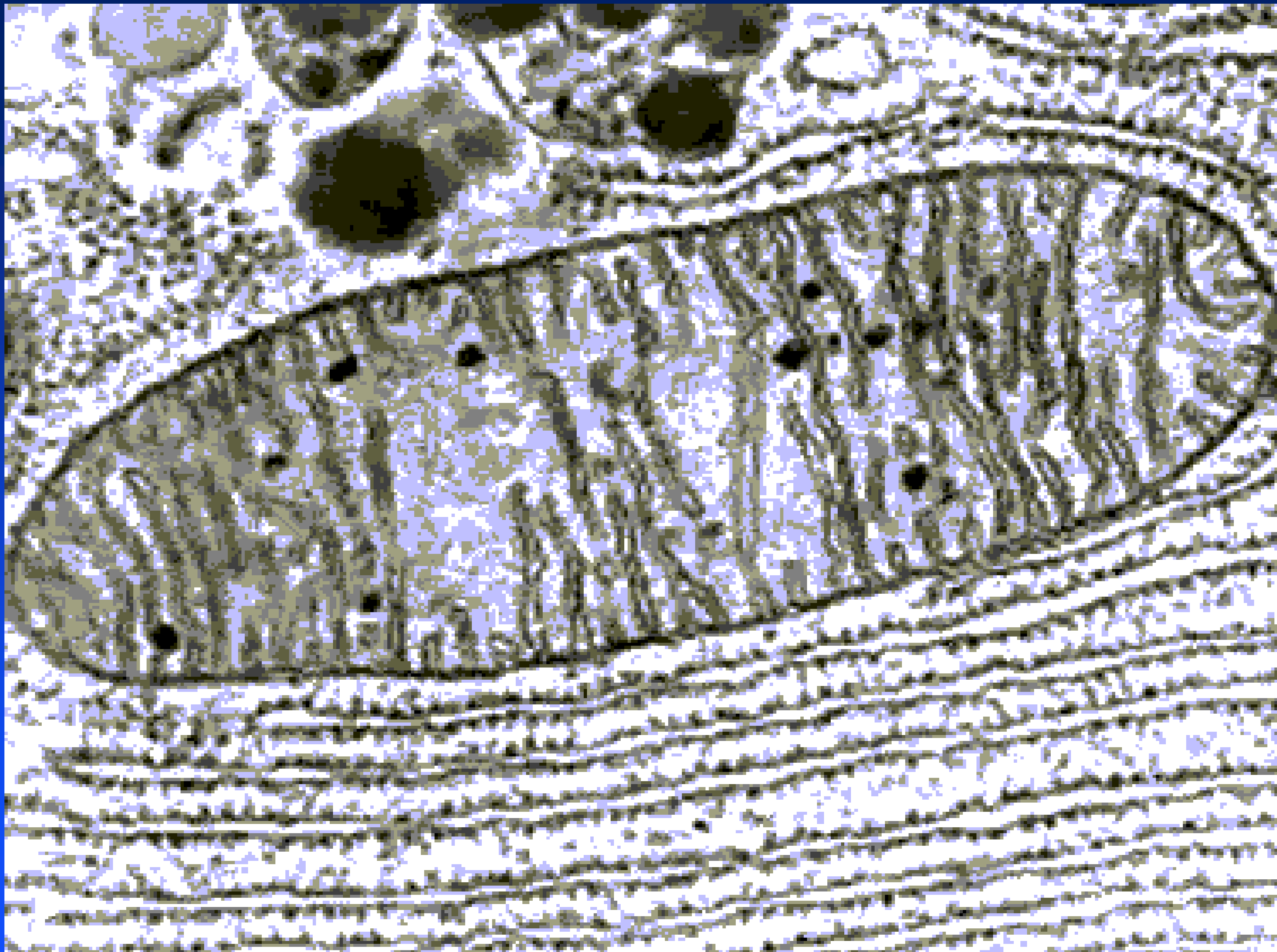
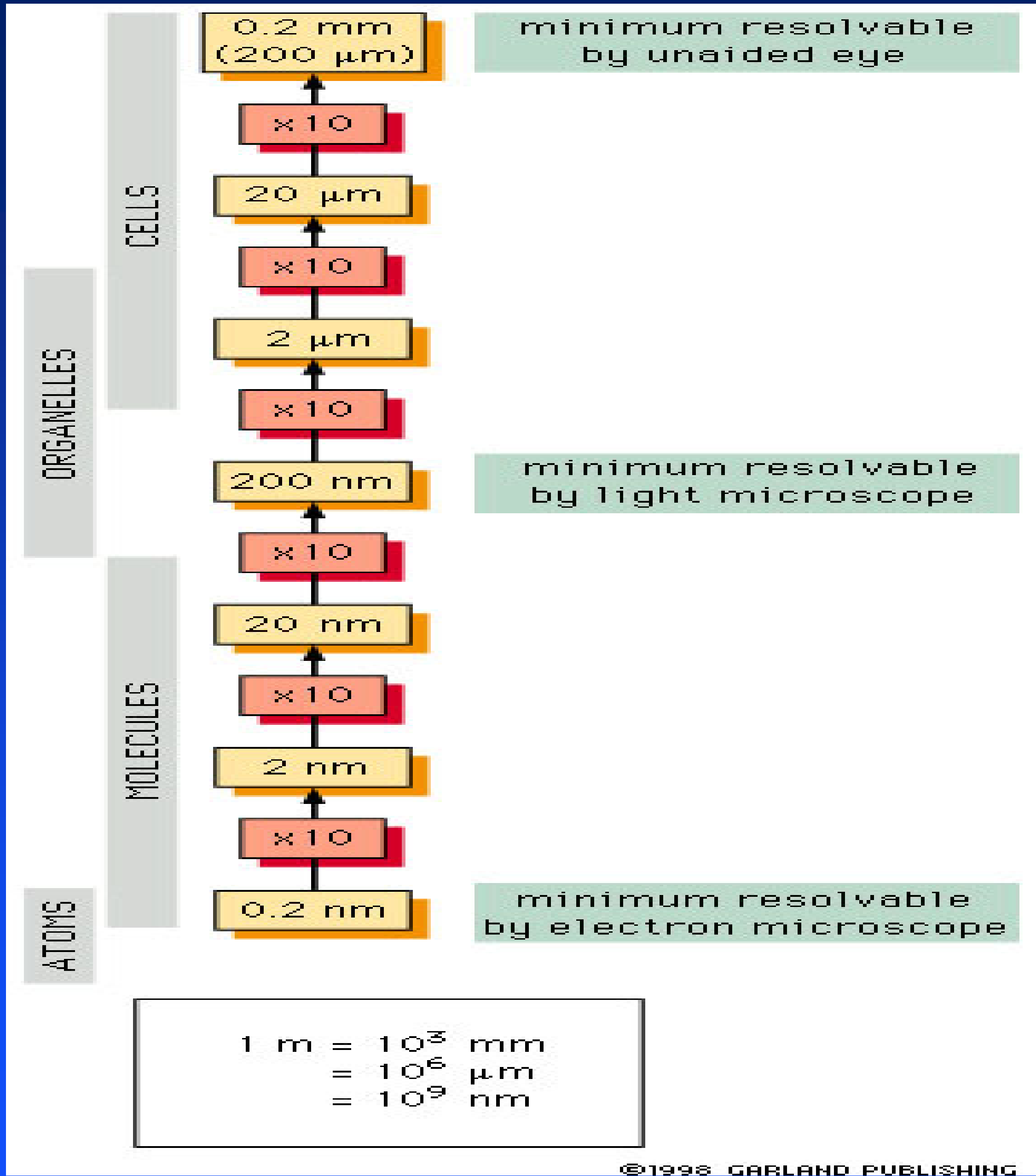
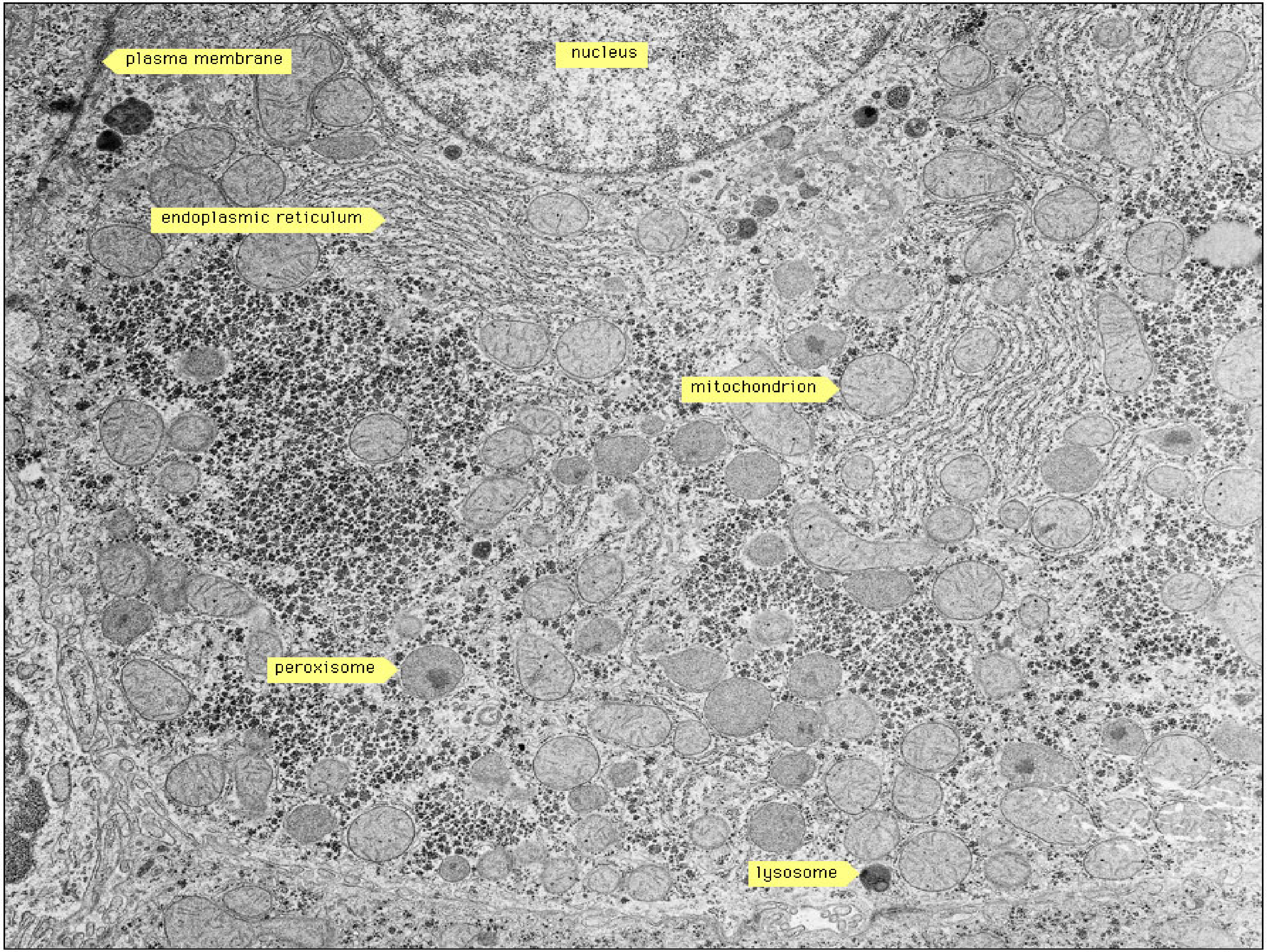


Scale in the biological world



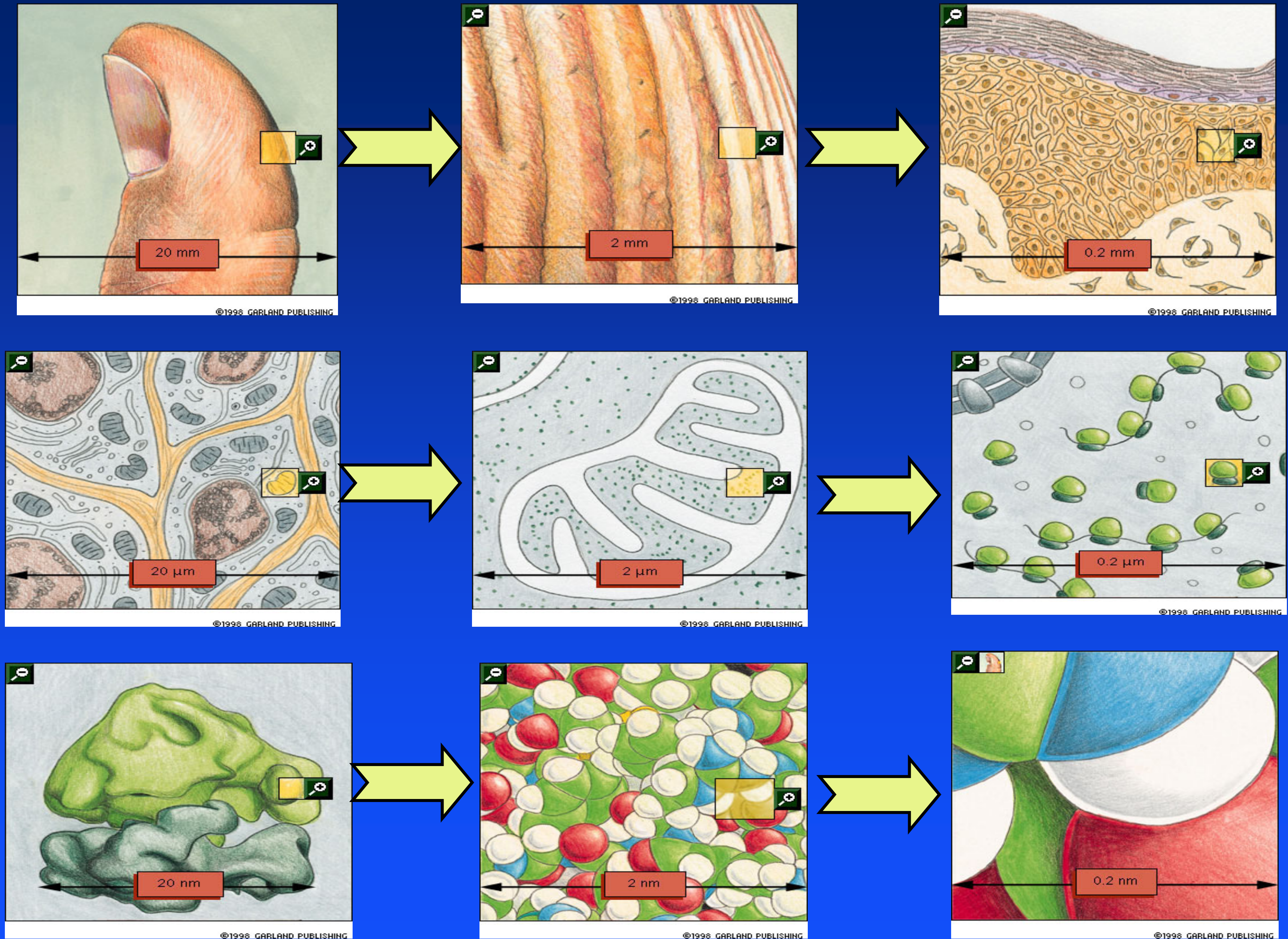
A cell seen by TEM



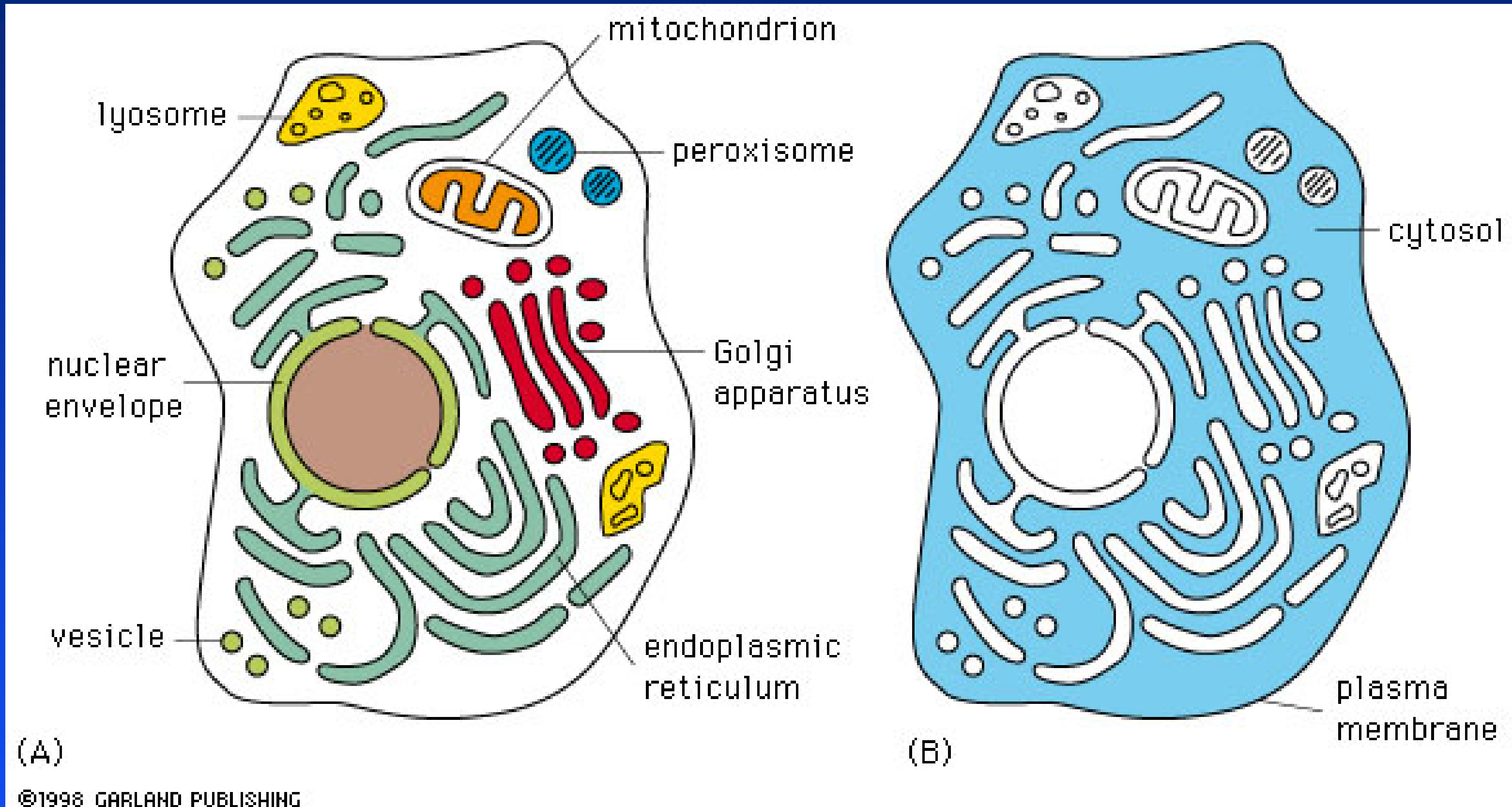
(A)

2 µm

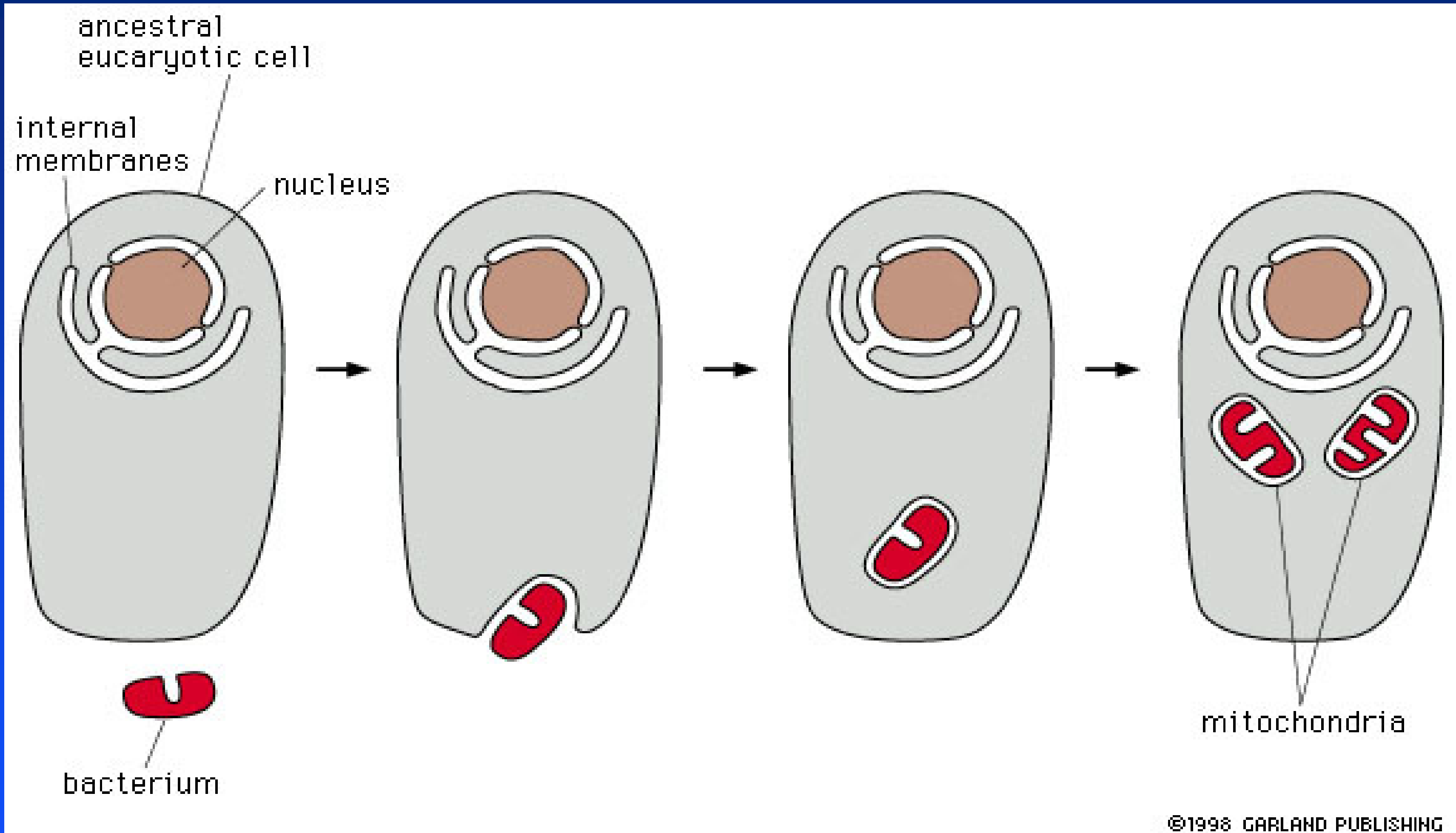
From living cells to atoms



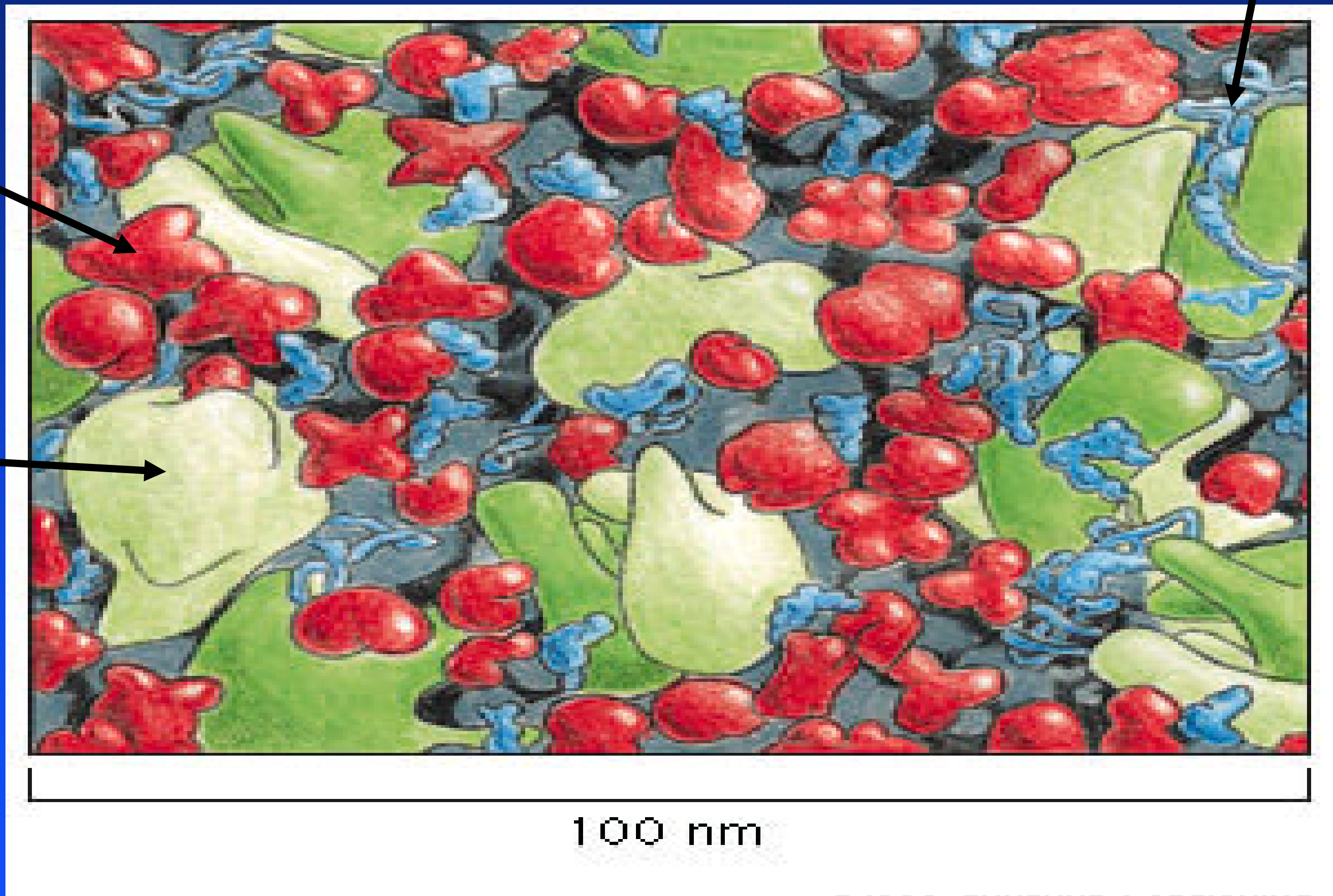
Compartmentalisation in the cell: internal membranes and the cytosol



The Origin of mitochondria: The endosymbion hypothesis



The cytosol:
more than just H2O



Living cells obey the laws of thermodynamics

Living cells are NOT isolated systems:

Cells take energy from the environment (chemicals ie foodstuffs or photons ie sunlight) to generate order ie assemblies

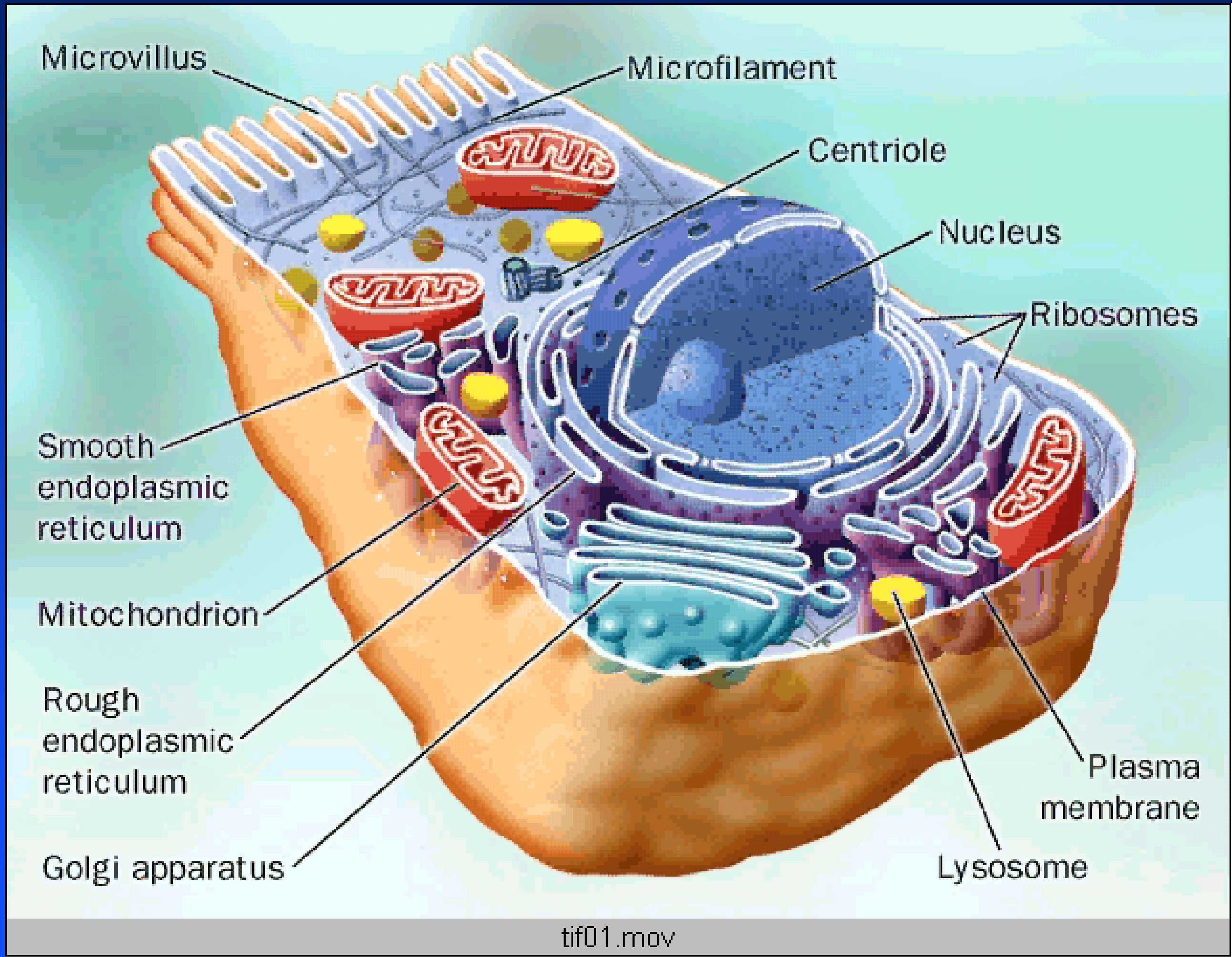
In doing so, they dischagre HEAT to the environment

This increases the total ENTROPY

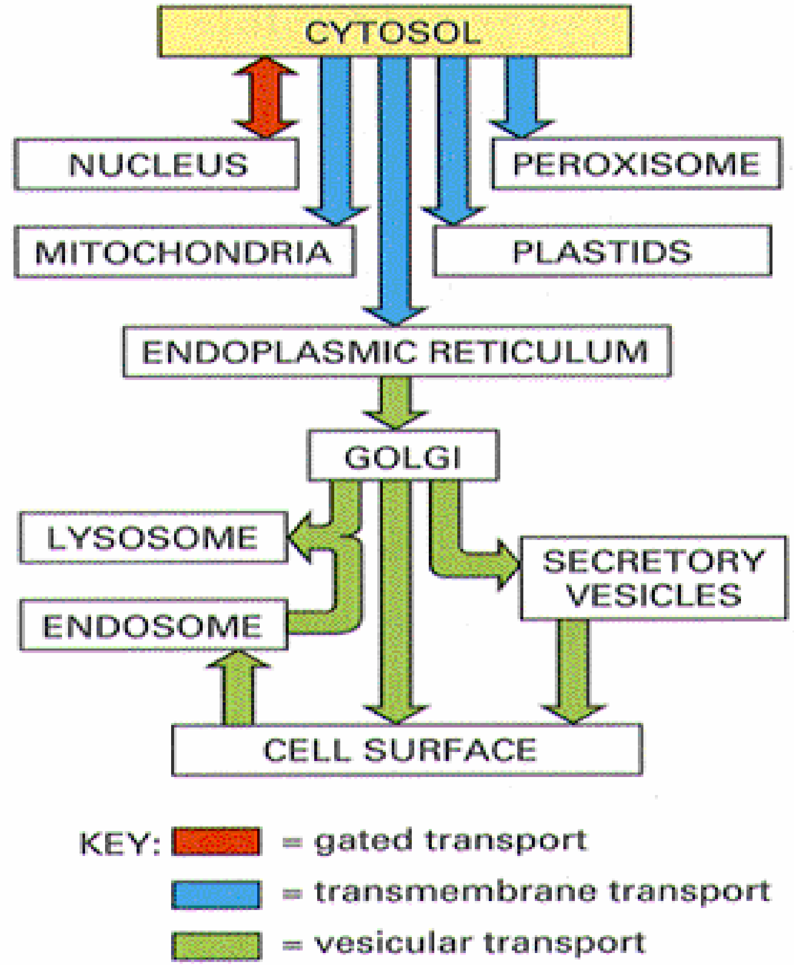
Energy conversion is vital for the cell

Mitochondria biogenesis and function

The Eukaryotic Cell



Different ways to target proteins in the cell

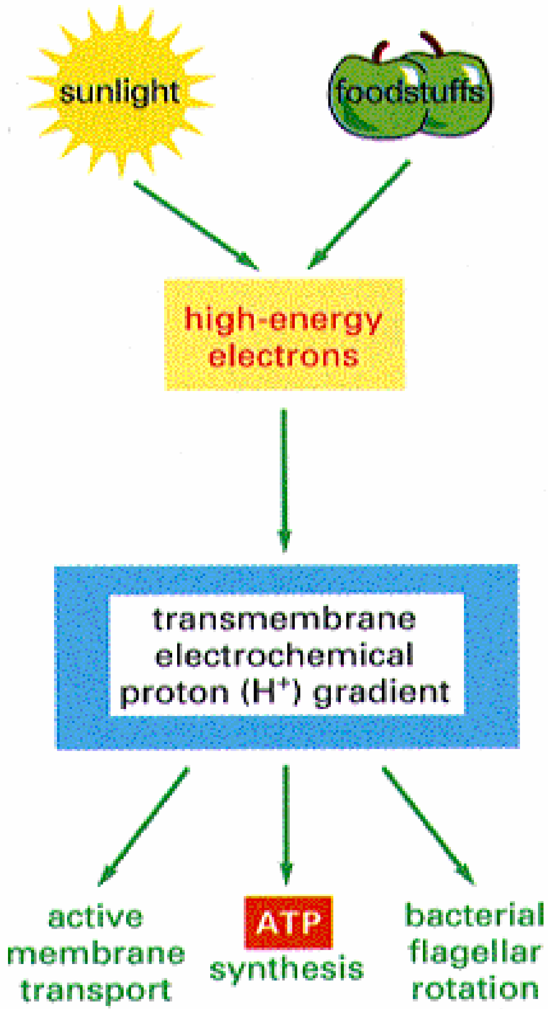


Mitochondria are <u>essential</u> for life

Energy Conversion: Mitochondria and Chloroplasts

- Membrane-bounded
- Occupy a major fraction of cell volume
- Large amount of <u>internal</u> membrane
- Common pathway for energy conversion: Chemiosmotic coupling

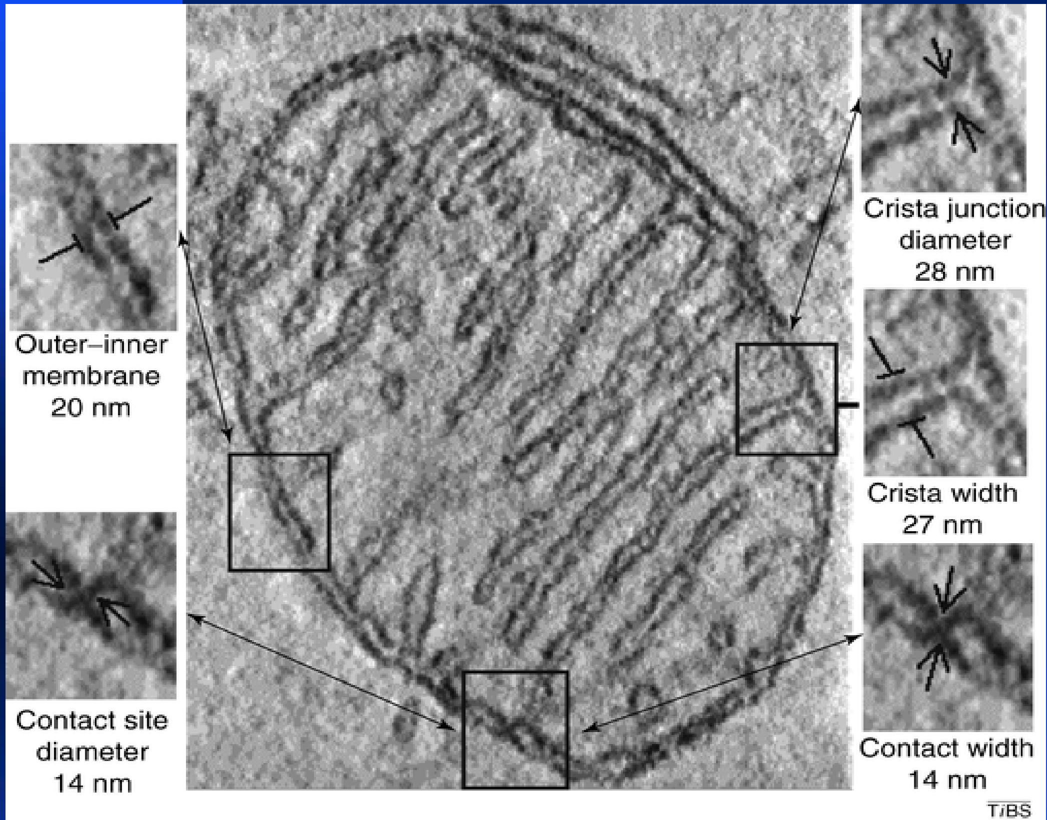
Chemiosmotic coupling



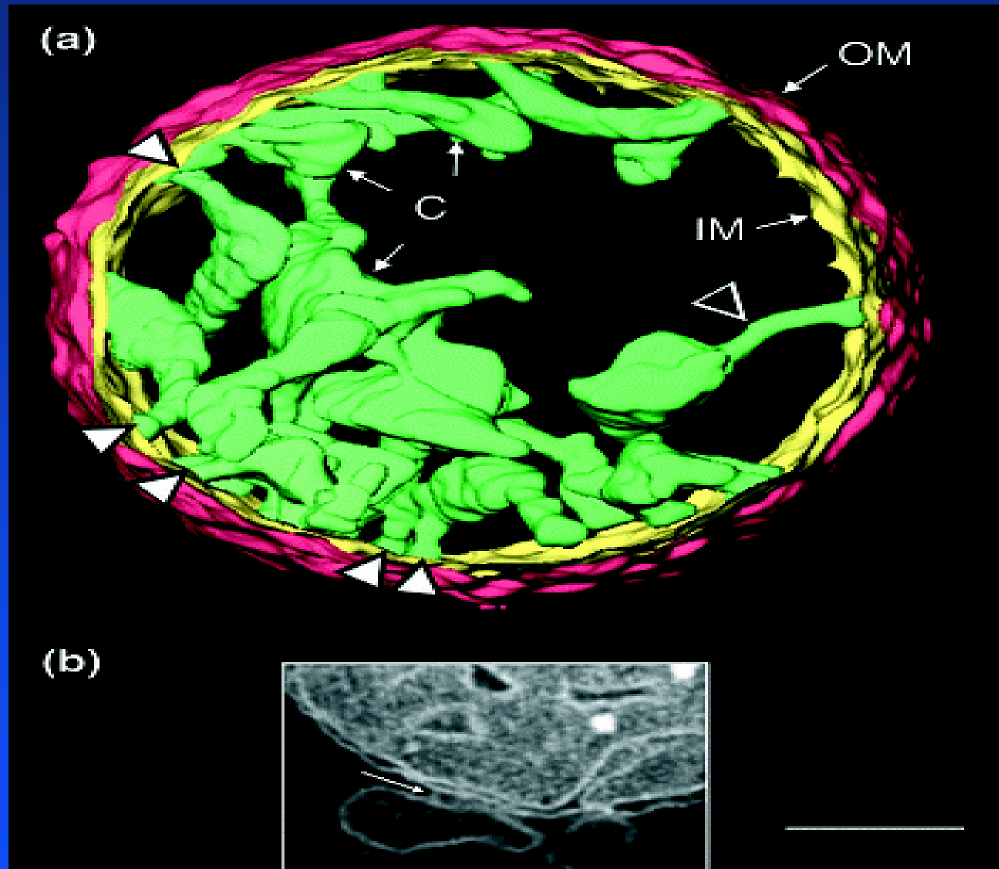
The Mitochondrion

- 1. Substantial portion of cell volume
  - About 20% of the volume of a eukaryotic cell
  - Mitochondrial IM is 1/3 of total cell membrane
- 2. Mitochondrial function supplies 30 ATP molecules (only 2 ATP from anaerobic (cytosolic) glycolysis
- 3. Mobile, shape-changing,fusion/separation

EM view of a Mitochondrion



3D Reconstruction



mobile, shape-changing

Large GTPases control mitochondrial

fusion (Fzo1, OM)

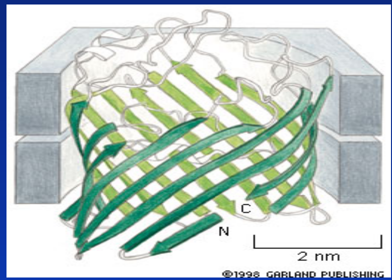
fission (Dnm1, OM)

and

Inner membrane remodelling
(Mgm1, IMS)

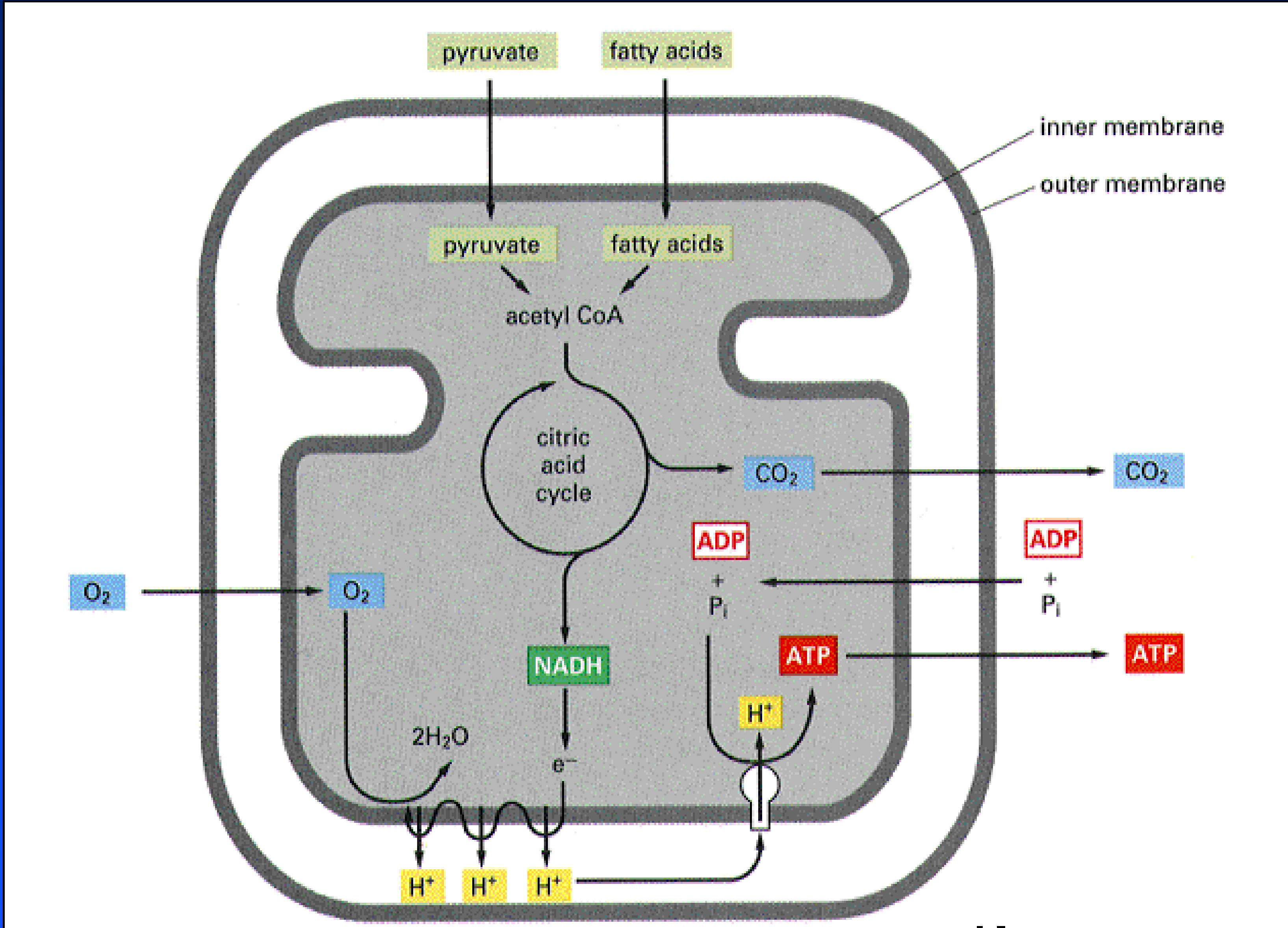
Mitochondrial Structure

- **Outer Membrane (semipermeable)**
  - 6% of total mit.protein
  - lipid metabolism enzymes, porin

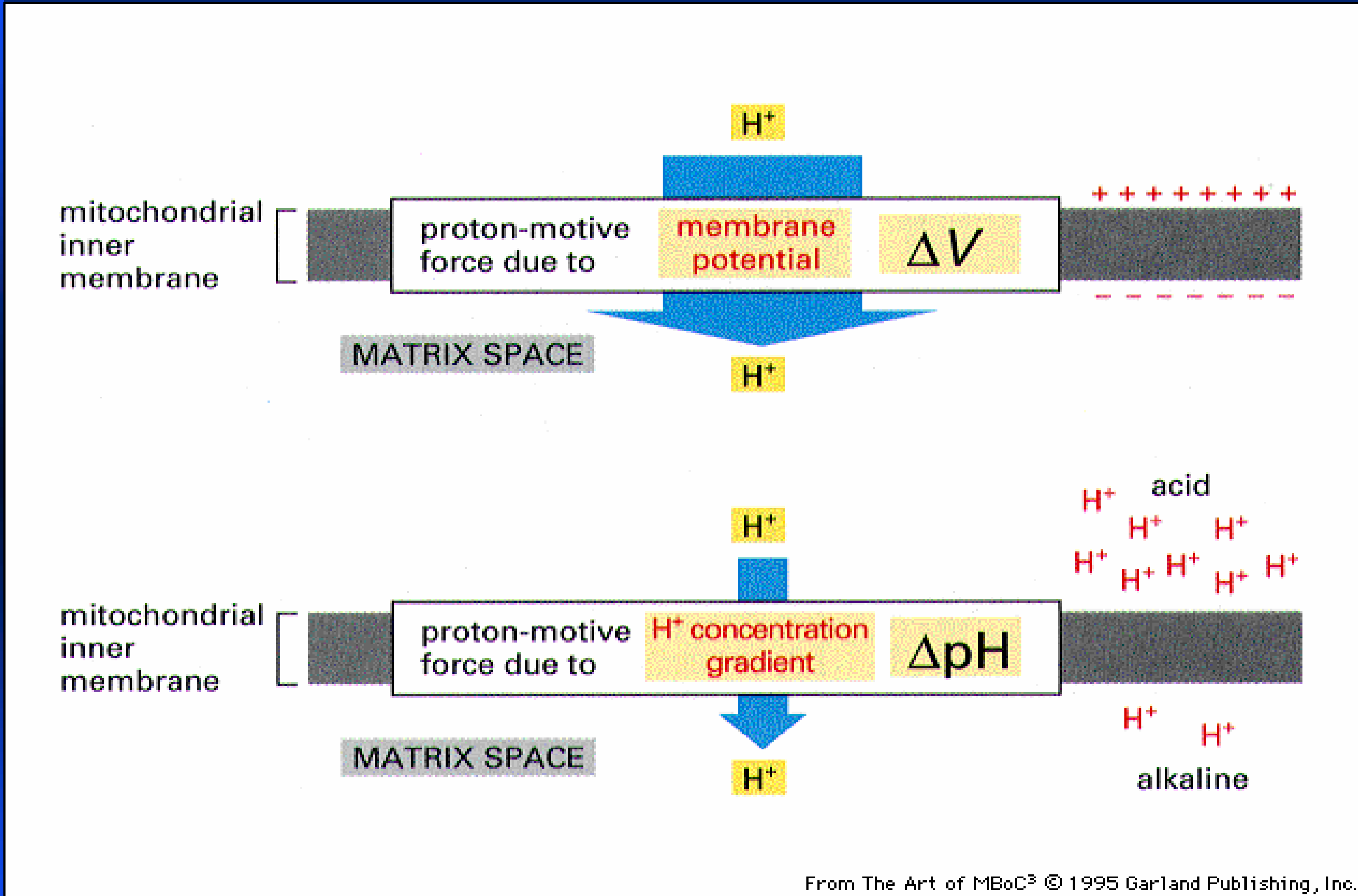


- **IMS**
  - 6% of total mit.protein
  - enzymes that use ATP to phosphorylate other nucleotides
- **Inner Membrane (impermeable)**
  - 21% of total mit.protein
  - ATP synthase, respiratory chain enzymes, transport proteins
- **Matrix**
  - 67% of total mit.protein
  - Hundreds of enzymes, DNA, ribosomes, tRNAs

Mitochondrial Energy Metabolism



The Electrochemical Proton Gradient

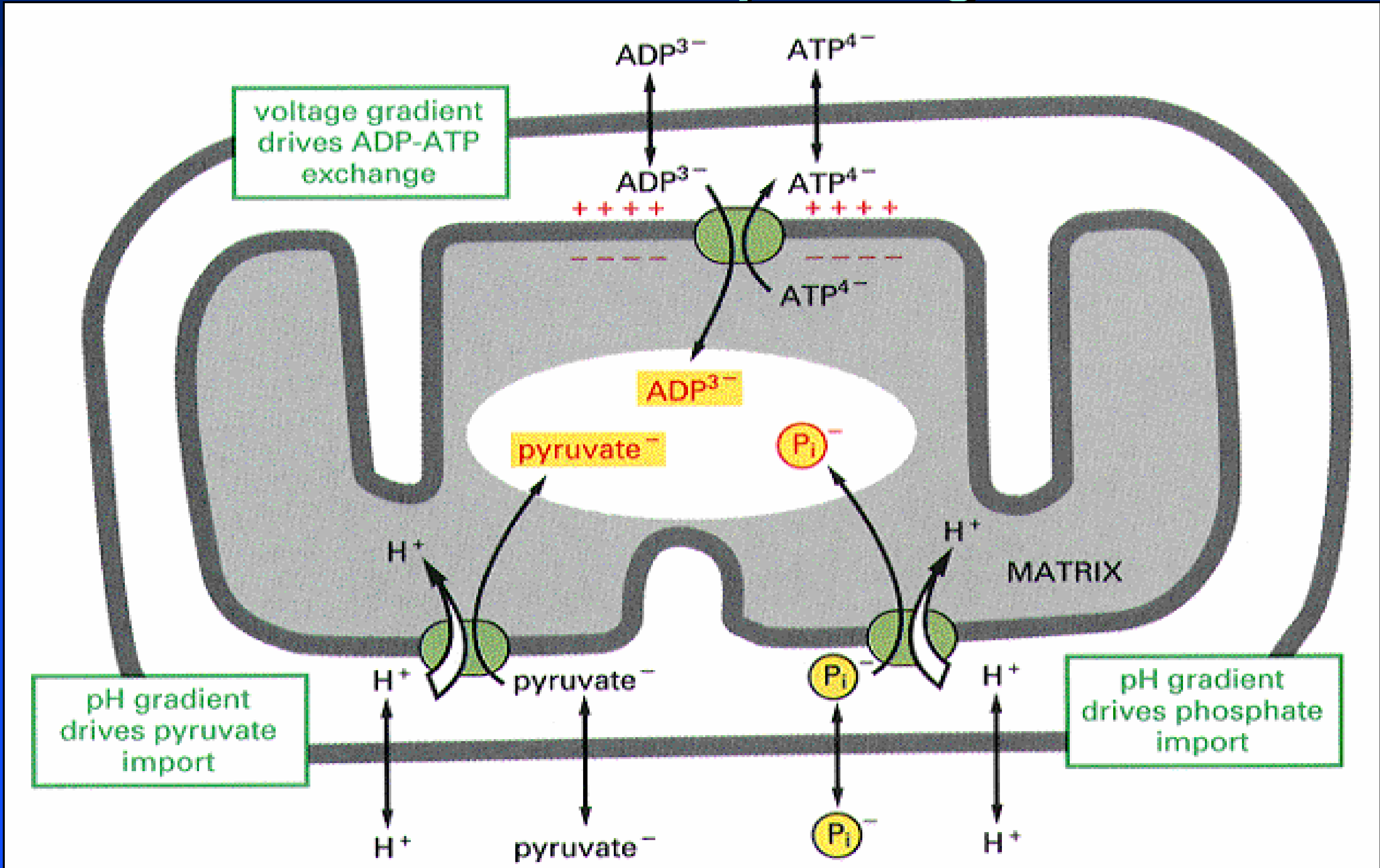


140 mV

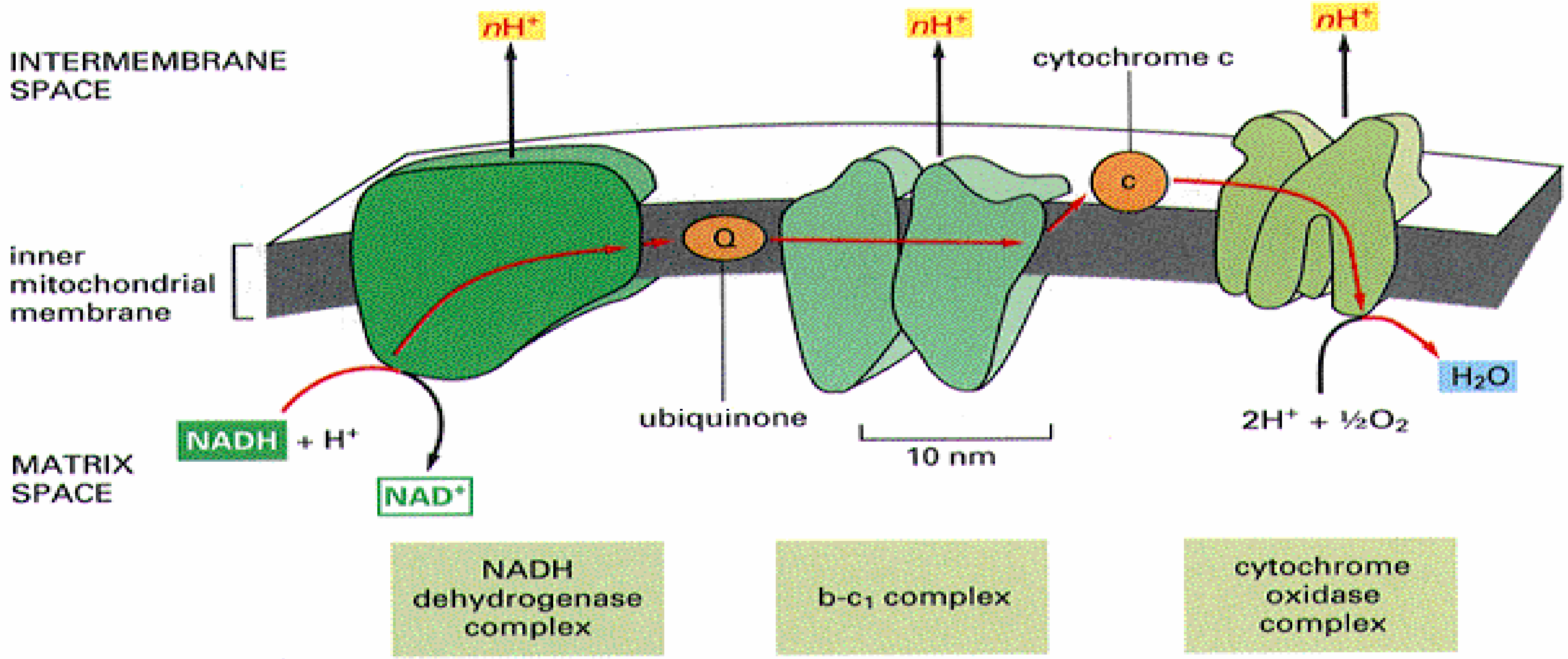
60 mV
(-1 pH unit)

TOTAL
200 mV

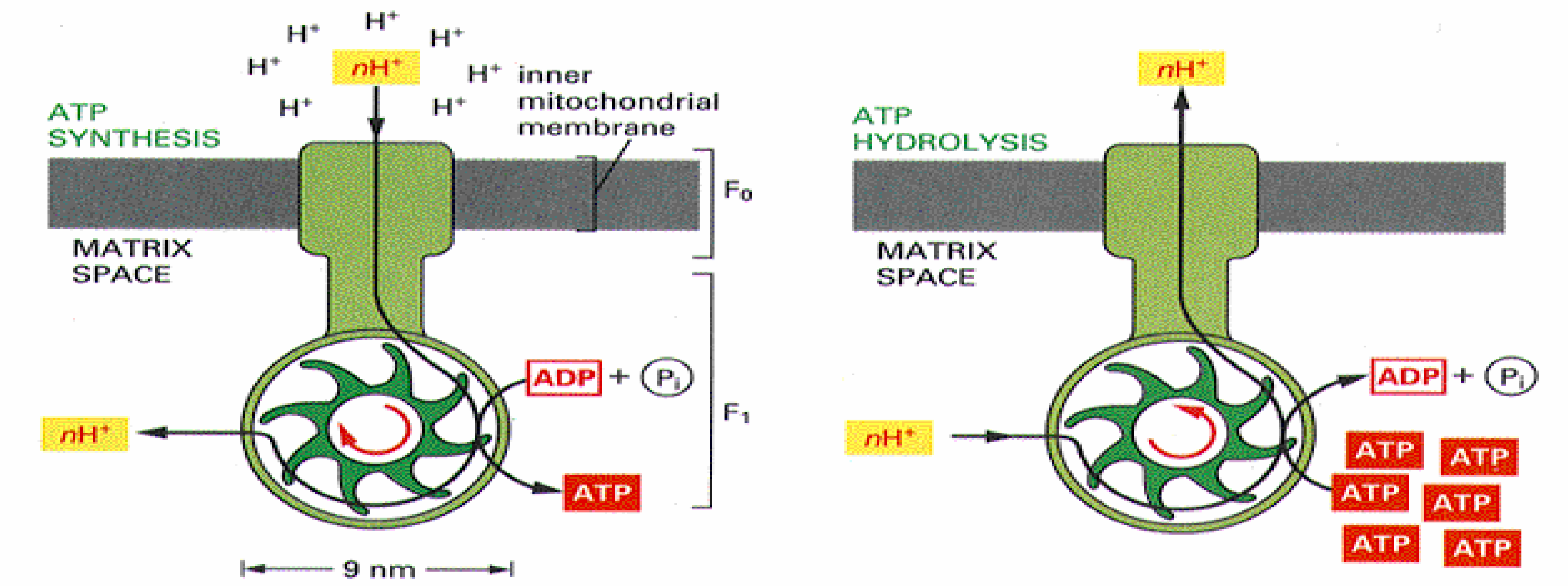
Active transport processes are driven by the electrochemical proton gradient



The Respiratory chain consists of 3 large membrane-embedded enzyme complexes



ATP Synthase is a reversible coupling device: It interconverts the energies of the electrochemical proton gradient and chemical bonds



Functional Complexity

Respiration and ATP Synthesis

Synthesis of heme, lipids, amino acids and nucleotides

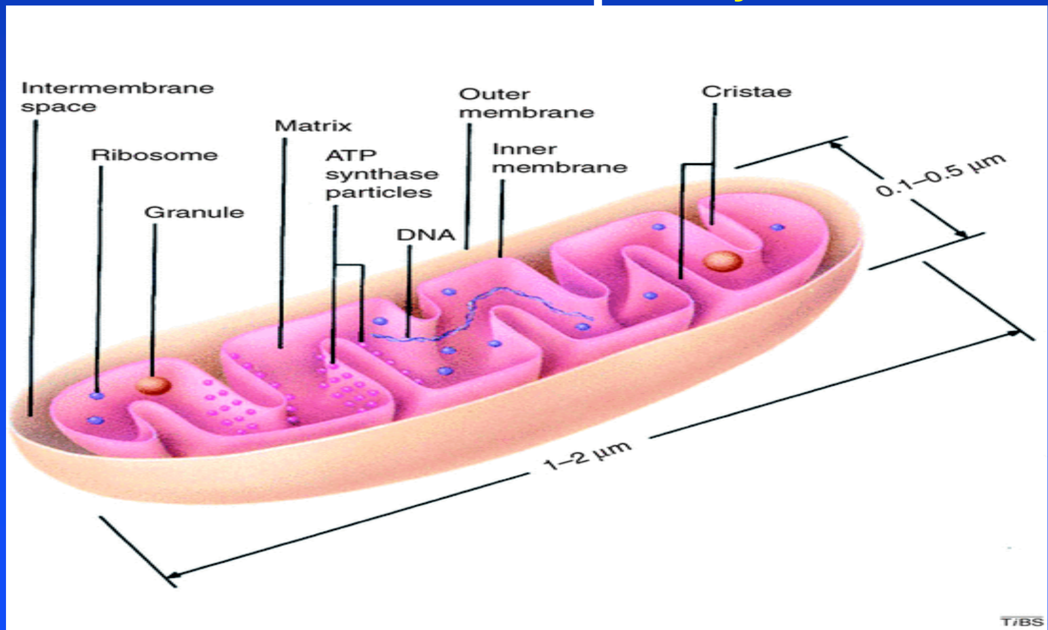
Intracellular homeostasis of inorganic ions

Structural Complexity

5-15% of total cell protein
20% volume of eukaryotic cell
IM is 1/3 of total cell membrane

About 1000 different polypeptides (600 in yeast)

Only a dozen encoded by mtDNA

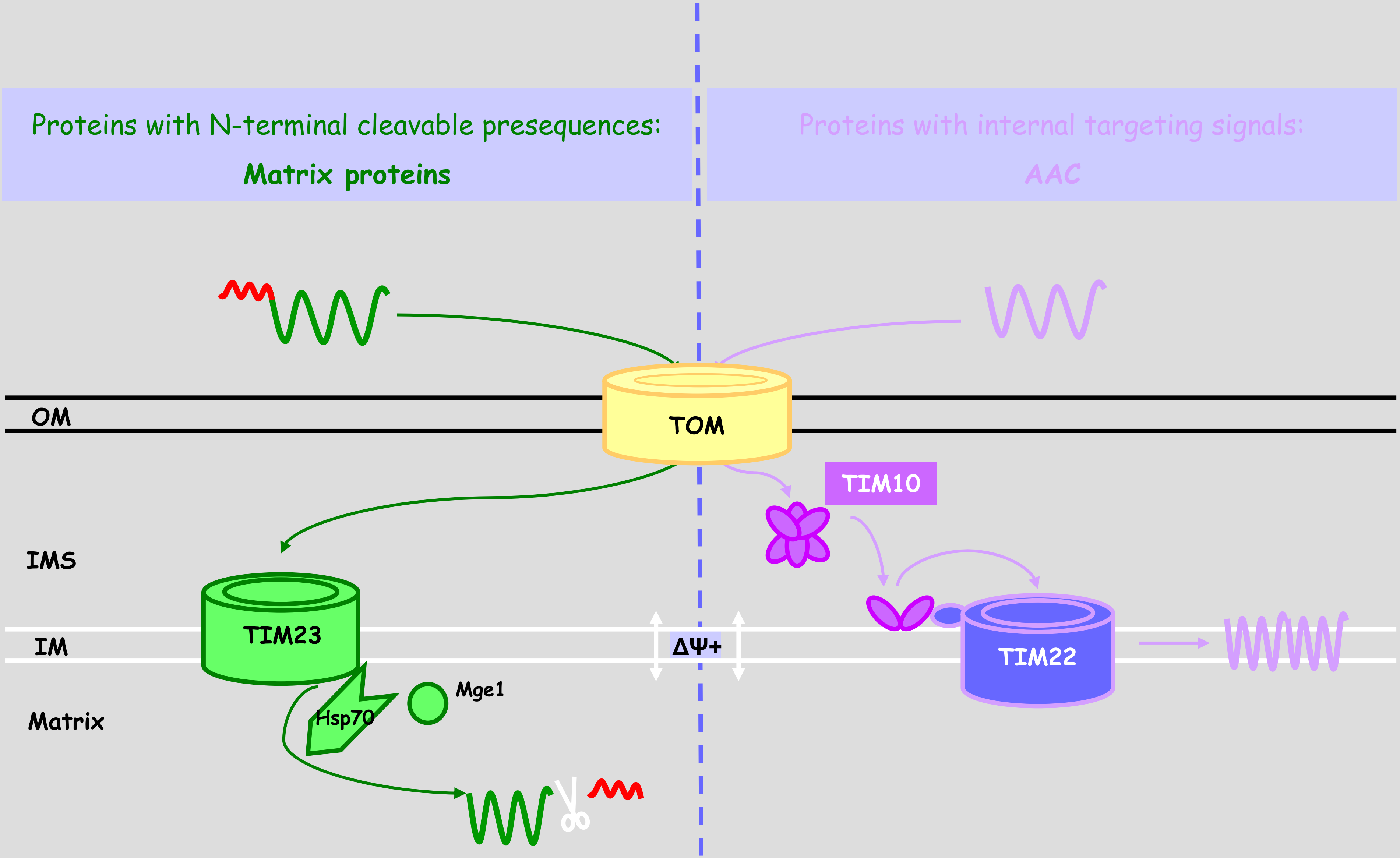


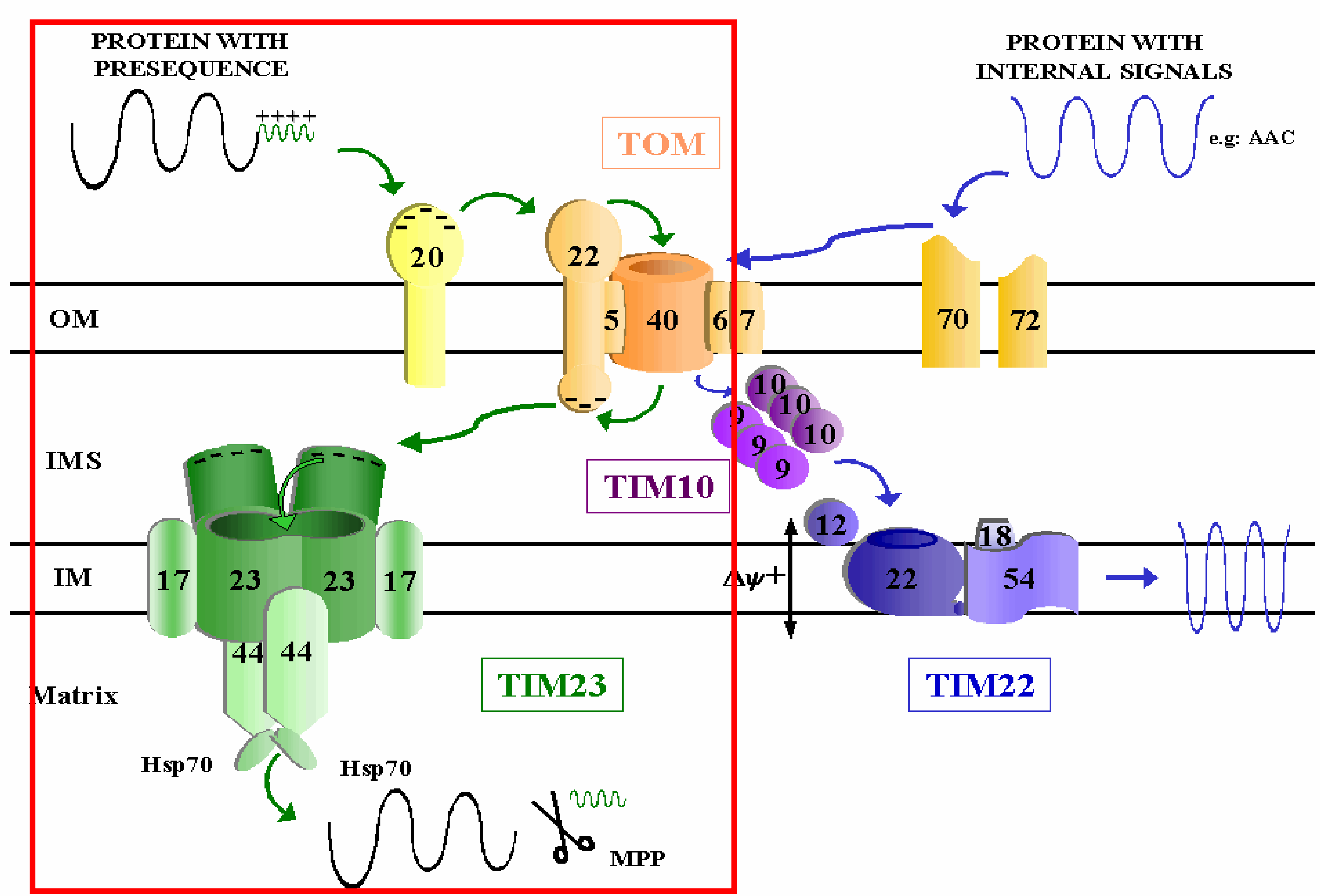
Protein import is the major mechanism of mitochondria biogenesis

Identification of components of the Mitochondrial Protein Import System

- Genetic analyses (fungal genetics)
- In vitro import assay system with isolated functional mitochondria
  - Chemical Crosslinking
  - Biochemical reconstitution

IMPORT INTO YEAST MITOCHONDRIA

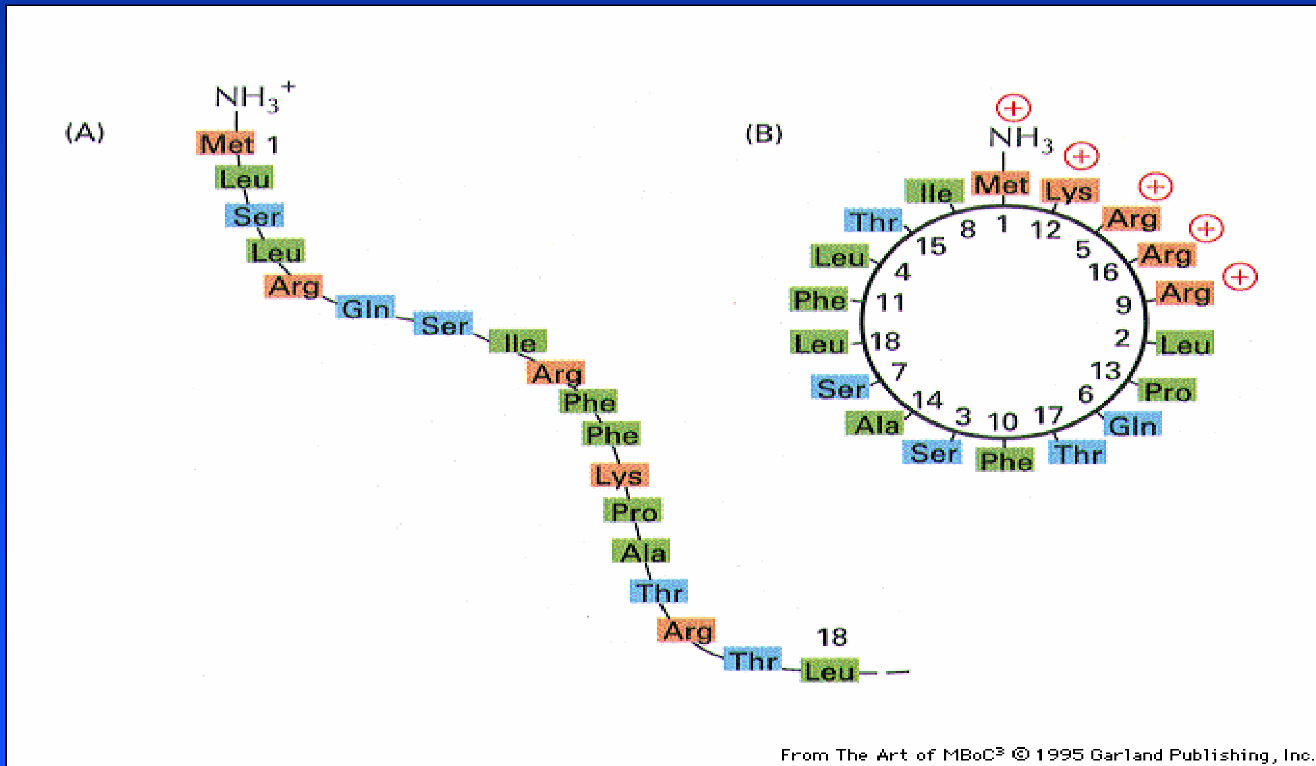


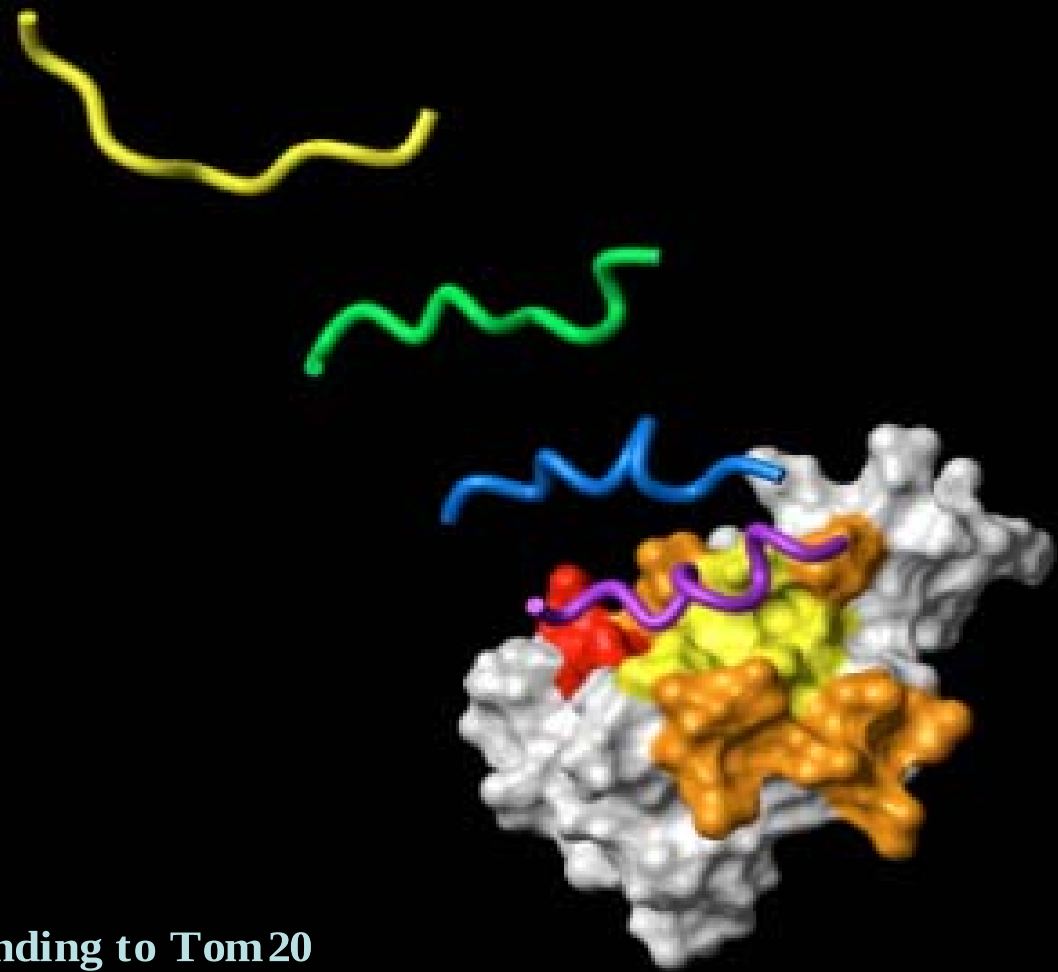


PROTEIN WITH PRESEQUENCE
++++
TOM
PROTEIN WITH INTERNAL SIGNALS
e.g: AAC
20
22
5
40
6
7
70
72
OM
IMS
IM
Matrix
TIM10
10
9
12
18
22
54
$\Delta\psi$+
17
23
44
TIM23
TIM22
Hsp70
MPP

Import into the matrix - 1

- **Depends on a matrix-targeting signal: The presequence**
  - Cleavable, usually located at the N-terminus
  - usually 12-15 residues long
  - amphiphilic, with positively charged residues on one side of an a-helix





Presequence binding to Tom20

Endo and Kohda

Import into the matrix - 2

- This is a multistep process
- Interactions with chaperones in the cytosol keep the precursor in an unfolded conformation (“import-competent”)
- Different import complexes in the OM (TOM complex) and the IM (TIM complex).
- Electrostatic interactions between the positive presequence and negative patches of receptors along the import pathway: The Acid chain hypothesis- Gradation of affinities leads the presequence along the import pathway
- The electrophoretic function of the potential across the IM draws the precursor across the IM
- The pulling force of the translocation motor mHsp70/Tim44 actively draws the precursor to complete translocation

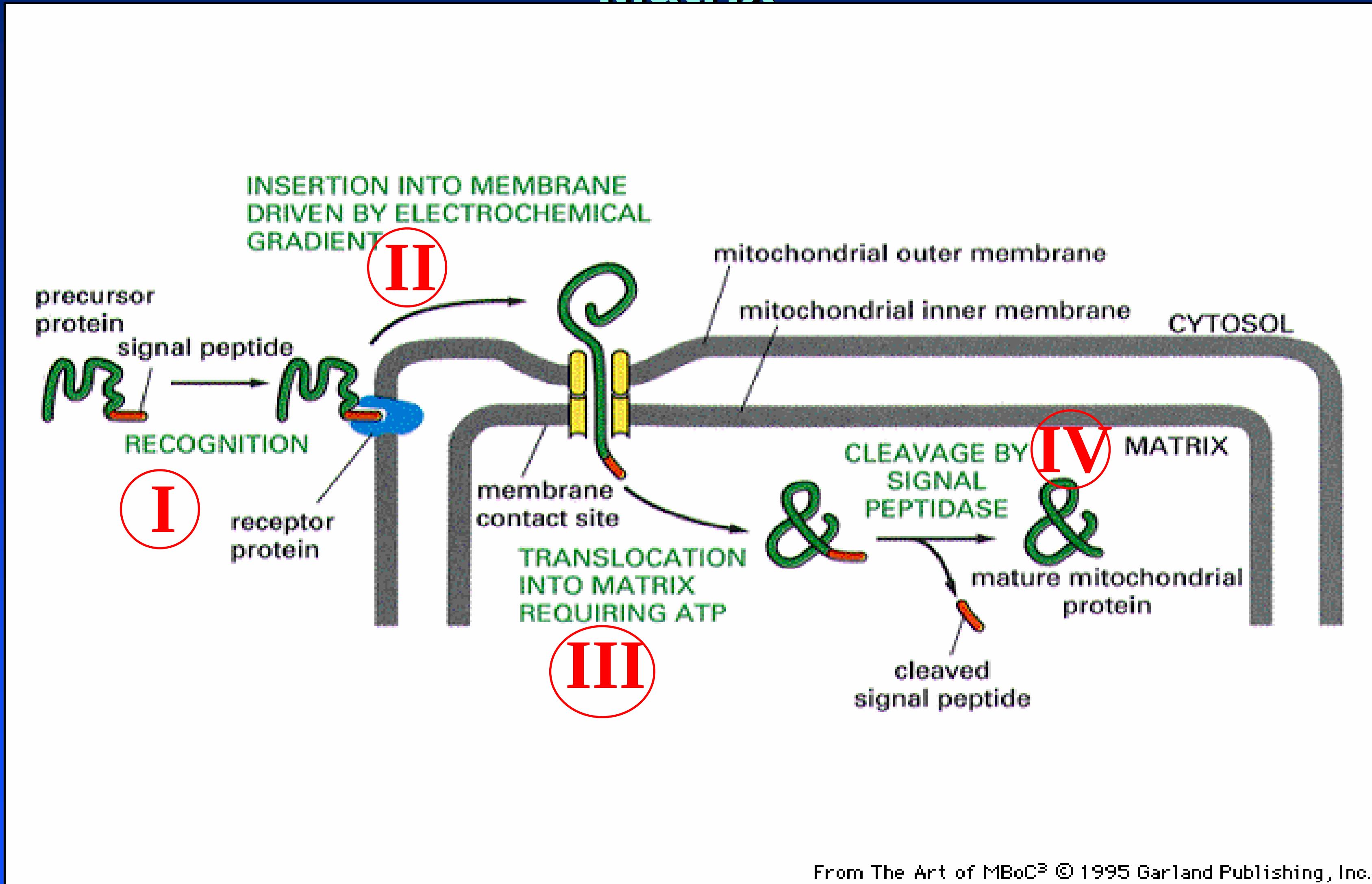
Import into the matrix - 3

- **Energy requirements:**
  - **ATP Hydrolysis**
    - In the cytosol (function of ATPase chaperones)
    - In the mitochondrial matrix (Hsp70 translocation motor)
  - **Electrochemical potential across the inner membrane**

Import into the matrix - 4

- **Components**
  - **TOM Complex:**
    - Receptors: Tom70, Tom20, Tom37, Tom22
    - Channel-forming: Tom40, Tom5
    - Channel modulating: Tom6, Tom7
  - **TIM Complex:**
    - Receptor: Tim23
    - Channel-forming: Tim23, Tim17
    - Translocation motor: Tim44, Hsp70, GrpE (co-chaperone)

Summary of Protein Import into the Mitochondrial Matrix



Import into the IMS

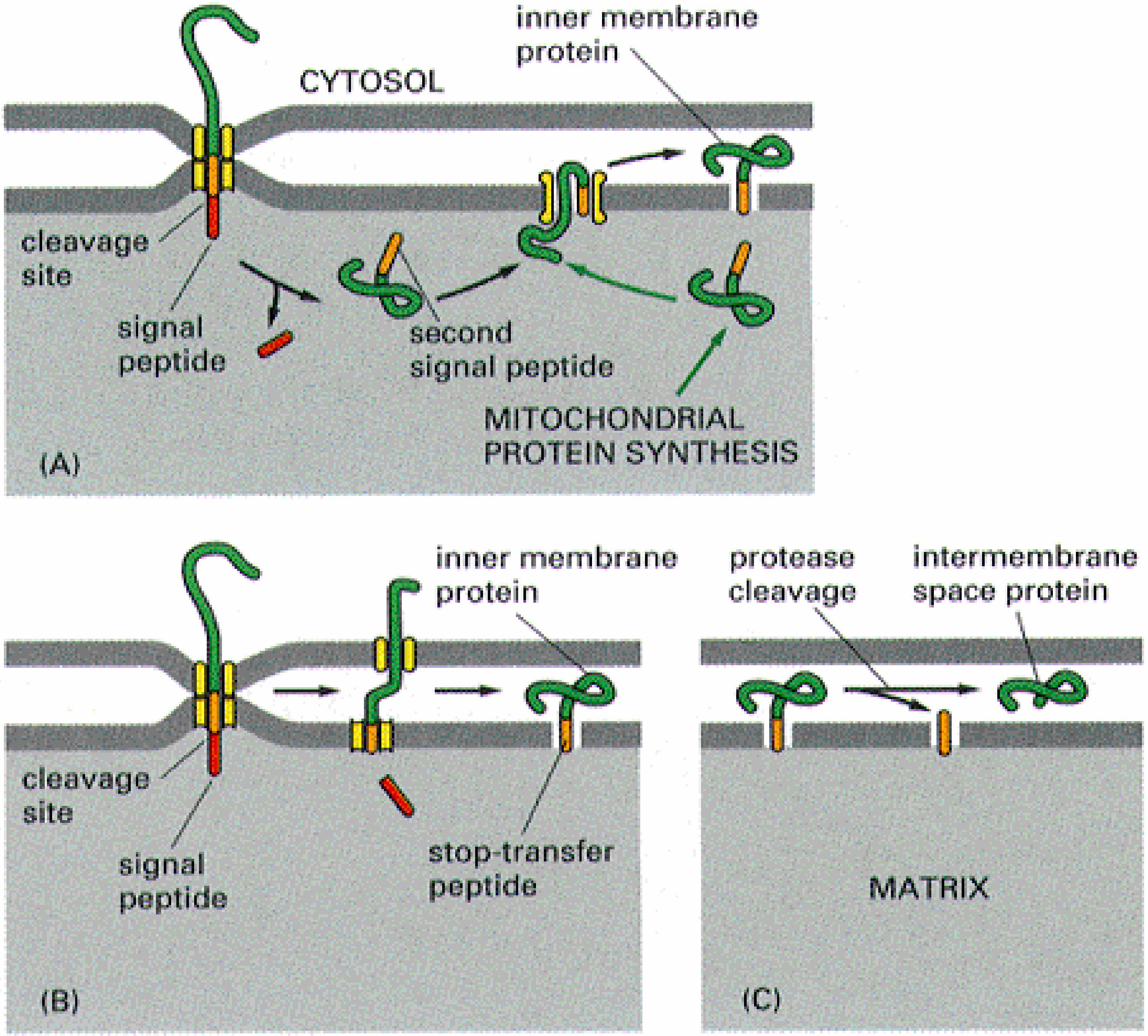
- A. Variation of the matrix targeting pathway
  - example: cytochrome b2

- B. Distinct pathway involving a specific IMS targeting signal
  - example: mitochondrial heme lyases

Cytb2 IMS targeting - 1

The Signal:

- Bipartite nature: matrix targeting signal followed by an IMS sorting signal
- IMS sorting signal contains mainly uncharged residues
- cleaved by specific IMS protease
- NOT very highly conserved



inner membrane
protein
CYTOSOL
cleavage
site
signal
peptide
second
signal peptide
MITOCHONDRIAL
PROTEIN SYNTHESIS
(A)
inner membrane
protein
protease
cleavage
intermembrane
space protein
cleavage
site
signal
peptide
stop-transfer
peptide
MATRIX
(B)
(C)

Cytb2 IMS targeting - 2

Energetics:

- Electrochemical potential absolutely essential but ATP hydrolysis NOT required

Components:

- Known Subunits of the TOM complex
- TIM23 complex
- IMS sorting peptidase

Mechanism:

Stop-transfer mechanism: the hydrophobic sorting signal is stuck at the TIM23 complex and laterally diffuses out into the lipid bilayer of the IM

Import into the IMS

- A. Variation of the matrix targeting pathway
  - example: cytochrome b2

- B. Distinct pathway involving a specific IMS targeting signal
  - example: mitochondrial heme lyases

Heme Lyase IMS targeting - 1

The Signal:

- Internal
- about 60 residues long
- highly conserved sequence
- highly hydrophilic (30% charged residues, similar number of + and - charged residues, distributed throughout the sequence)
- mainly a-helical, but NOT amphiphilic

Key reference: Diekert et al Proc. National Acad. Sci. USA 1999, 96, 11752-11757

The Mitochondrial Carrier Family

- Function as metabolite transporters

- 37 proteins in yeast
(10-15% of the total mitochondrial protein)

- 30-35 kDa

- Common topology: 3 similar repeated motifs
(3X2 helix model)