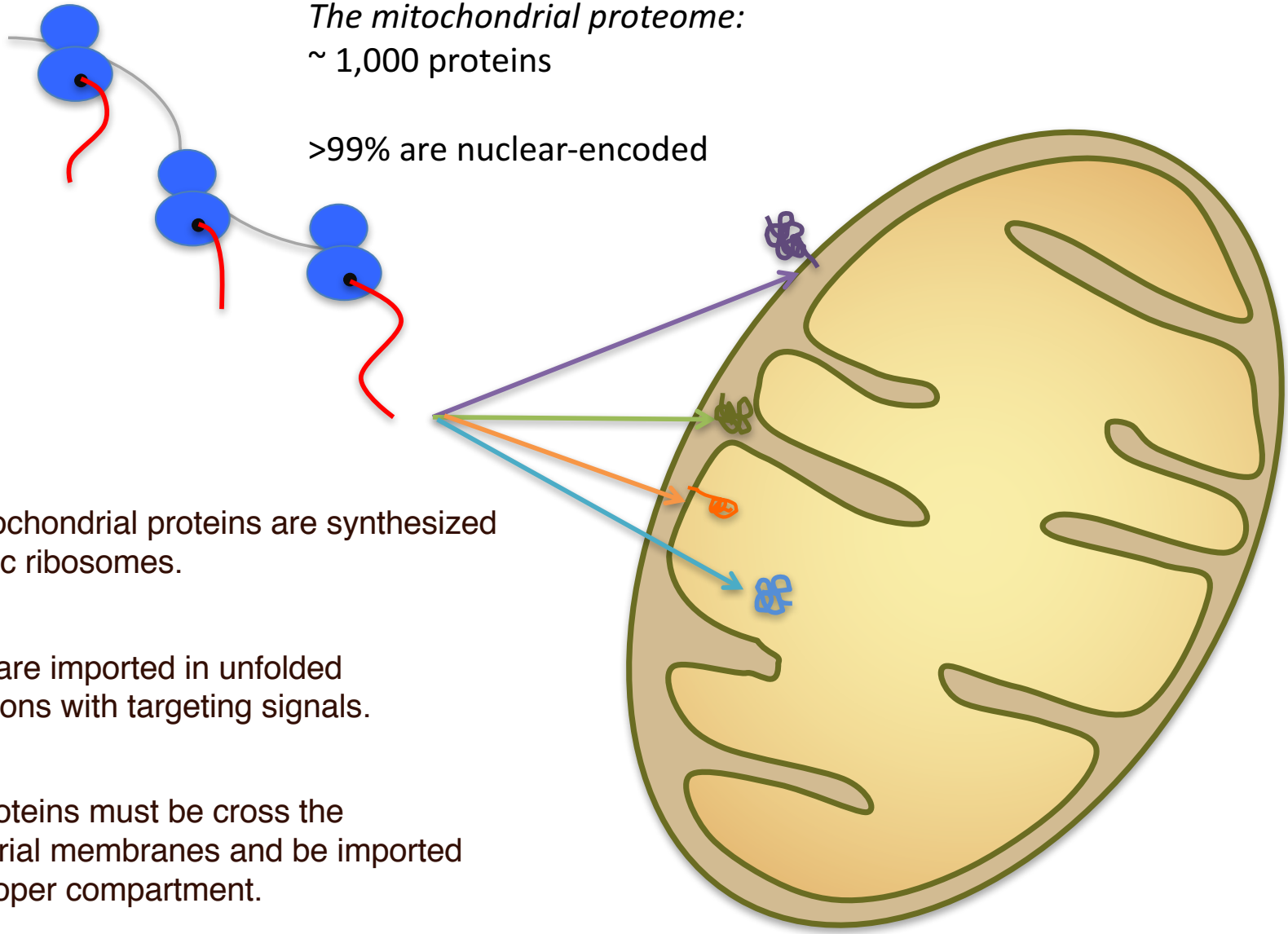
- mtDNA encoded polypeptides are hydrophobic multi-TM proteins.
- Mitochondrial ribosomes (in matrix) are associated with the IM and with the OXA (oxidase assembly) import machinery.
- Proteins are inserted co-translationally into the IM. Nascent polypeptide tunnel exit is aligned with OXA.
- OXA is also used in the insertion of some nuclearly encoded mitochondrial proteins.

Schmidt et al (2010) Nat Rev MCB

Only 13 mitochondrial polypeptides do not need to be imported from the cytosol

The mitochondrial proteome:
~ 1,000 proteins

>99% are nuclear-encoded



• Most mitochondrial proteins are synthesized by cytosolic ribosomes.

• Proteins are imported in unfolded conformations with targeting signals.

• These proteins must be cross the mitochondrial membranes and be imported into the proper compartment.

Examples of mitochondrial proteins that must be imported

TABLE 17-2 Selected Mitochondrial Proteins That Are Synthesized in the Cytosol

Mitochondrial Location	Protein*
Matrix	Alcohol dehydrogenase (yeast)
	Carbamoyl phosphate synthase (mammals)
	Citrate synthase and other citric acid enzymes
	DNA polymerase
	F_1 ATPase subunits α (except in plants), β, γ, and δ (in certain fungi)
	Mn^{2+}-superoxide dismutase
	Ornithine aminotransferase (mammals)
	Ornithine transcarbamoylase (mammals)
	Ribosomal proteins
	RNA polymerase
Inner membrane	ADP/ATP antiporter
	$CoQH_2$–cytochrome c reductase complex: subunits 1, 2, 5 (Fe-S protein), 6, 7, and 8
	Cytochrome c oxidase subunits 4, 5, 6, and 7
	Phosphate/OH^- antiporter
	Proteolipid of F_0 ATPase
	Thermogenin (brown fat)
Intermembrane space	Cytochrome c
	Cytochrome c peroxidase
	Cytochrome b_2 and c_1 (subunits of $CoQH_2$–cytochrome c reductase complex)
Outer membrane	Mitochondrial porin (P70)

**Most proteins (except the ADP-ATP antiporter, cytochrome c, and porin) are fabricated as longer precursors.*

Lodish et al, 2000

Specific uptake-targeting sequences in newly made proteins are recognized by different organelles

TABLE 17-1 Properties of Uptake-Targeting Signal Sequences That Direct Proteins from the Cytosol to Organelles

Target Organelle	Usual Signal Location within Protein	Signal Removal*	Nature of Signal
Endoplasmic reticulum	N-terminal	(+)	"Core" of 6–12 mostly hydrophobic amino acids, often preceded by one or more basic amino acids
Mitochondrion†	N-terminal	(+)	3–5 nonconsecutive Arg or Lys residues, often with Ser and Thr; no Glu or Asp residues
Chloroplast†	N-terminal	(+)	No common sequence motifs; generally rich in Ser, Thr, and small hydrophobic amino acid residues and poor in Glu and Asp residues
Peroxisome	C-terminal	(−)	Usually Ser-Lys-Leu at extreme C-terminus
Nucleus	Internal	(−)	One cluster of 5 basic amino acids, or two smaller clusters of basic residues separated by ~10 amino acids

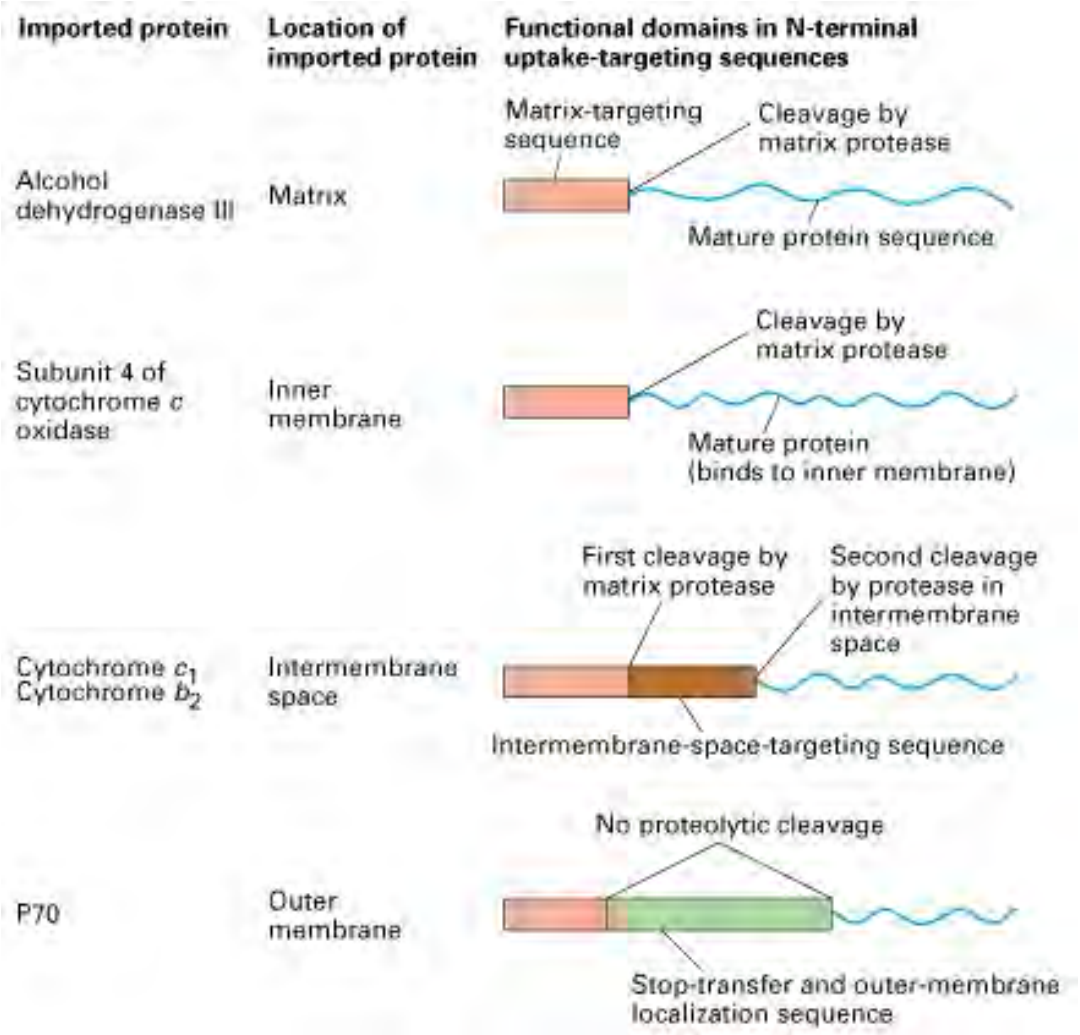
**Indicates whether signal sequence usually is (+) or is not (−) removed after a protein enters its target organelle.*
†These signals direct the protein from the cytosol into the matrix space of the mitochondrion or the corresponding stroma of the chloroplast; other signals discussed in the text redirect proteins into other subcompartments of these organelles.

Uptake-targeting sequences of imported mitochondrial proteins

4 possible mitochondrial localizations

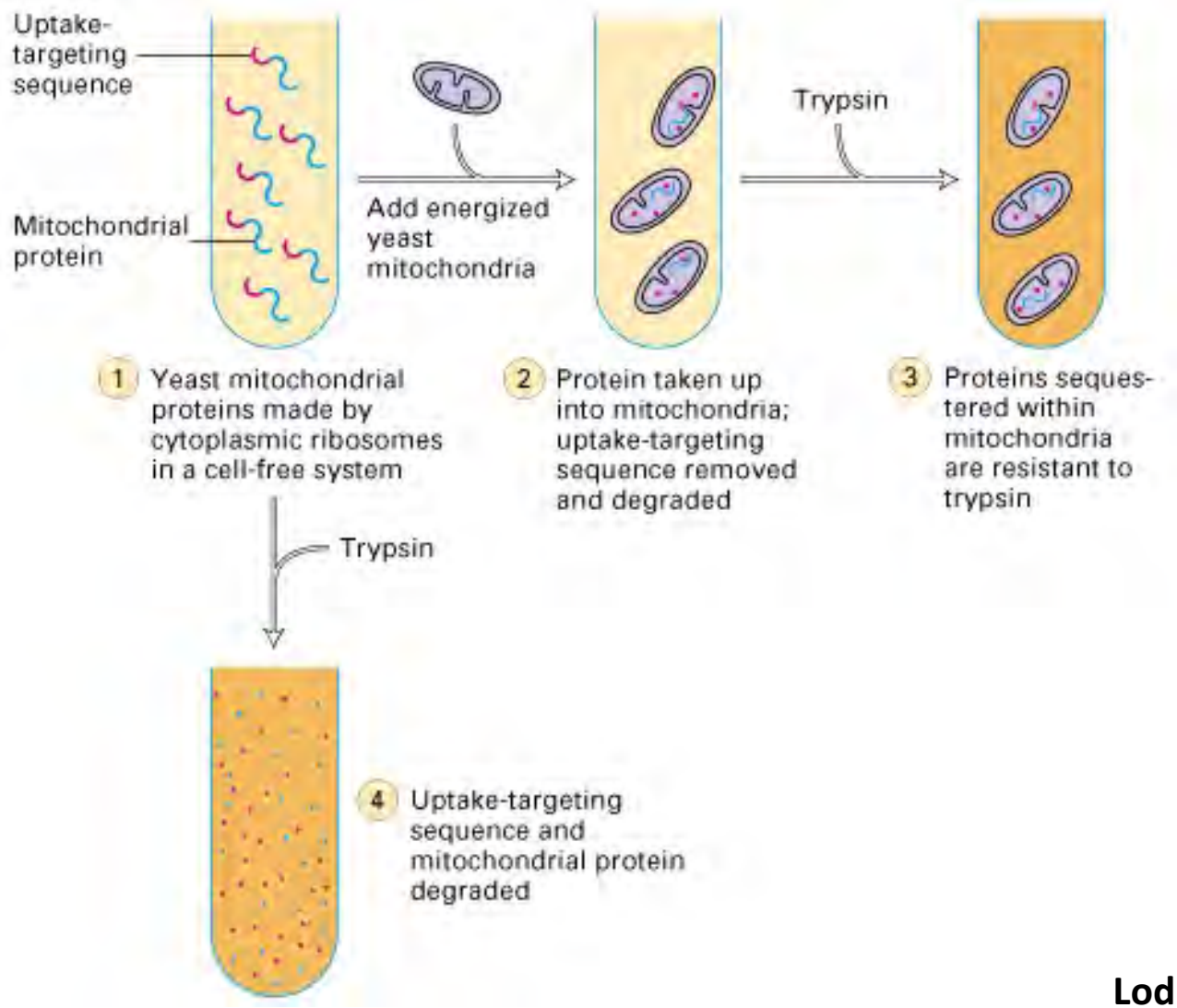
Two types of mitochondrial targeting sequences:

- Classical N-terminal MTS that are cleaved upon import. ~60% of mitochondrial proteins use this route.
- Internal targeting sequences that remain in the mature protein



Lodish et al, 2000

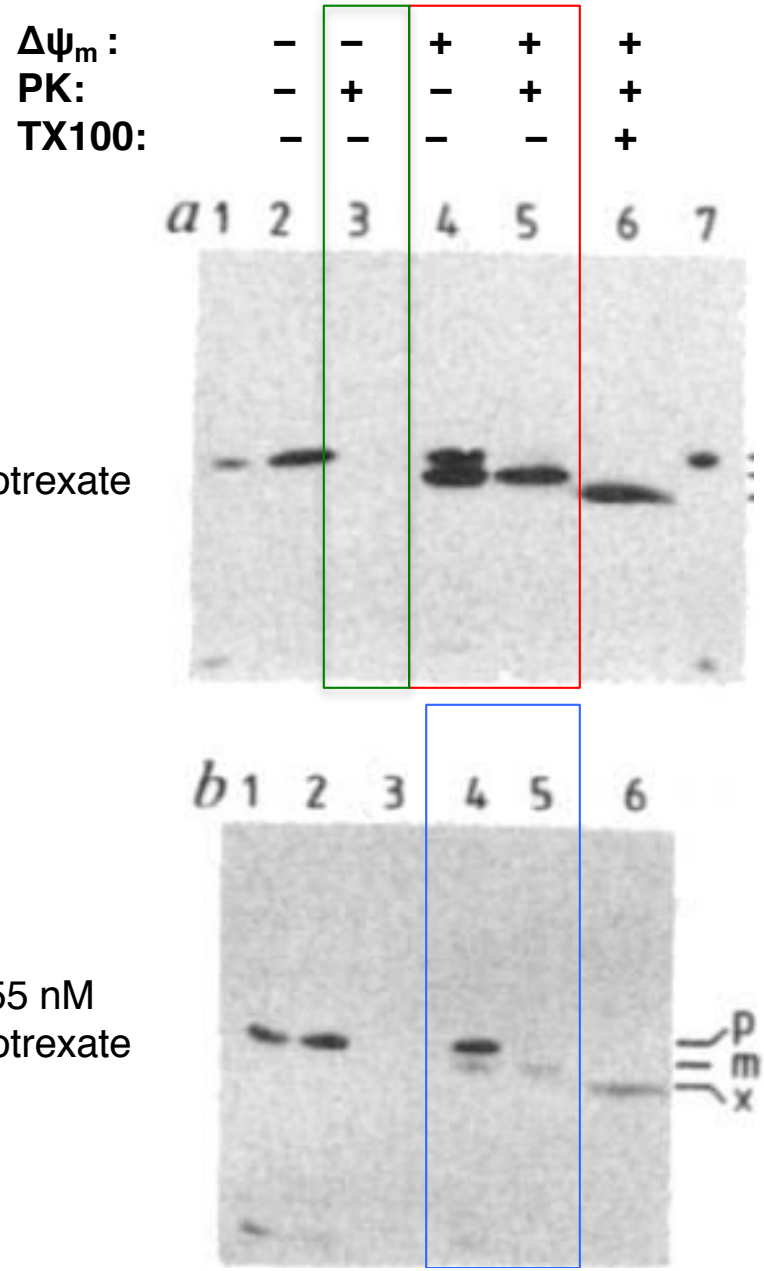
A cell-free system for studying post-translational uptake of mitochondrial proteins



Lodish et al, 2000

In vitro analysis of mitochondrial import

Lanes 1 and 7: control loading of precursor



- Substrate= mouse DHFR with yeast cytochrome oxidase subunit IV presequence; recombinant protein purified from E. coli and used for in vitro import studies with yeast mitochondria.
- Import results in protease resistant mature form. The MTS has been cleaved from the precursor.
- Import depends on membrane potential.
- Methotrexate binds to DHFR and stabilizes the folded conformation.
- Methotrexate inhibits import.
- Conclusion: import process required unfolding of precursor protein
- Is Methotrexate a general inhibitor of import?
- Perhaps Methotrexate can pass through membranes?

Eilers & Schatz (1986) Nature

Targeting of integral membrane proteins

• Hydrophobic transmembrane (TM) domains are synthesized in cytosol and must be transported to more energetically favorable lipid bilayer. Several problems to solve:

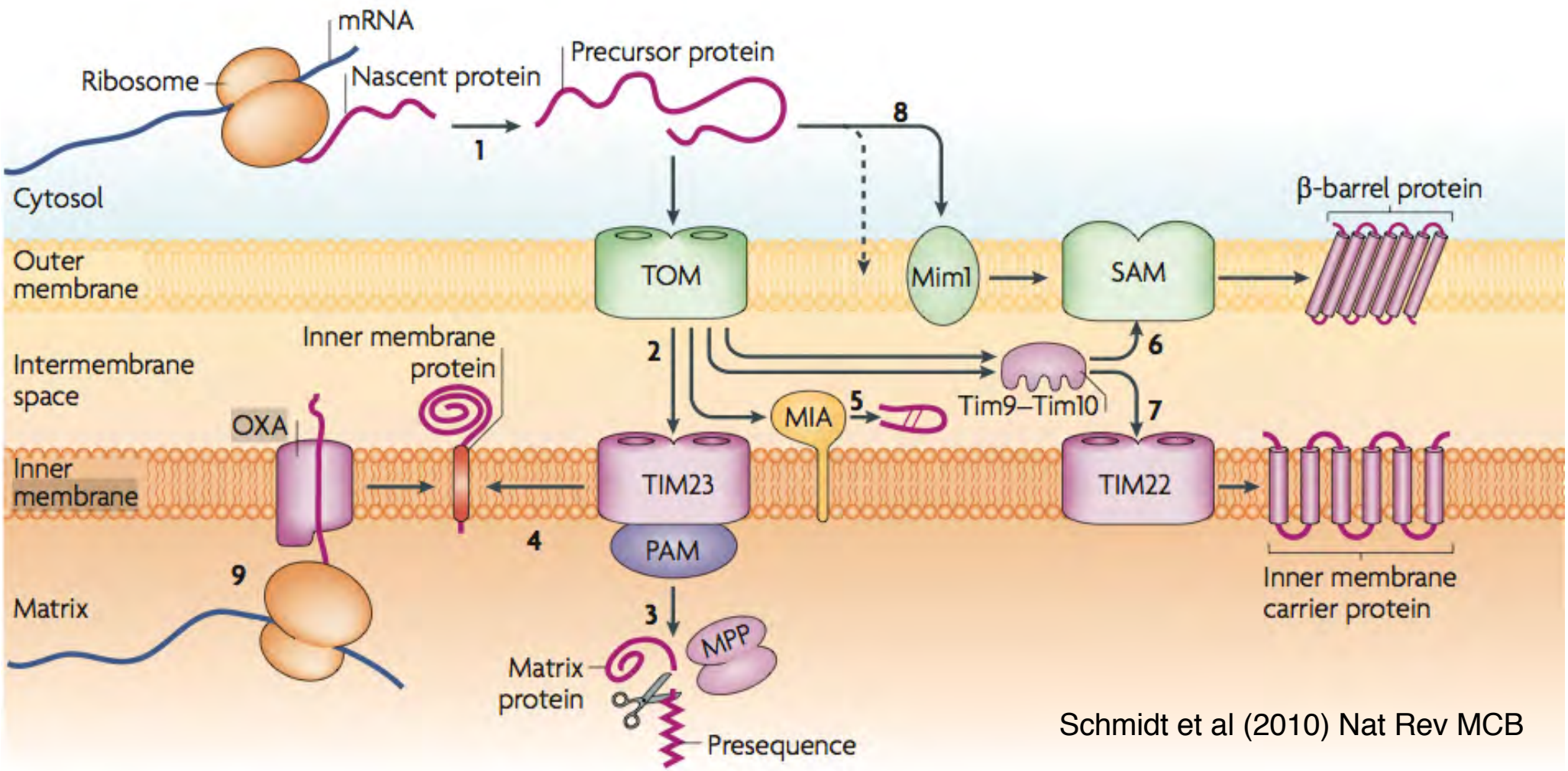
• Recognize TM domains

• Shielding/stabilizing TM domains in the cytosol: for the most part, mitochondrial proteins are translated by cytosolic ribosomes and imported post-translationally--chaperones (e.g., Hsp70, Hsp90) stabilize precursors in unfolded conformation in cytosol

• Targeting to the correct membrane: targeting sequences recognized by import receptors attached to import pores.

• Integration of the TM domain into the membrane in the correct topology

Overview of mitochondria import pathways



Schmidt et al (2010) Nat Rev MCB

Five pathways:

A, B: Presequence containing proteins (matrix and IM proteins): TOM + TIM23

C: Carrier pathway for IM proteins: TOM + TIM22

D: Oxidative folding pathway for IMS proteins with Cys motifs: TOM + MIA (mitochondrial IMS assembly machine)

E: OM β-barrel pathway: TOM + SAM (sorting and assembly machine)

Import of pre-proteins containing a cleavable presequence into the matrix and IM

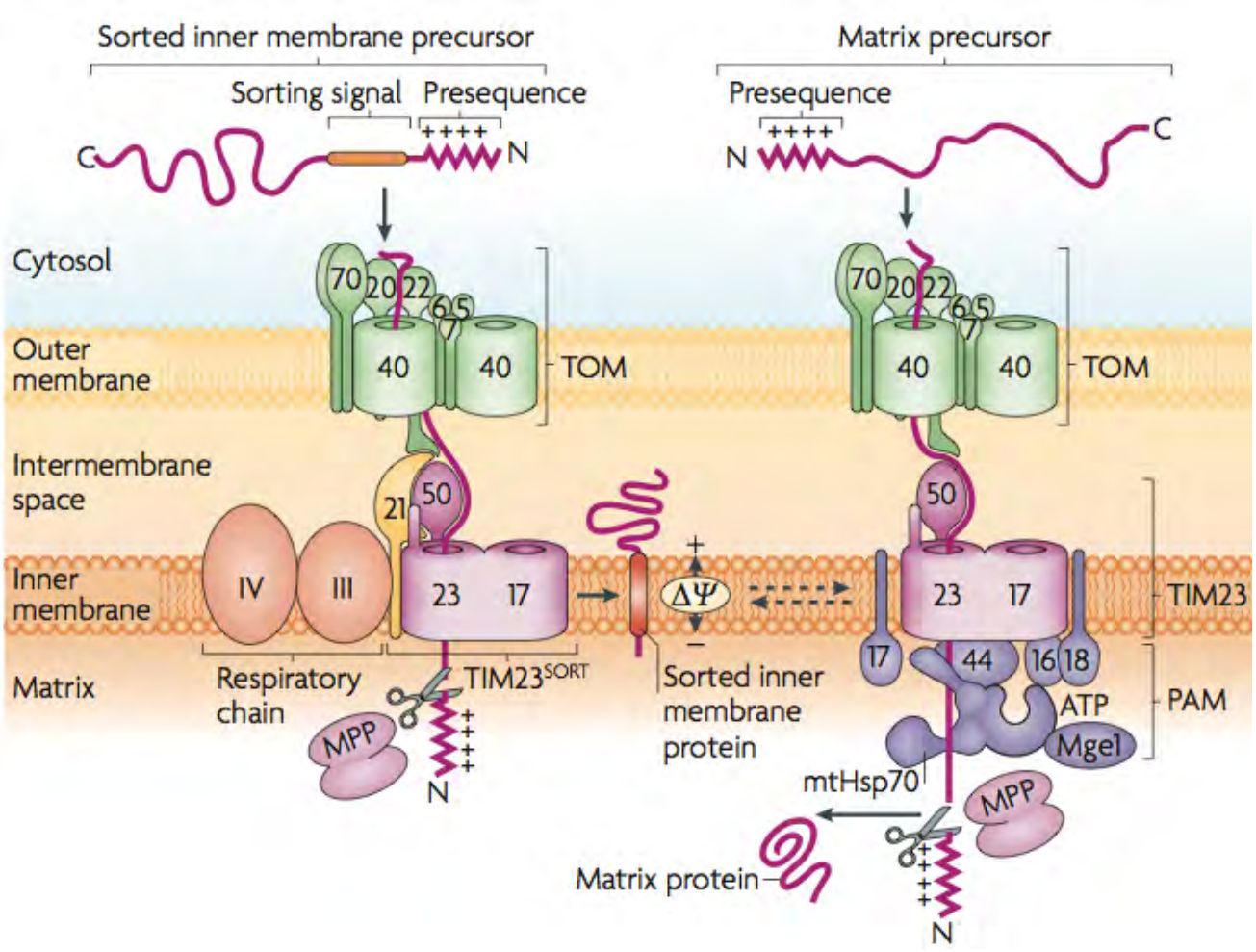
Matrix targeting signal:
-No clear consensus sequence
-N-terminal residues, 10-80 AAs.
-forms amphipathic helix with one side hydrophobic and one side positive.
-can adopt extended conformation
-cleaved by mitochondrial processing peptidase (MPP) in matrix

Matrix proteins: Cleavable precursor imported via TOM and then TIM23, with the help of the PAM (presequence translocase associated motor).

IM proteins: Imported via TOM and then exit laterally from TIM23. They contain hydrophobic segment.

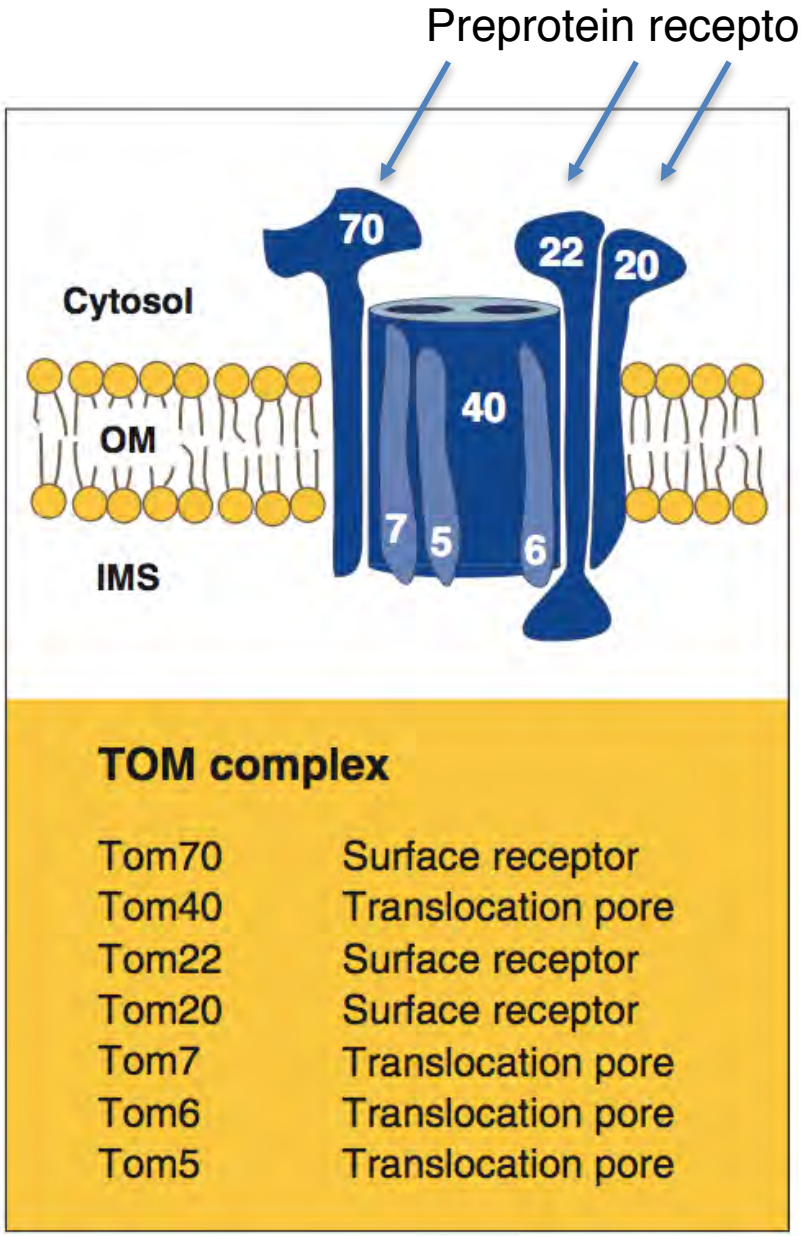
Note that 2 types of TIM23 complexes are found.

This pathway accounts for ~60% of mitochondrial proteins.



Schmidt et al (2010) Nat Rev MCB

TOM: translocator of the outer membrane



Neupert (2007) Ann Rev Biochem

General import pore of TOM formed by **TOM40**, and 3 smaller subunits (Tom5, Tom6,Tom7); Tom40 is a β-barrel protein that forms pores in the membrane.

Tom20: main receptor for N-terminal MTS; binding hydrophobic face of the MTS

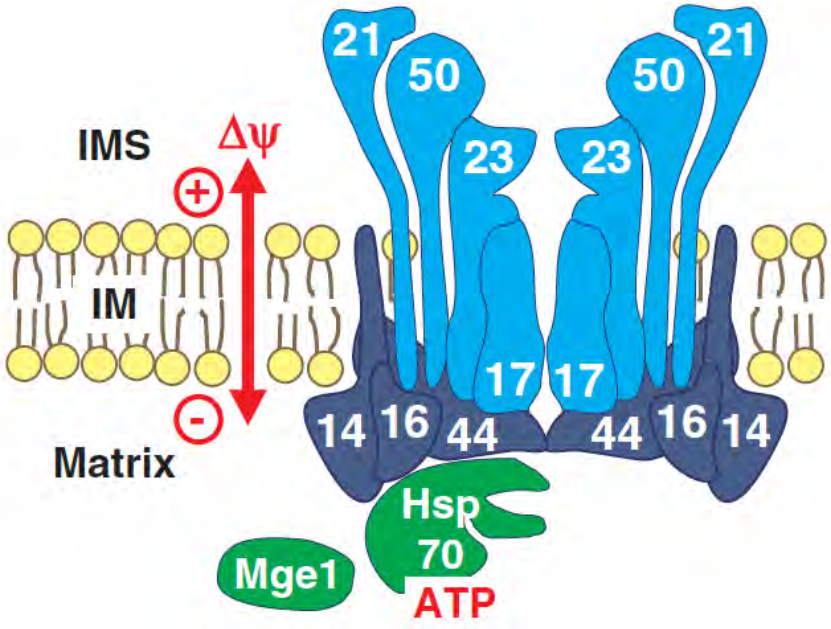
Tom70: receptor for proteins with internal targeting signal and also hydrophobic proteins.

Tom20 and Tom70 also bind to the chaperones, facilitating their release of substrate

Different substrates show different sensitivities to loss of Tom20 or Tom70.

TOM complex required for import of all internal mitochondrial proteins. TOM complex required for some, but not all, OM proteins.

TIM23: translocator of the inner membrane



TIM23 complex

Tim50	Membrane sector
Tim44	Import motor
Tim23	Membrane sector
Tim21	Membrane sector
Tim17	Membrane sector
Tim16/Pam16	Import motor
Tim14/Pam18	Import motor
mtHsp70	Import motor
Mge1	Import motor

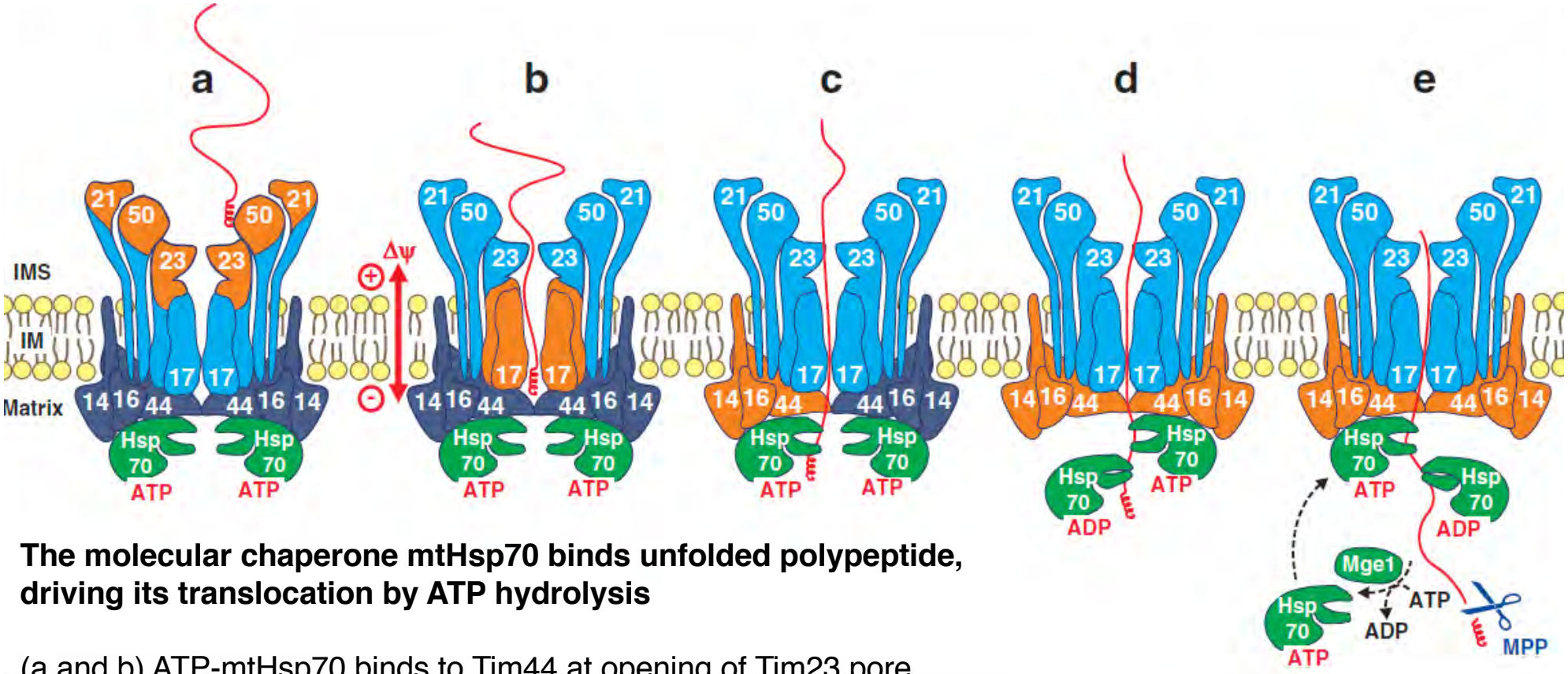
TIM23 translocase:
-major translocase of IM
-Transports all matrix proteins, most IM proteins, and some IMS proteins.
-utilizes membrane potential + ATP hydrolysis as energy input.
-may be associated with TOM complex.

Membrane sector: membrane pore composed of Tim23/Tim17/Tim50/Tim21.

Import motor of TIM23 complex:
-Without this motor, the membrane sector can only import the MTS (driven by membrane potential).
-Tim44/Tim14/Tim16/mtHsp60/Mge1.
-Tim44 recruits ATP-bound mtHsp60 to the site of the incoming polypeptide.
-mtHsp60 binds translocating unfolded polypeptide.
-ATP hydrolysis by mtHsp60 results in release from Tim44.

Neupert (2007) Ann Rev Biochem

The presequence translocase-associated motor (PAM) drives translocation into the matrix



The molecular chaperone mtHsp70 binds unfolded polypeptide, driving its translocation by ATP hydrolysis

(a and b) ATP-mtHsp70 binds to Tim44 at opening of Tim23 pore.
(c) Emerging polypeptides binds ATP-mtHsp70.
(d) ATP hydrolysis by mtHsp70 (stimulated by peptide binding? or Tim14?) results in release from Tim44.
(e) Bound mtHsp70 prevents backward sliding of polypeptide.
New ATP-mtHsp70 can bind to Tim44 and polypeptide.
ADP-ATP nucleotide exchange facilitated by Mge1.
ATP hydrolysis powers directional import.

Neupert (2007) Ann Rev Biochem

Brownian rachet model vs. power-stroke/pulling/lever arm

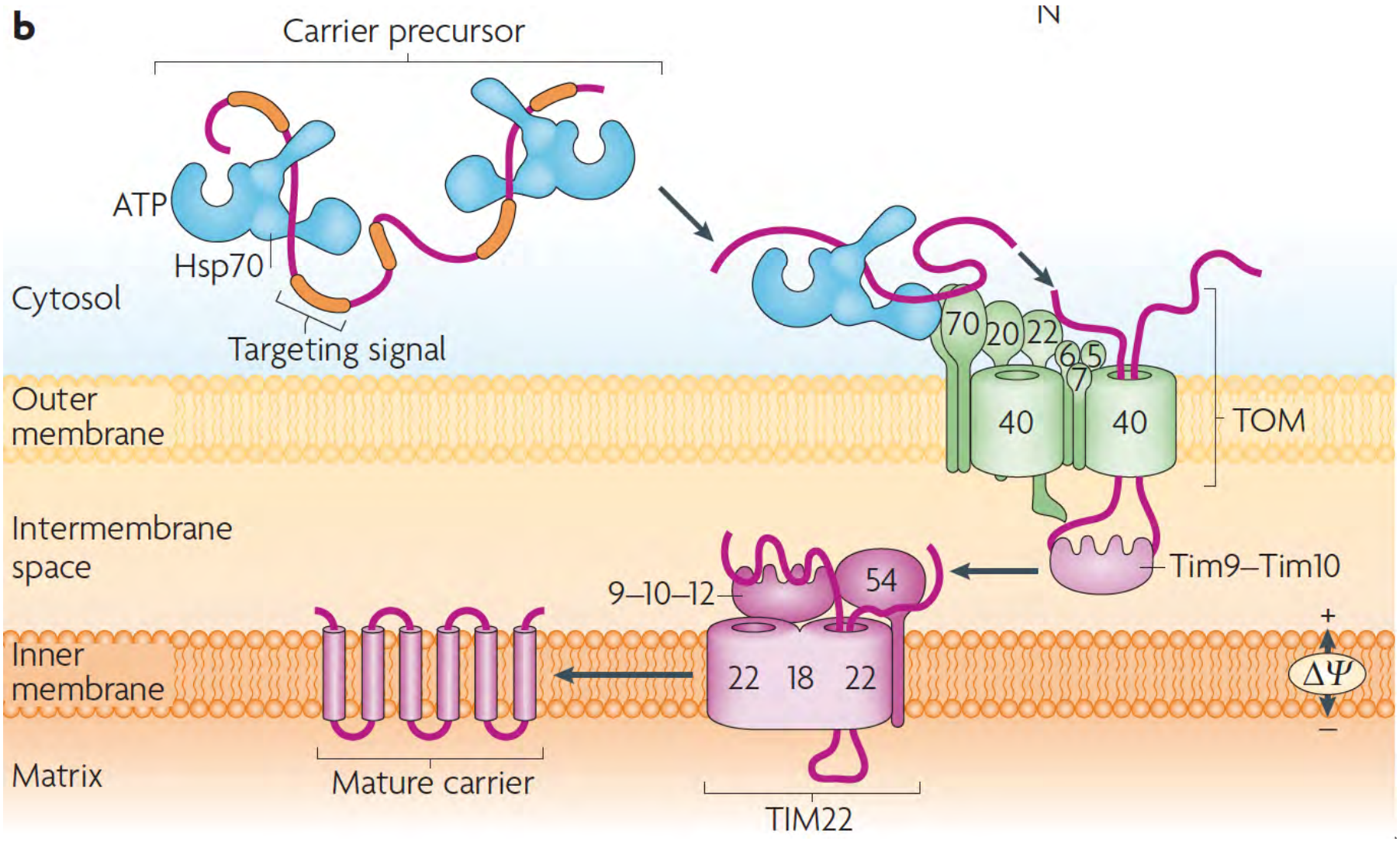
Brownian rachet:

- precursor undergoes positional fluctuations in the pore
- binding of mtHsp70 prevents retrograde movement, making the overall movement directional
- ATP hydrolysis needed to coordinate precursor binding and release from Tim44

Power stroke:

- Upon ATP hydrolysis, mtHsp70 undergoes conformational change, pulling on the precursor

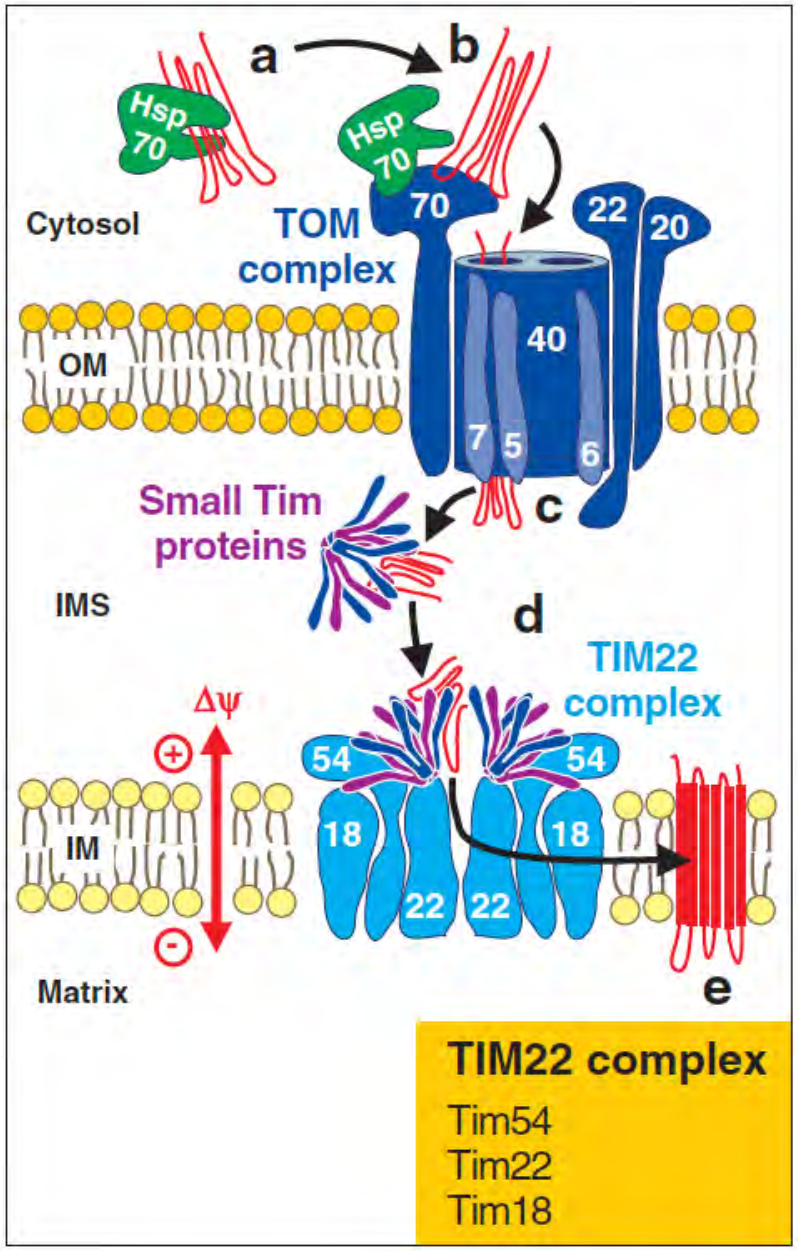
TOM and TIM22 are used in the carrier pathway



- Imported proteins have internal targeting sequences and multiple TM segments (e.g., ADP/ATP carrier).

Schmidt et al (2010) Nat Rev MCB

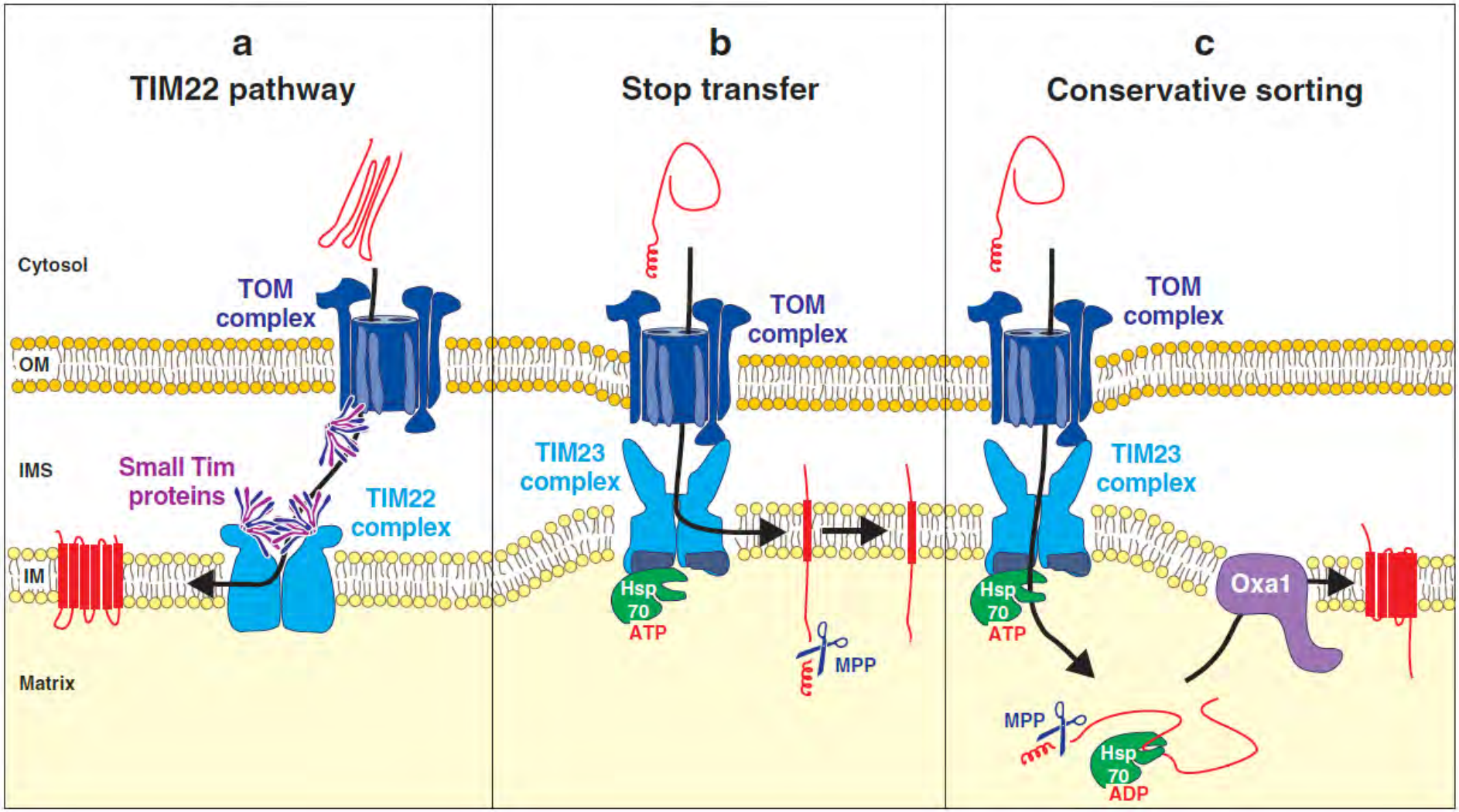
Small TIMs in the carrier pathway



- For some IM proteins with multiple TM segments, the "carrier pathway" involving TIM22 is used.
- Many of these proteins are metabolic carriers like the ADP-ATP carrier and are non-cleavable.
- Multiple α-helical transmembrane segments.
- The small Tim proteins (hexameric complex of Tim9/10 or Tim 8/13) are chaperones that transfer the precursor from TOM to TIM22.

Neupert (2007) Ann Rev Biochem

Three pathways to the inner membrane



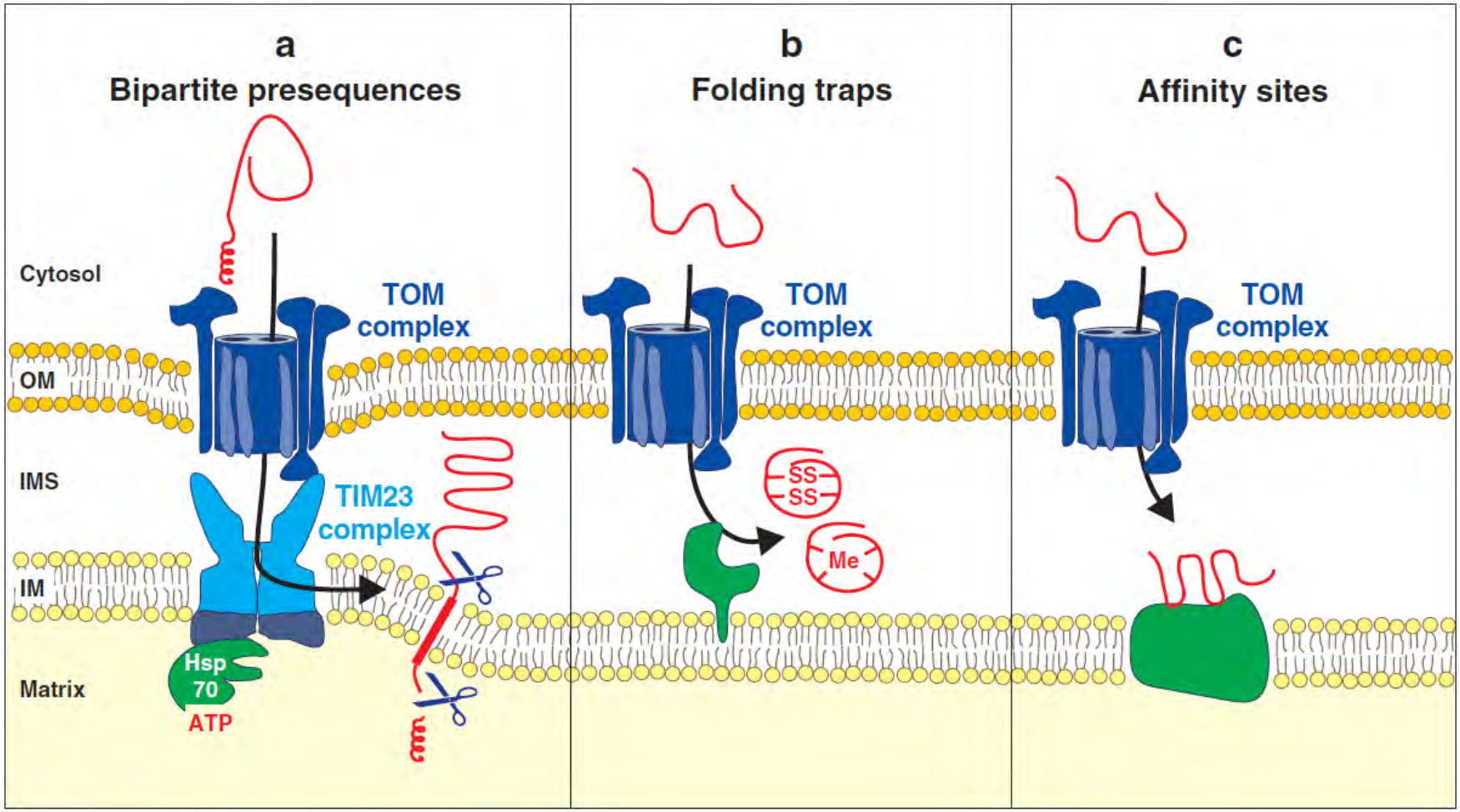
Tim22 is used by solute carriers of the IM and some membrane-bound TIM subunits. Small TIMs function to escort substrate from TOM to TIM22.

Sequences in and around the TM regions likely provide the signal for this pathway. Typical signal TM and N_{in}-C_{out} topology.

Resembles the export process of procaryotes; probably most ancient pathway.

Neupert (2007) Ann Rev Biochem

Pathways to the IMS



Dependent on membrane potential.
Lateral exit from TIM23 and proteolytic cleavage.

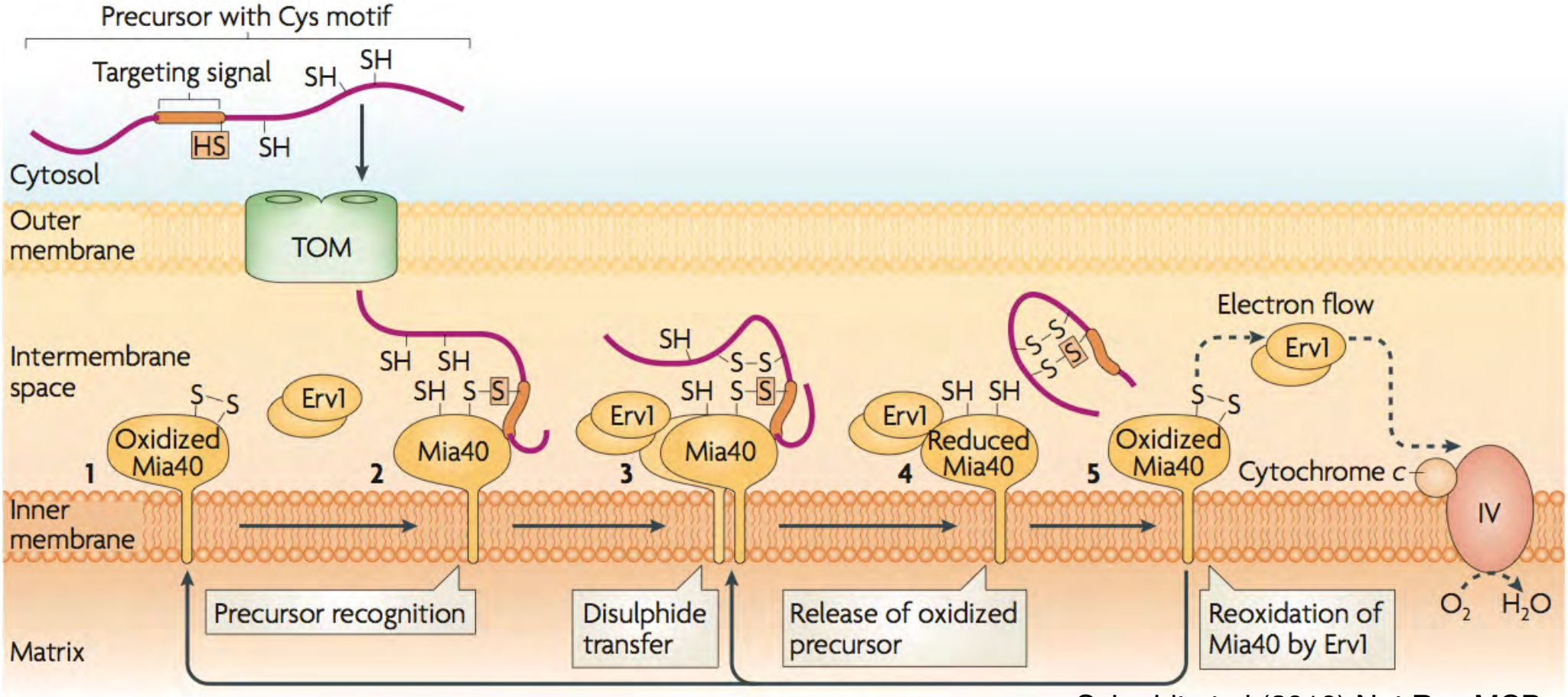
Bipartite presequence: (1) matrix MTS (2) hydrophobic stop-transfer segment
Cleavage by IMP1/2; Ex: Cyt*b2*, Smac

Folding leads to trapping of protein; therefore import is directional.
Ex: 1) cytochrome *c* has a heme cofactor (that coordinates iron)
2) disulfide bond formation catalyzed by Mia40 and Erv1.

Import is driven by binding to components in the IMS.

Neupert (2007) Ann Rev Biochem

Oxidative folding of IMS proteins



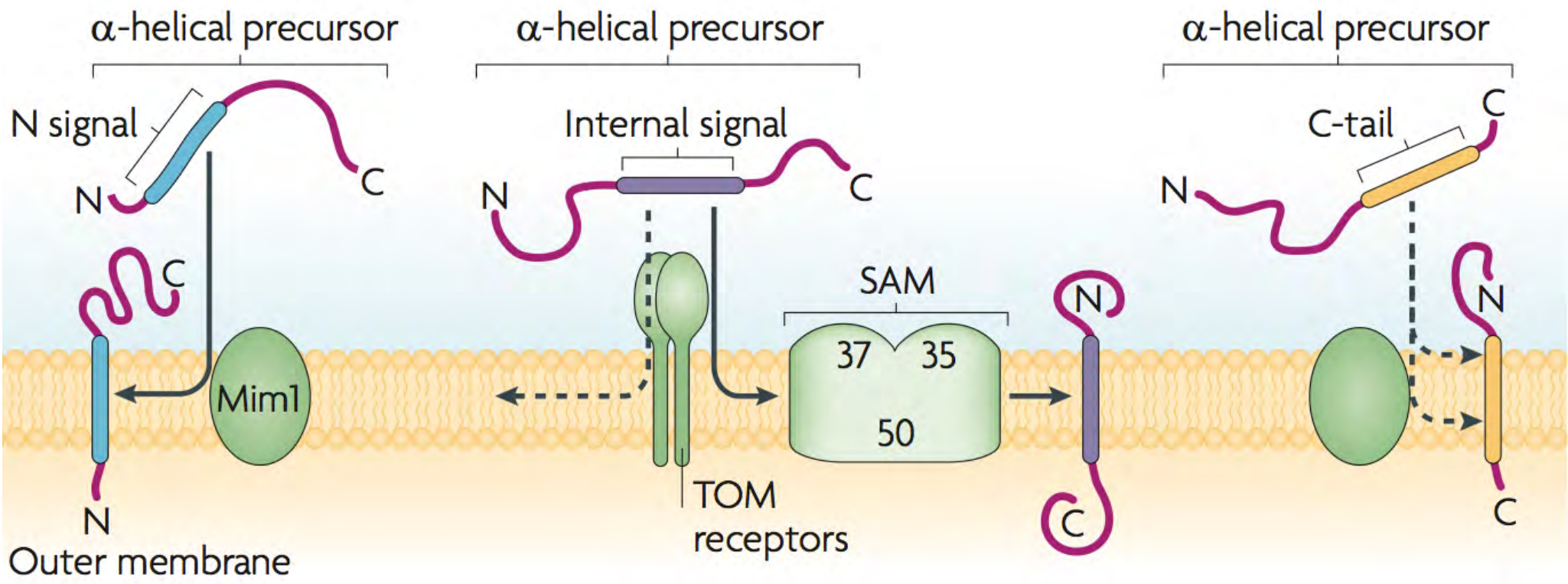
Schmidt et al (2010) Nat Rev MCB

Most IMS contain Cys and are translocated through TOM in an unfolded and reduced state. Mia40 is a protein disulfide carrier that facilitates disulfide bond formation of the imported protein.

Mia40 and Erv1 constitute a disulfide relay system.

Electrons go from imported protein to Mia40 to Erv1 to cytochrome *c* to the respiratory chain.

Import of OM proteins with α-helical TM segments



Some proteins use Mim1 for membrane insertion.

Import of many OM proteins requires the TOM complex, though not its pore.

Some go from TOM to SAM.

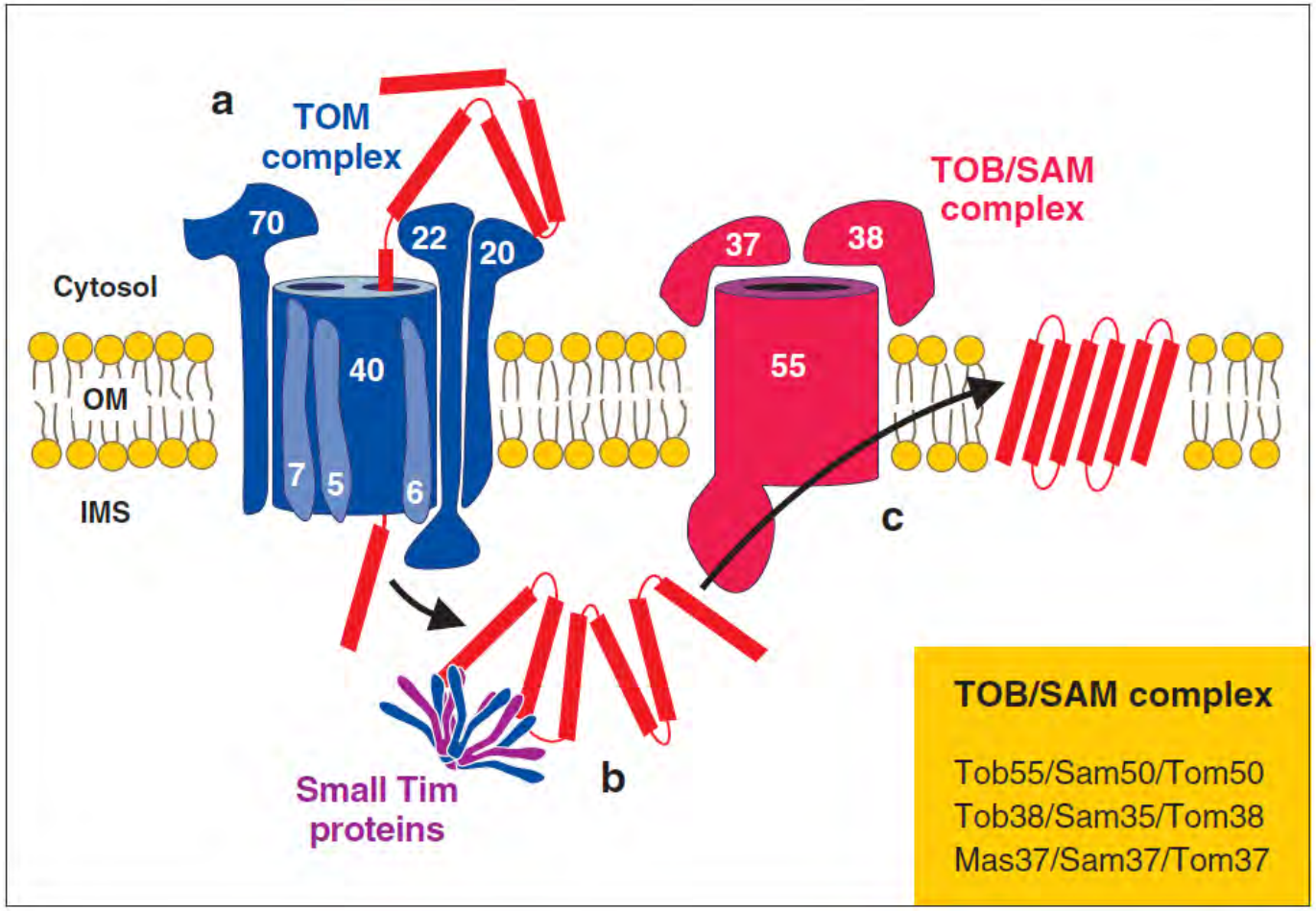
Some tail-anchors proteins seem to be TOM-independent.

Schmidt et al (2010) Nat Rev MCB

β-barrel proteins in the outer membrane

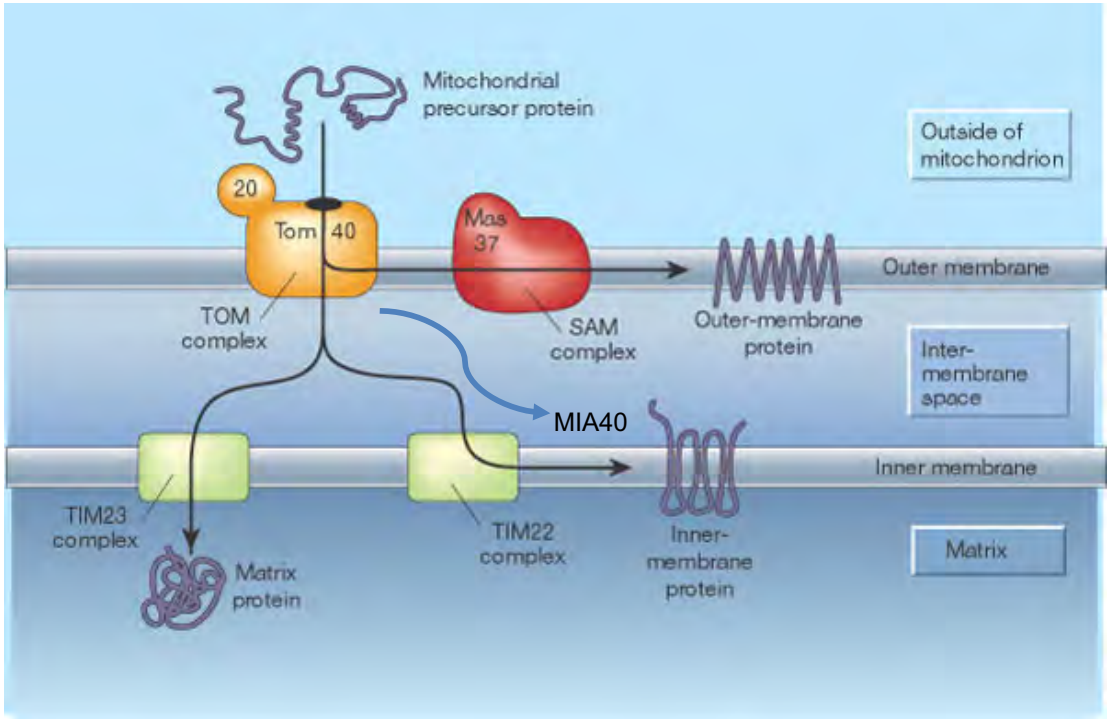
β-barrel proteins (transmembrane barrel of β-strands) are found only in mitochondrial OM, chloroplast OM, and bacterial OM. VDAC and porin are prototypical.

- β-barrel proteins depend on both TOM and SAM/TOB complexes for sorting to the OM.
- Small Tims chaperone the transfer.
- SAM=sorting and assembly machinery
- TOB=topogenesis of mitochondrial OM β-barrel



SAM50/TOB55 is the core of the SAM complex; TOB55 is homologous to bacterial BamA/OMP85, involved in insertion of bacterial β-barrel outer membrane proteins, which first go through periplasmic membrane through Sec Y (keeps same topology).
Bacterial PhoE expressed in yeast is imported into mito.

Distinct import complexes for mitochondrial proteins



TOM Complex

-Tom40 is the protein channel traversed by almost all mitochondrial proteins

-Tom20,22,70 are receptors with different but overlapping specificity

TIM Complexes

- TIM23 translocation of mediates matrix targeted proteins; some substrates exit laterally to go the IM; others are retrieved from the matrix to the IM

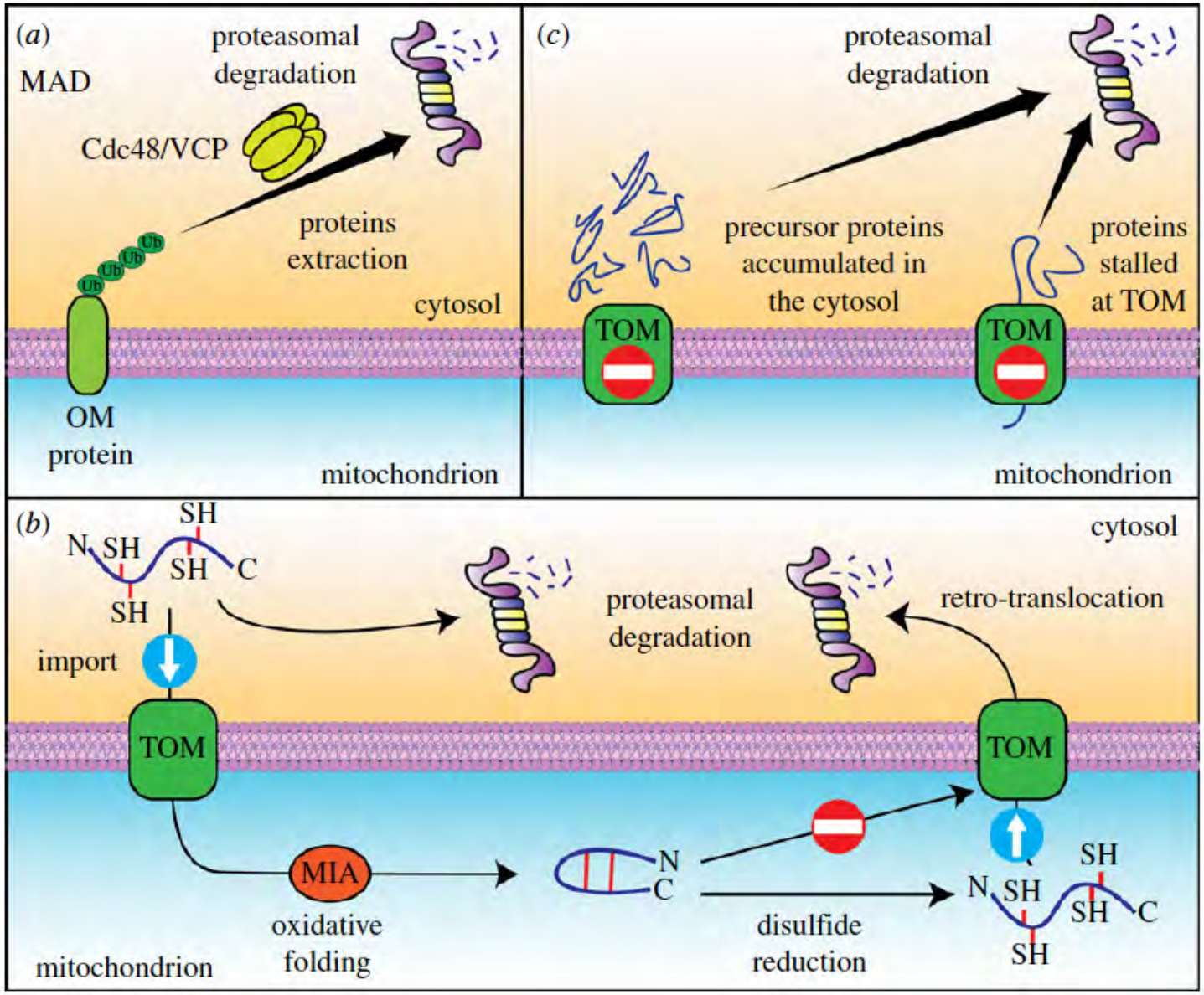
- TIM22 translocates proteins into IM

SAM Complex

- Contains Tob55/Mas37/Tob38 likely sorts/assembles polytopic OM proteins

Nature, vol 424:505

The UPS and protein quality control in mitochondria



UPS is involved in degrading:

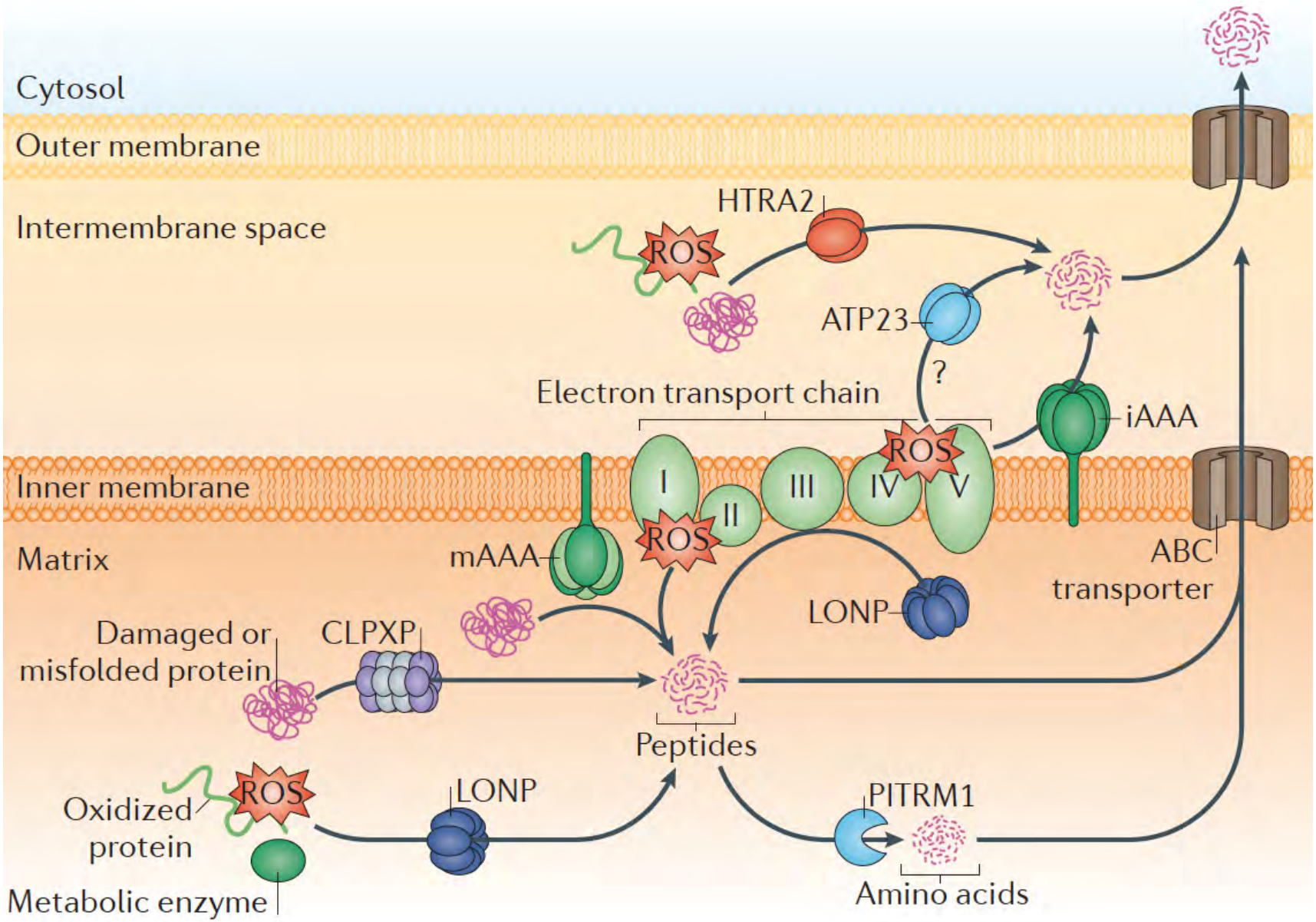
a. Unstable or misfolded proteins in the OM

b. Misfolded proteins in the IMS

c. Import failures/stalling on the OM

Bragoszewski et al (2017) Open Biology

Mitochondrial proteases in protein quality control



Quiros et al (2015) NRMCB