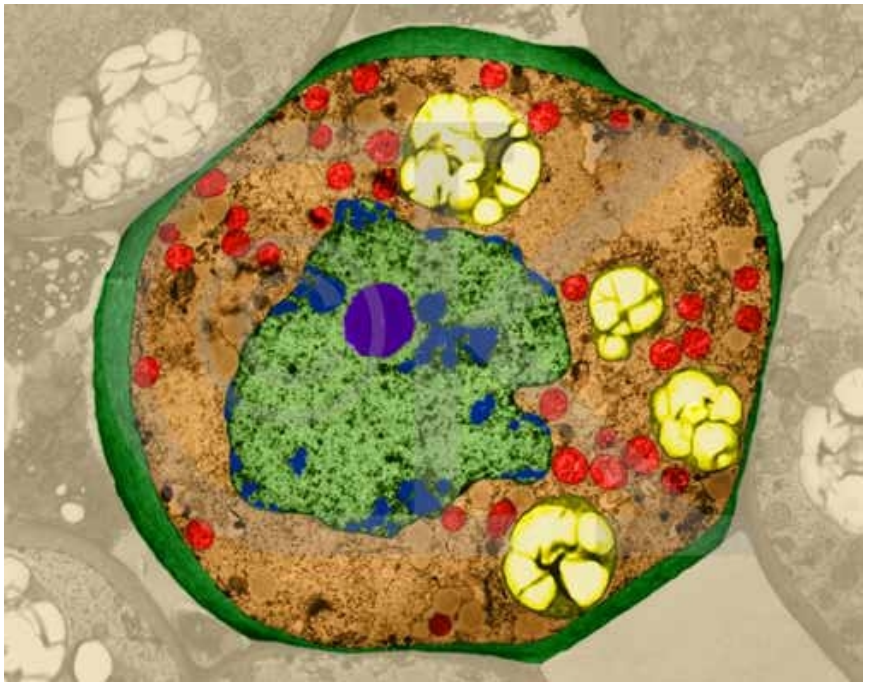
Summer term 2016
Nanobiophysics Module

Introduction to Molecular and Cellular Biology

LECTURES 7-8:

Cell organization I



LECTURES 7-8: CELL ORGANIZATION I

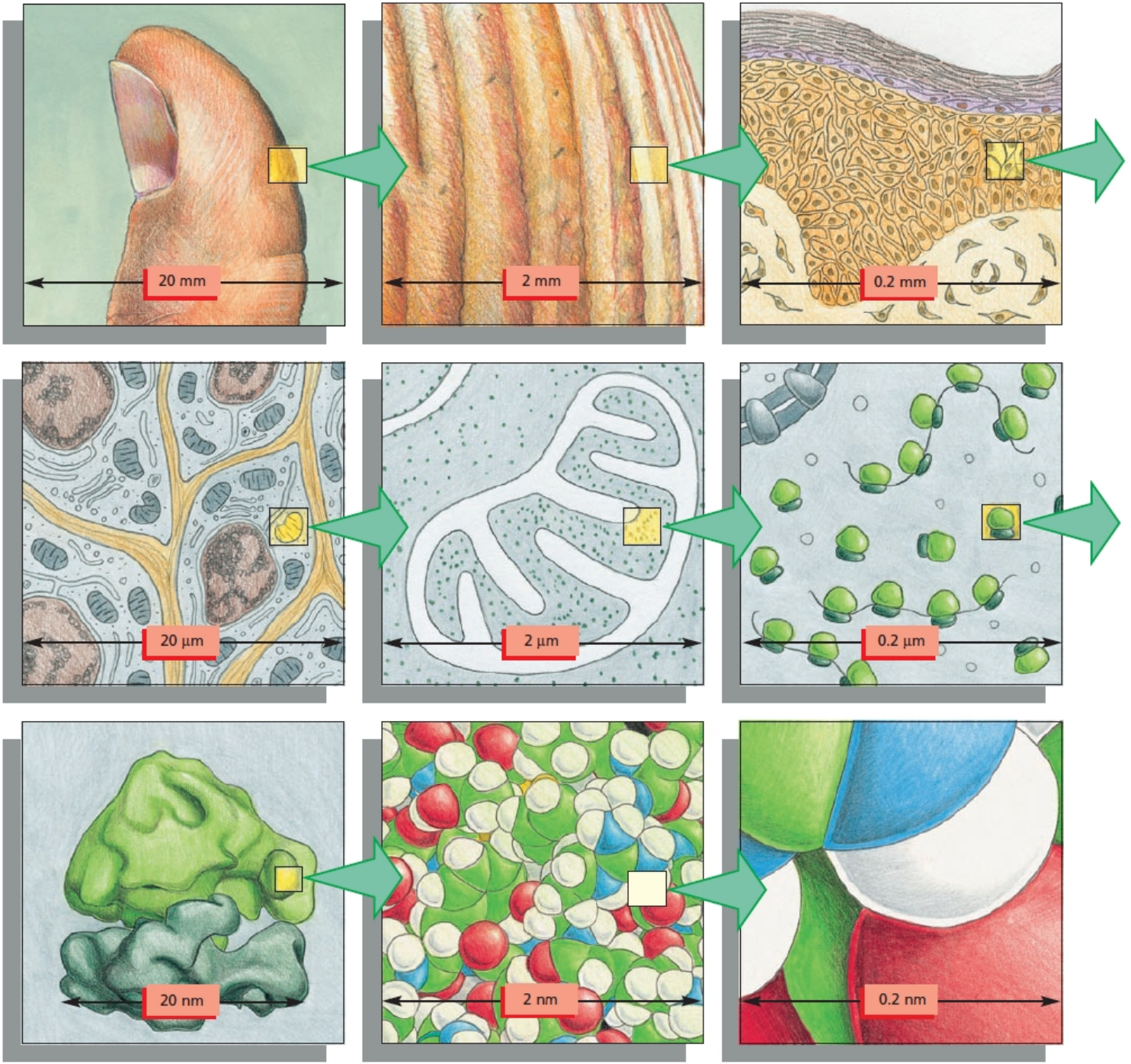
- Type of cells: eukaryotic (animal/plant) and prokaryotic
- Cellular compartmentalization
- Organelles and their functions:
  - cell wall
  - cytosol, cell mebrane, nucleus, ER, GA, peroxisomes, lysosomes, vacuole, ribosomes
  - chloroplasts, mitochondrion
  - oxidative phosphorylation
- Protein sorting

SOME FACTS

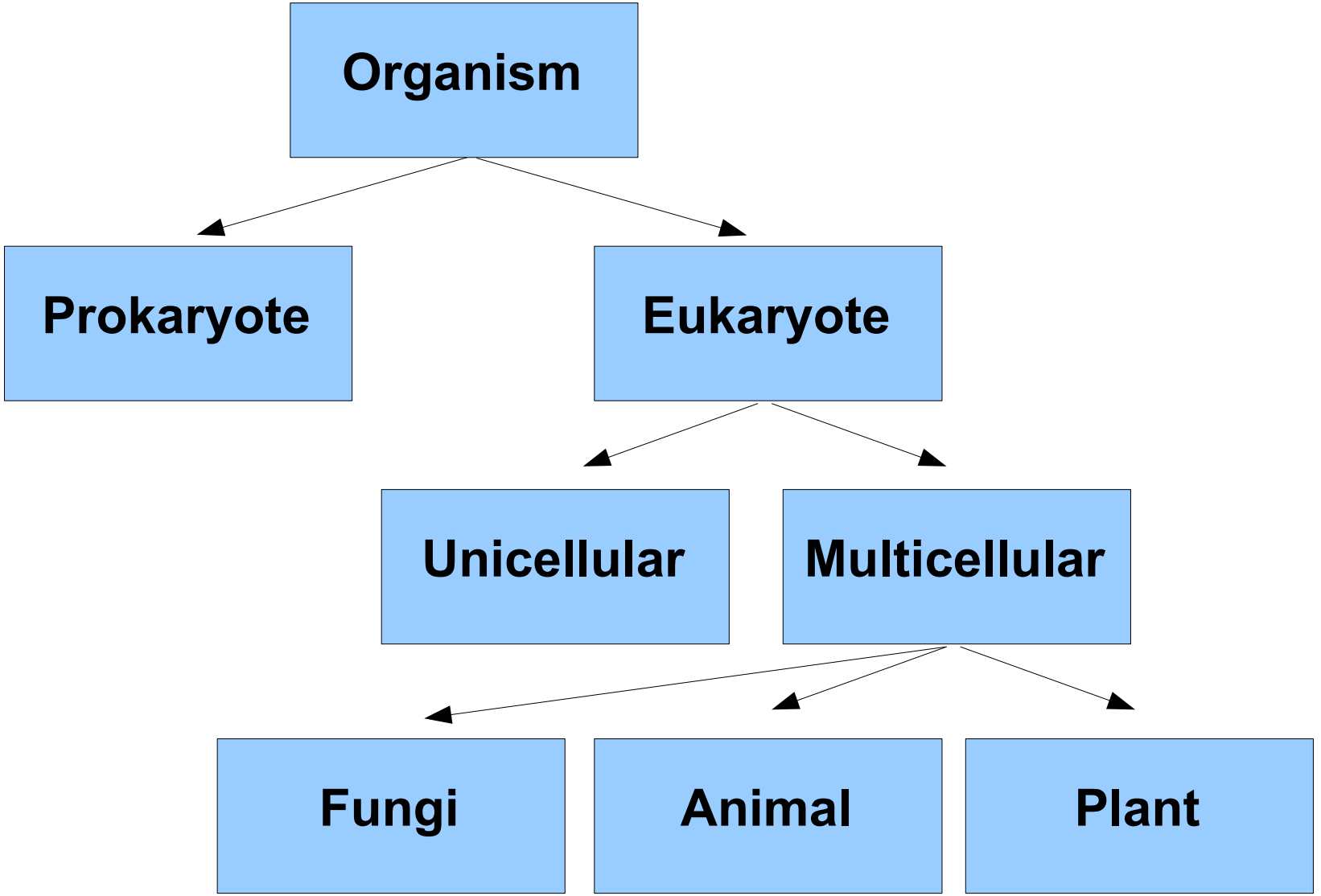
Cell: the basic structural, functional and biological unit

- 3.5 billion years ago: first cell
  - from the space
  - self-replicating RNA + lipid membrane
- Van Leeuwenhoek (1632-1723): described bacteria
- Robert Hooke: “cell”, 1665
- Most of organisms are unicellular
- Human body ~ 10^{13} cells
- Cells contain hereditary information as DNA
- Cells use RNA transcribed from DNA to produce proteins
- Proteins are used for all cellular machinery
- Fragment of genetic information is one gene => one protein
- Energy consume
- Production of molecular building blocks
- Membrane: the border and transport pathway of the cell
- Minimal cell has ~500 genes
- Human genome ~25 000 protein coding genes

HOW BIG ARE CELLS AND THEIR COMPONENTS?



CLASSIFICATION OF CELLS



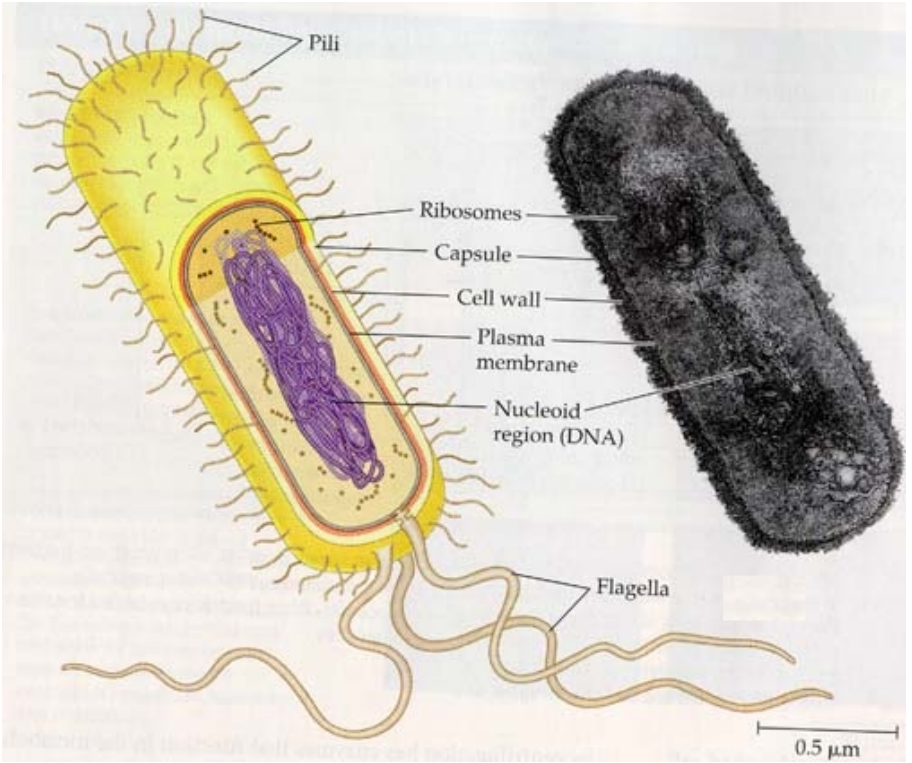
PROKARYOTIC VS. EUKARYOTIC CELL

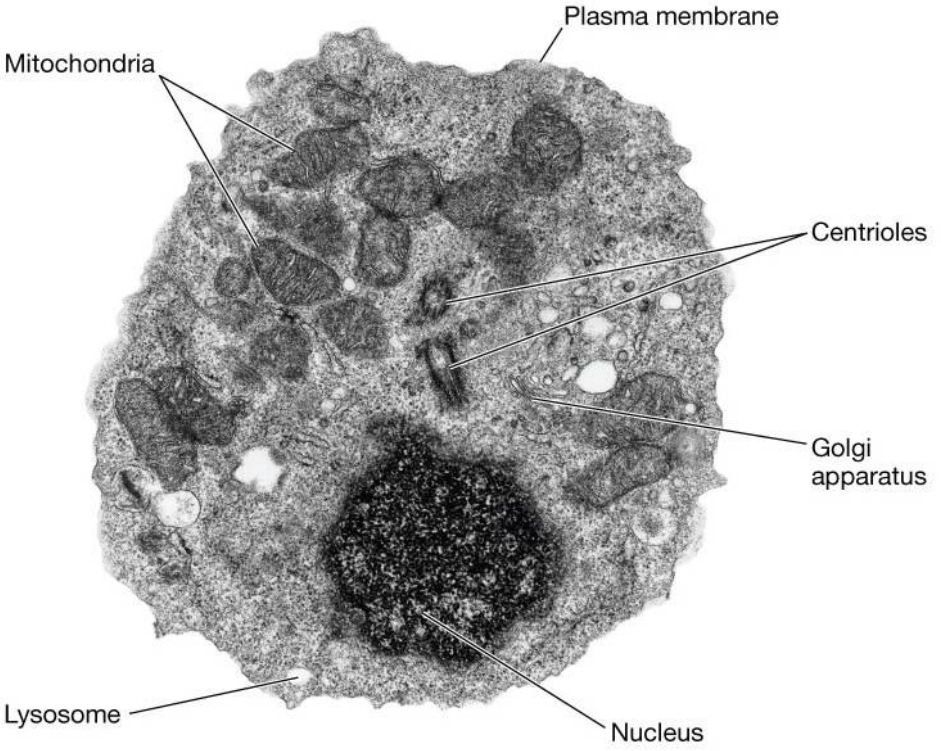
PROKARYOTIC

before **nucleus**

EUKARYOTIC

true **nucleus**





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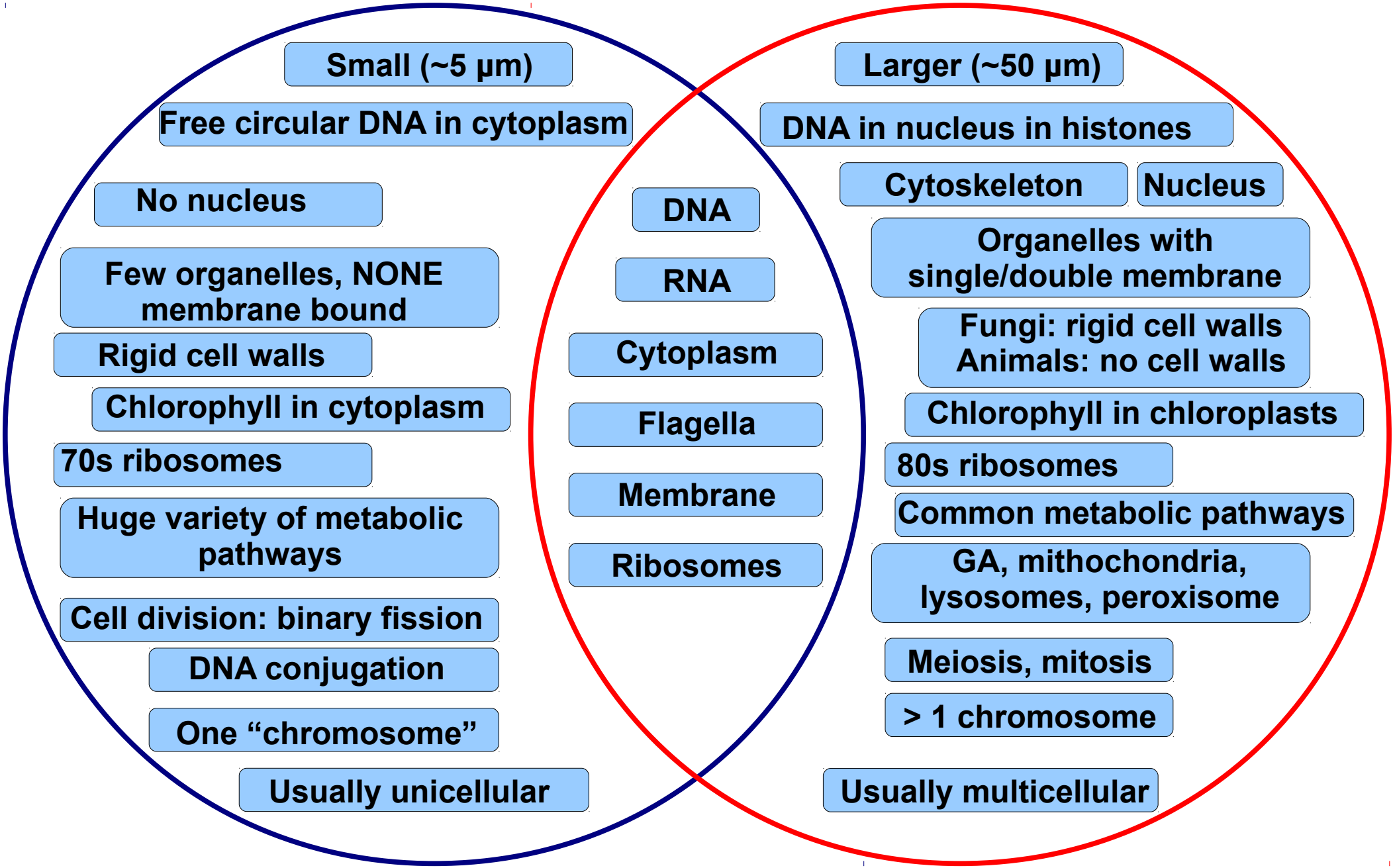


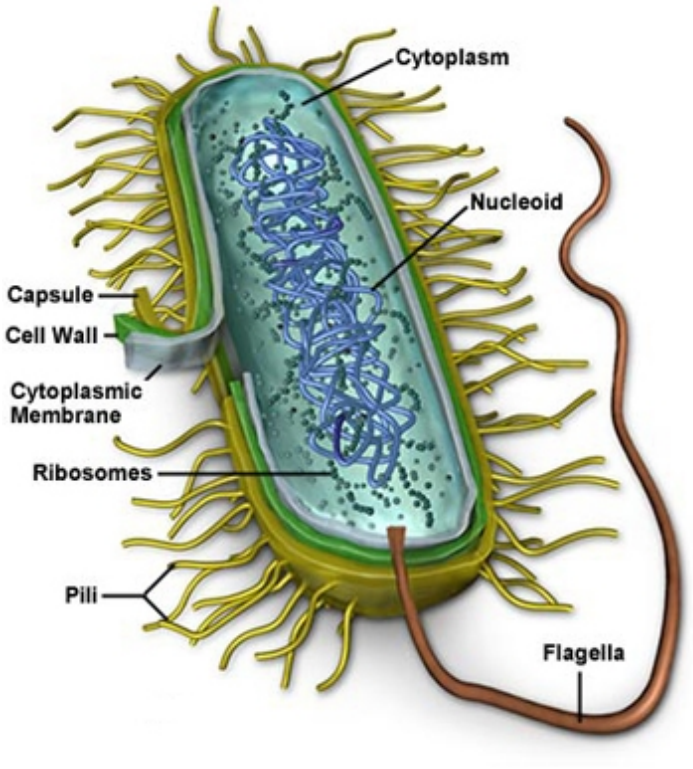
PROKARYOTIC VS. EUKARYOTIC CELL

PROKARYOTIC

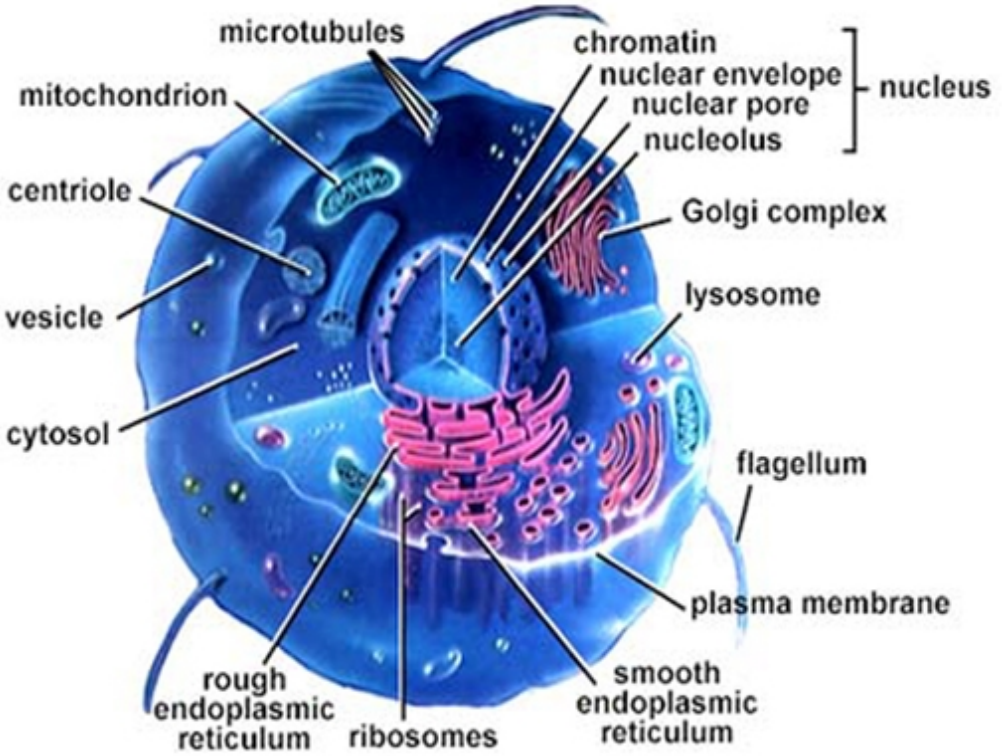
before **nucleus**

EUKARYOTIC

true **nucleus**

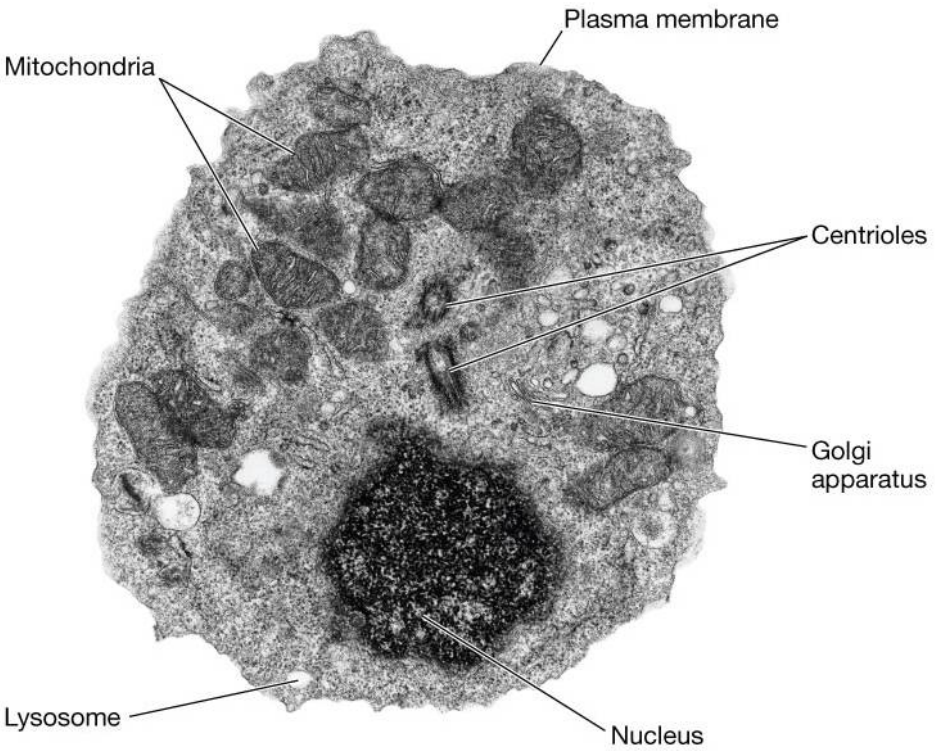


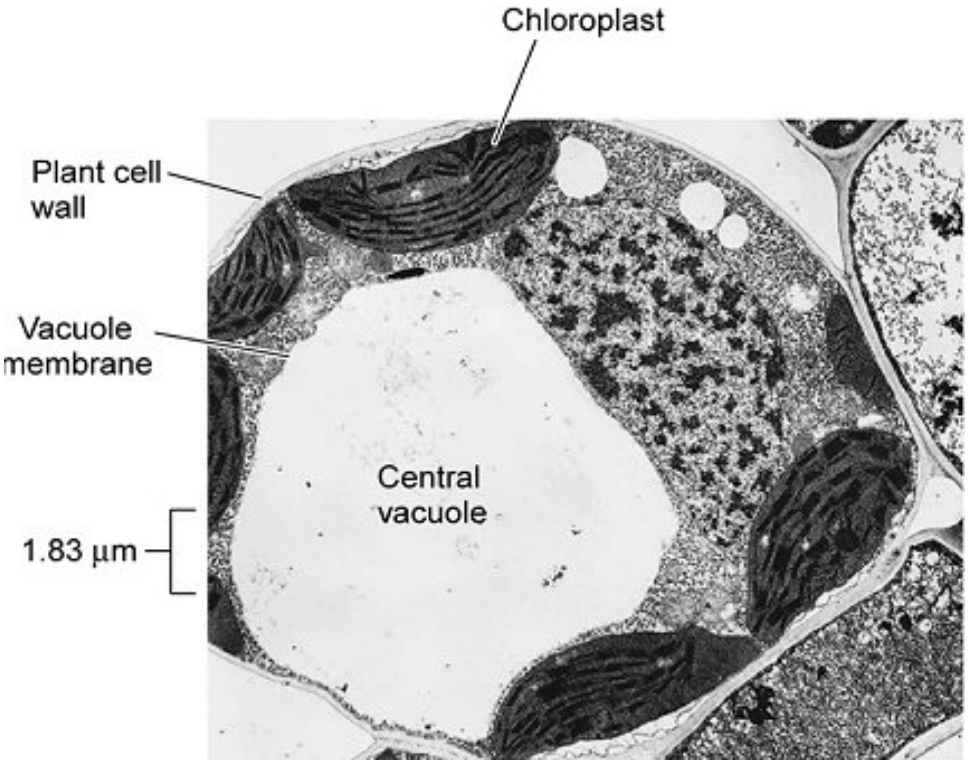
prokaryotic cell
(bacteria)



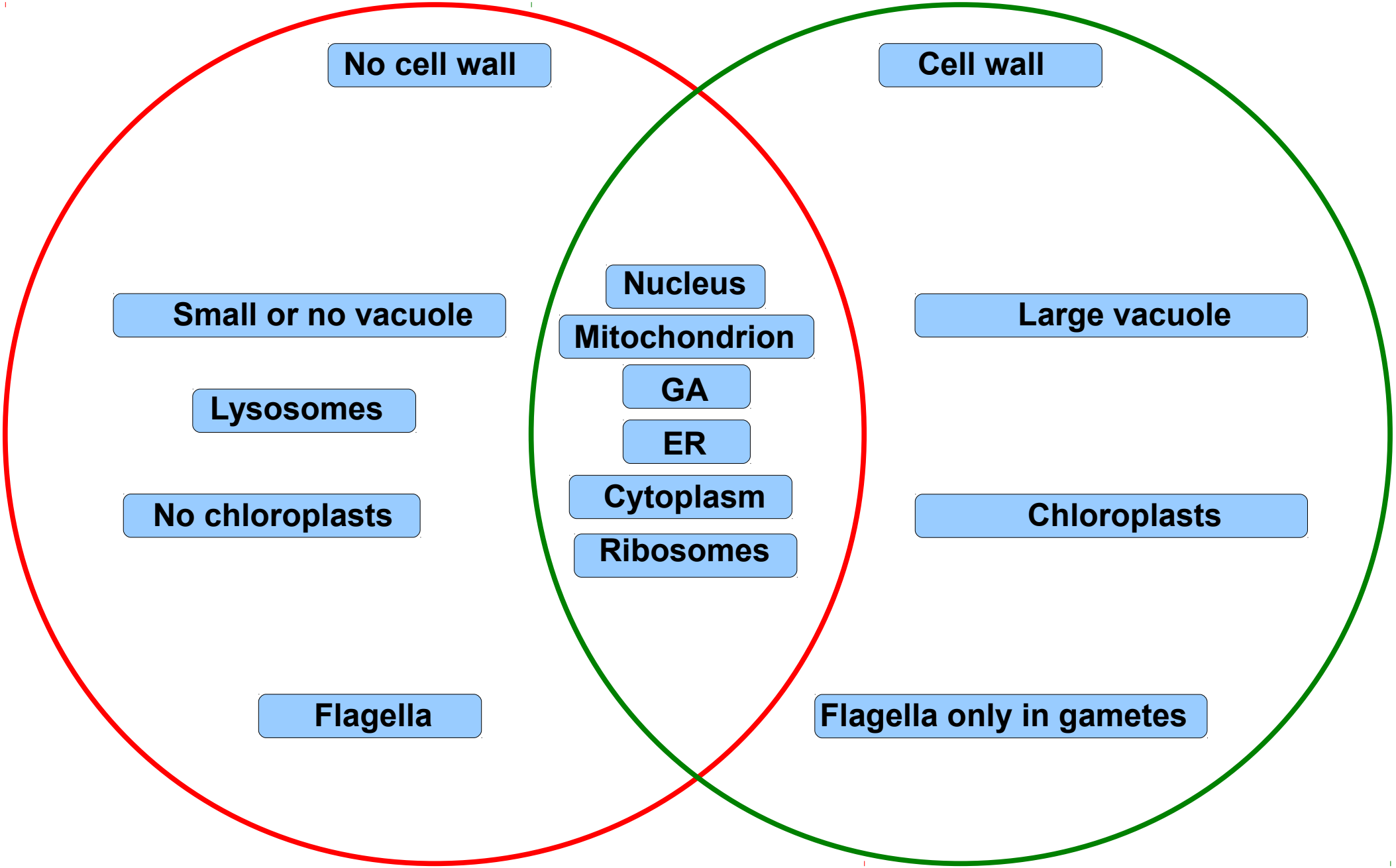
eukaryotic cell
(protists, fungi, animals, plants)

ANIMAL VS. PLANT CELL

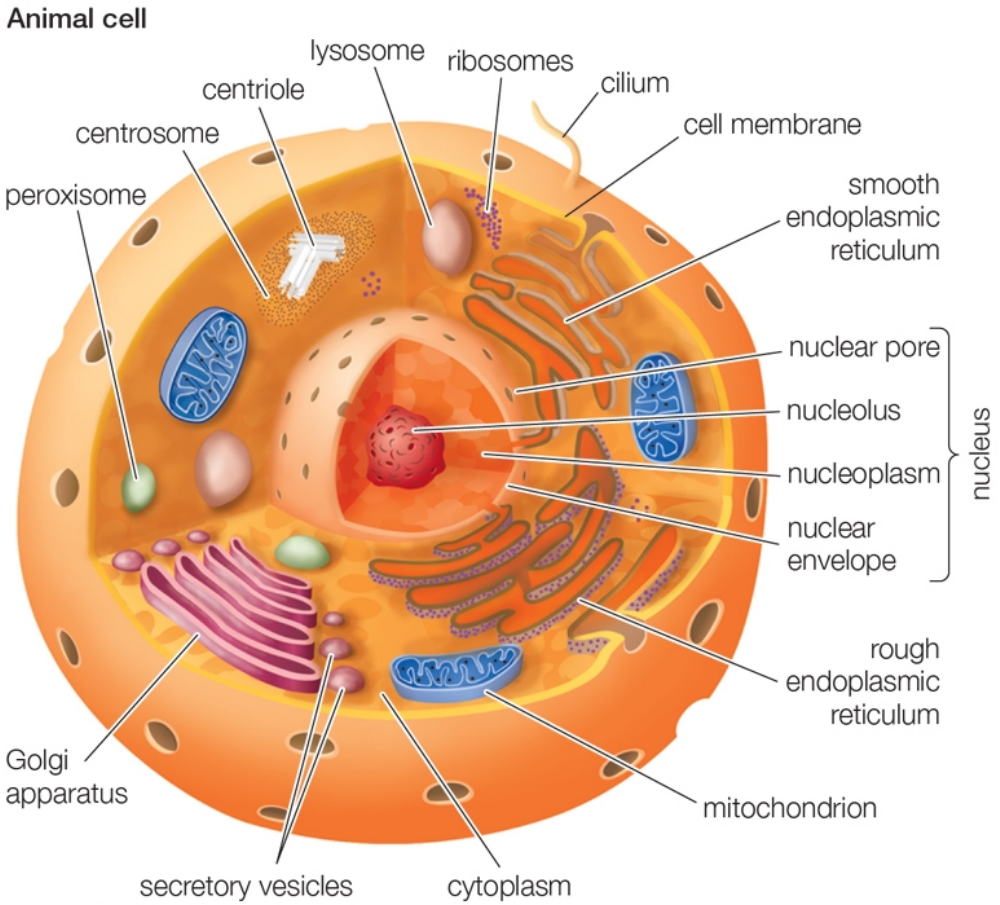


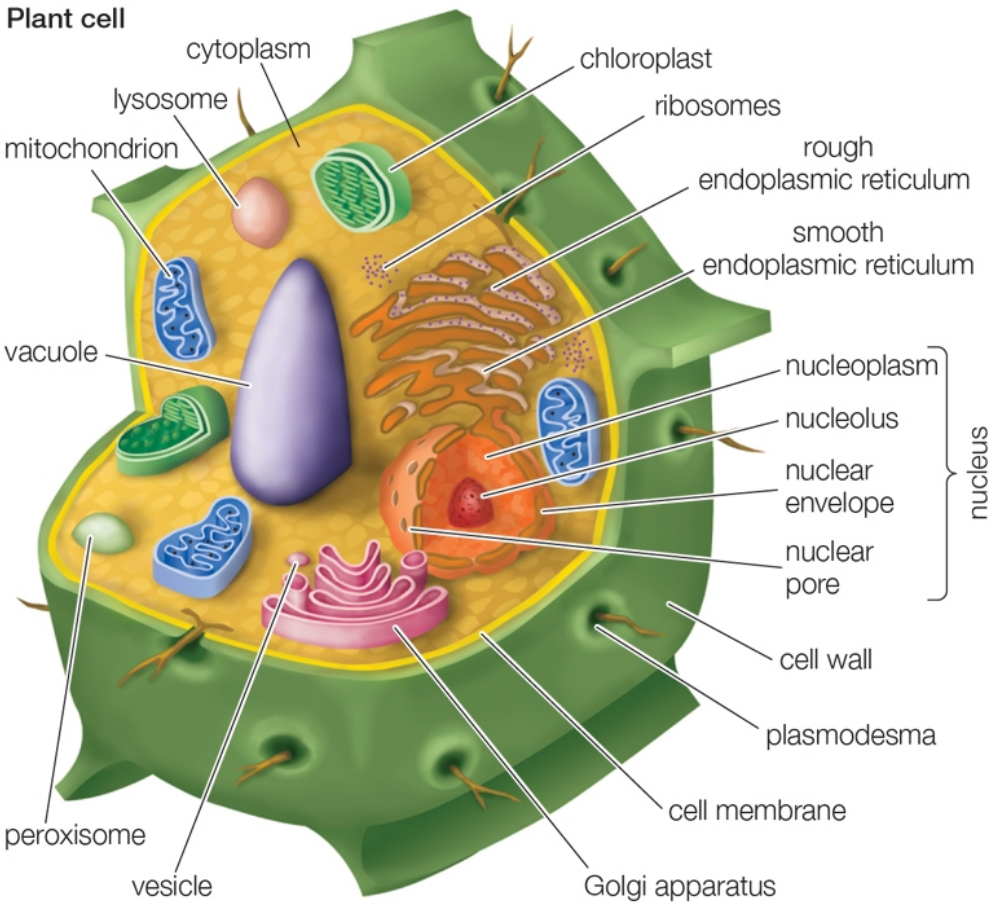


ANIMAL VS. PLANT CELL



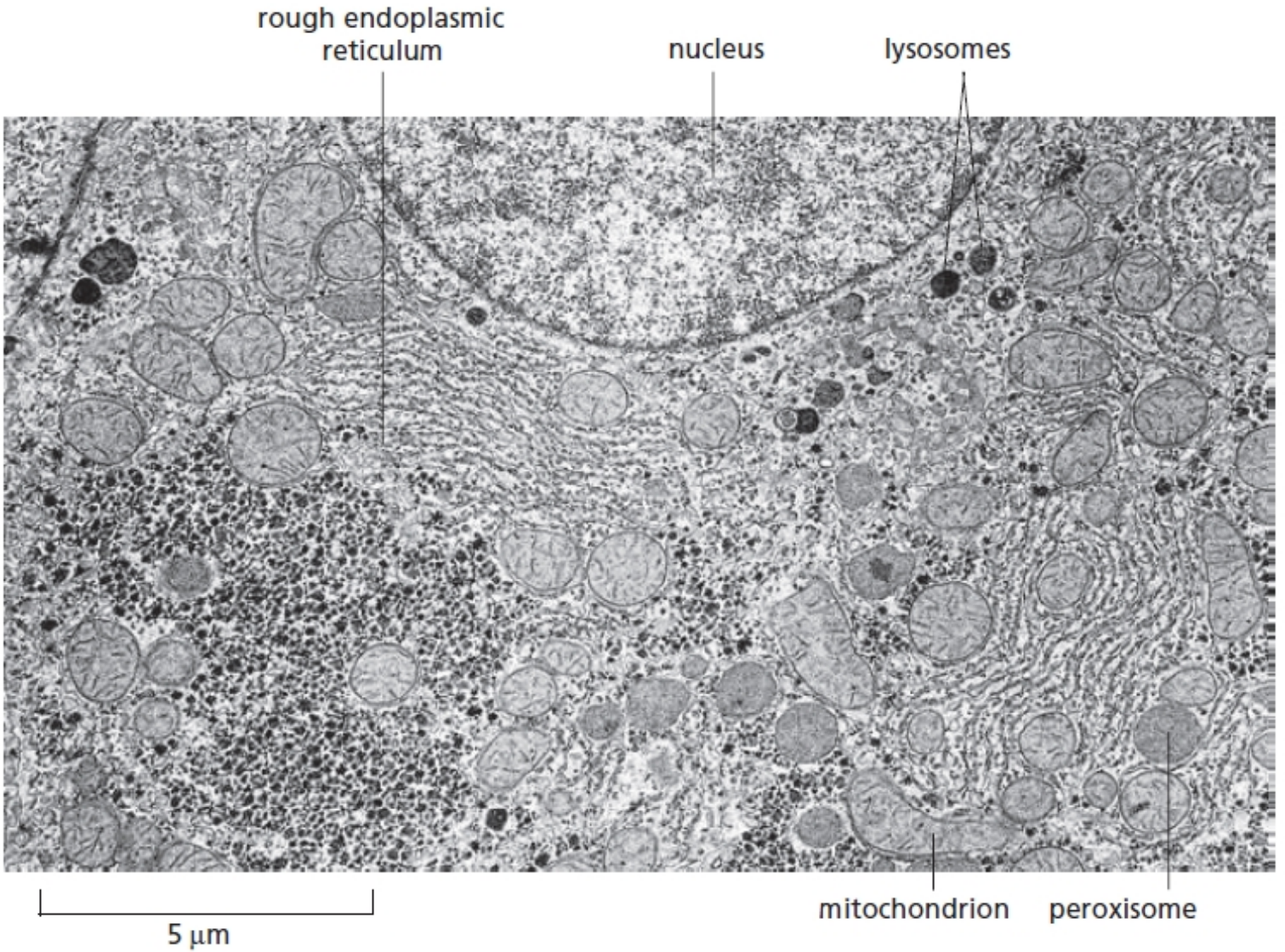
ANIMAL VS. PLANT CELL





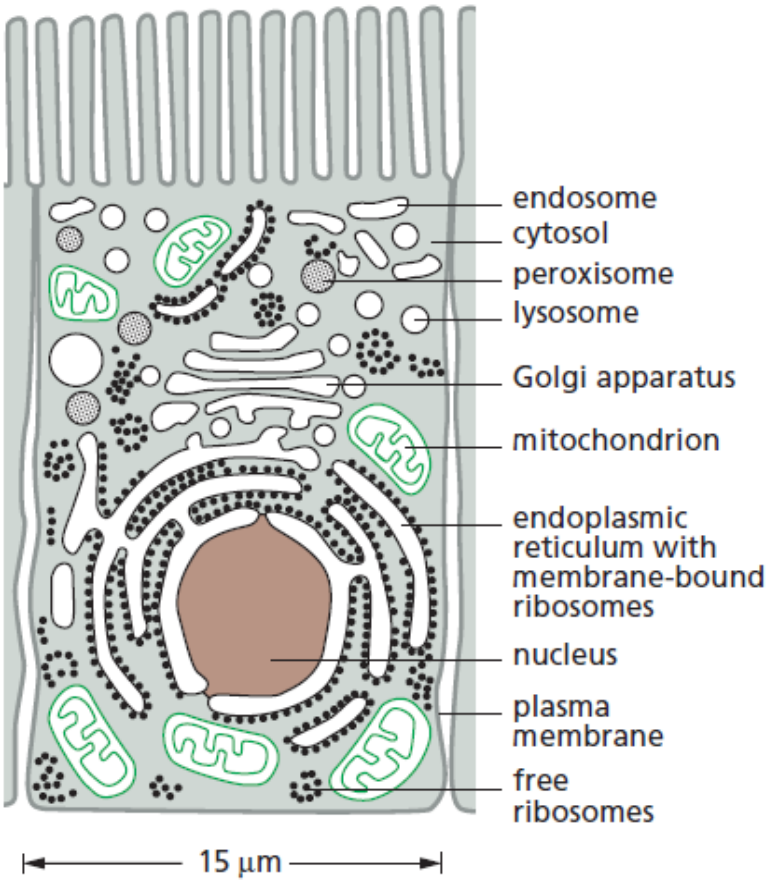
CELL COMPARTMENTATION

In eukaryotes: internal membranes create enclosed compartments and organelles in which different metabolic processes are segregated.



CELL COMPARTMENTATION

- Segregation of functions
- Local concentration of particular substances (proteins and enzymes)
- Membrane-enclosed organelles are selectively permeable
- Protein sorting: transfer of specific proteins to its compartment



FUNCTIONS OF DIFFERENT COMPARTMENTS

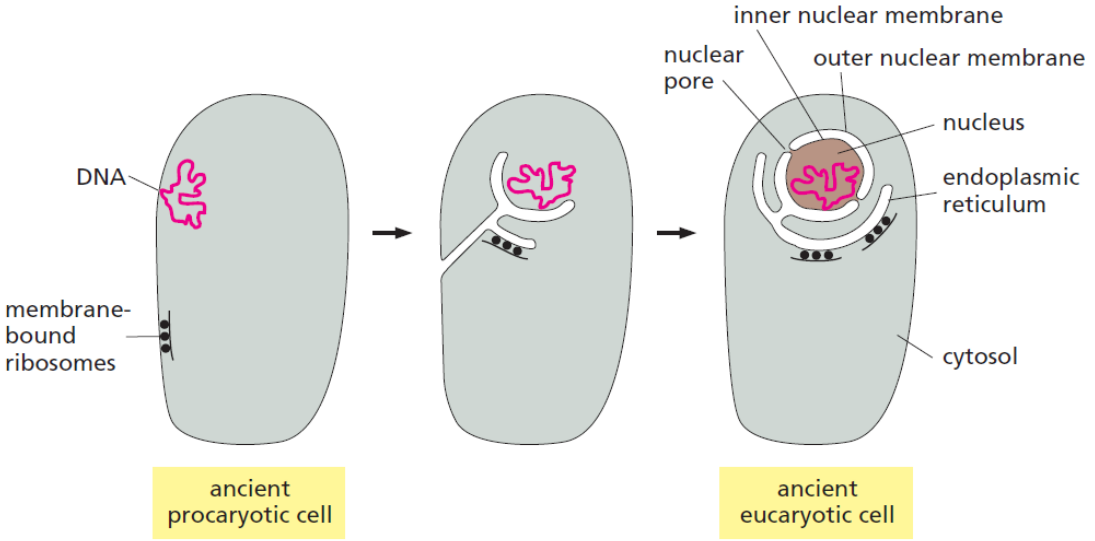
Compartment	Main function
Cytosol	Contains many metabolic pathways, protein synthesis
Nucleus	Contains main genome; DNA, RNA synthesis
Endoplasim reticulum (ER)	Synthesis of most lipids; synthesis of proteins for distribution to many organelles and to the plasma membrane
Golgi apparatus	Modification, sorting, and packaging of proteins and lipids for either secretion or delivery to another organelle
Lysosomes	Intracellular degradation
Endosomes	Sorting of endocytosed material
Mitochondria	ATP synthesis by oxidative phosphorylation
Chloroplasts (plants)	ATP synthesis and carbon fixation by photosynthesis
Peroxisomes	oxidation of toxic molecules

VOLUMES OF DIFFERENT COMPARTMENTS (HEPATOCYTE)

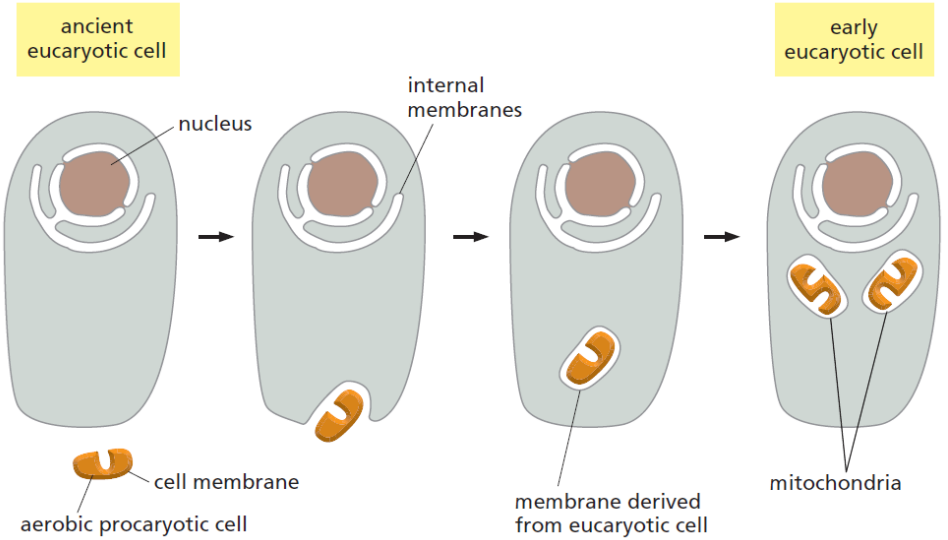
INTRACELLULAR COMPARTMENT	PERCENTAGE OF TOTAL CELL VOLUME	APPROXIMATE NUMBER PER CELL
Cytosol	54	1
Mitochondria	22	1700
Endoplasmic reticulum	12	1
Nucleus	6	1
Golgi apparatus	3	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

EVOLUTION OF MEMBRANE-ENCLOSED ORGANELLES

- From plasma membrane: nucleus, ER, GA, endosomes, lysosomes



- From prokaryotes: mitochondrion, chloroplasts

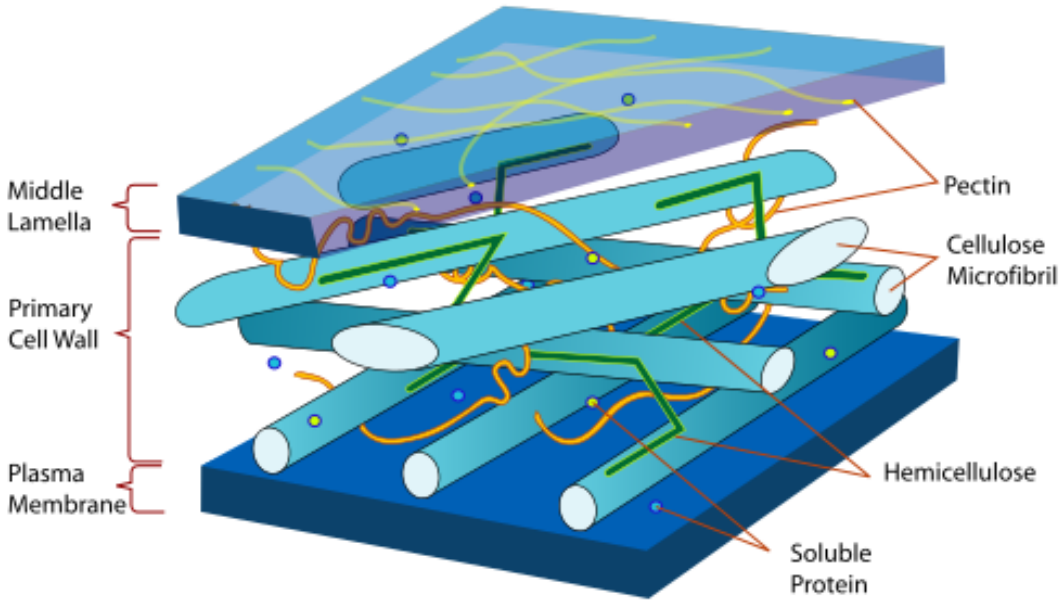


CELL WALL

Structural layer of cell outside the cell membrane

- **Prokaryotes, plants, fungi**
- **Functions: structural, defensive, maintaning the pressure**
- **Composition: glycoproteins, peptidoglycans, polysaccharide, proteins:**
  - **Plant (cellulose, hemicellulose, lignin)**
  - **Fungi (chitin, chitosan)**
  - **Algae (mammans, xylans, alginic acids)**
  - **Bacteria (proteoglycan: polysaccarides + D-amino acids)**

Plant cell wall



- **Bacretial classes:**
  - **Gram-positive (peptidoglycan, teichoic acids, thick walls)**
  - **Gram-negative (lipopolysaccharides, lipoproteins, thin walls)**

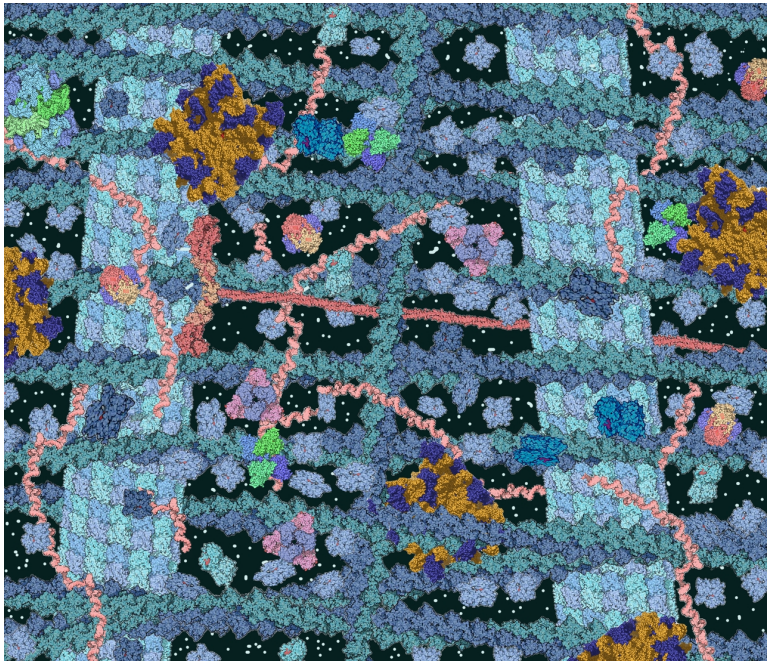
CYTOSOL

Part of cytoplasm, which is not separated by any membranes

- **The biggest volume of the cell**
- **Water + organelles + small molecules (~10^5)**
- **$[Ions]_{cytosol} \neq [Ions]_{extracellular}$**
- **Functions: osmogeneration, potentials, signaling, transport, creation of gradients**
- **Multiple levels of organization**
- **Water: 70% of cell, pH 7.0-7.4,**

  water clusters, high/low density,

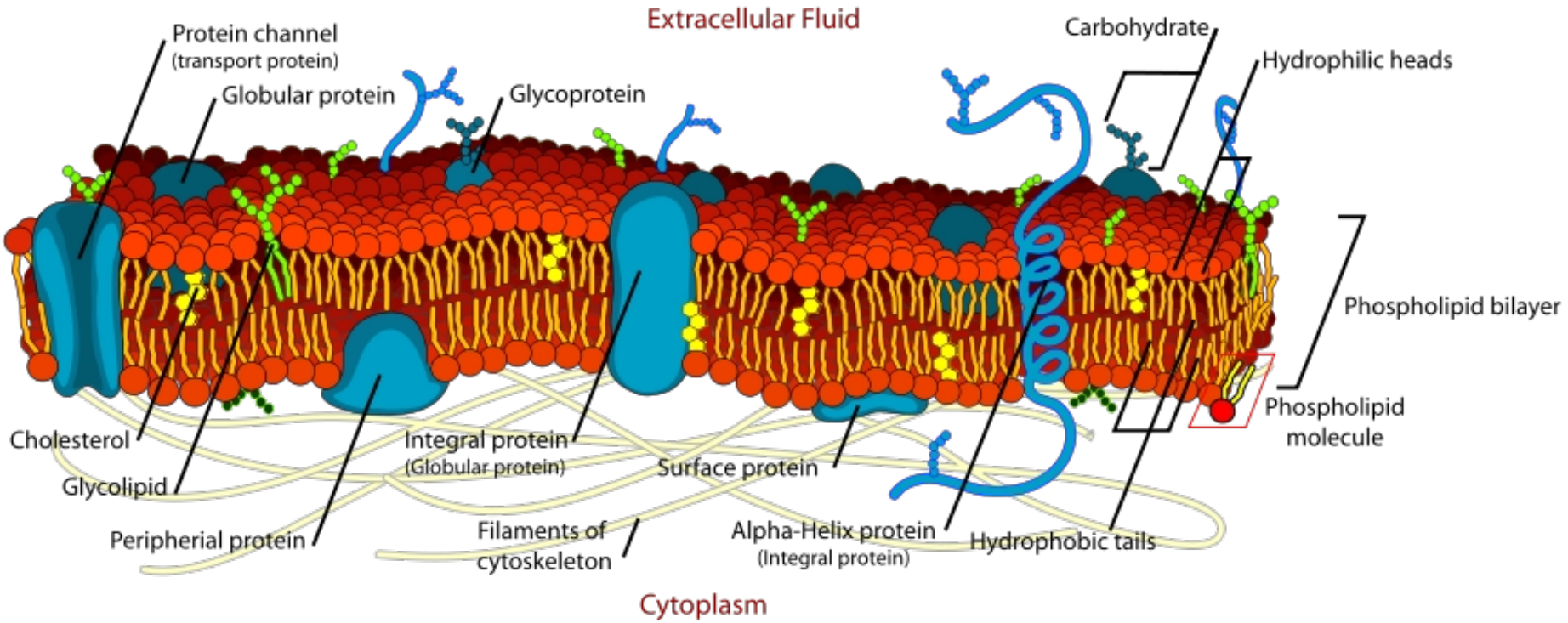
  5% => solvation shells



Ion	$[Ion]_{cytosol}$, mM	$[Ion]_{blood}$, mM
K^+	139	4
Na^+	12	145
Cl^-	4	116
HCO^{3-}	12	29
Amino acids in proteins	138	9
Mg^{2+}	0.8	1.5
Ca^{2+}	<0.0002	1.8

CELL MEMBRANE (L14-16)

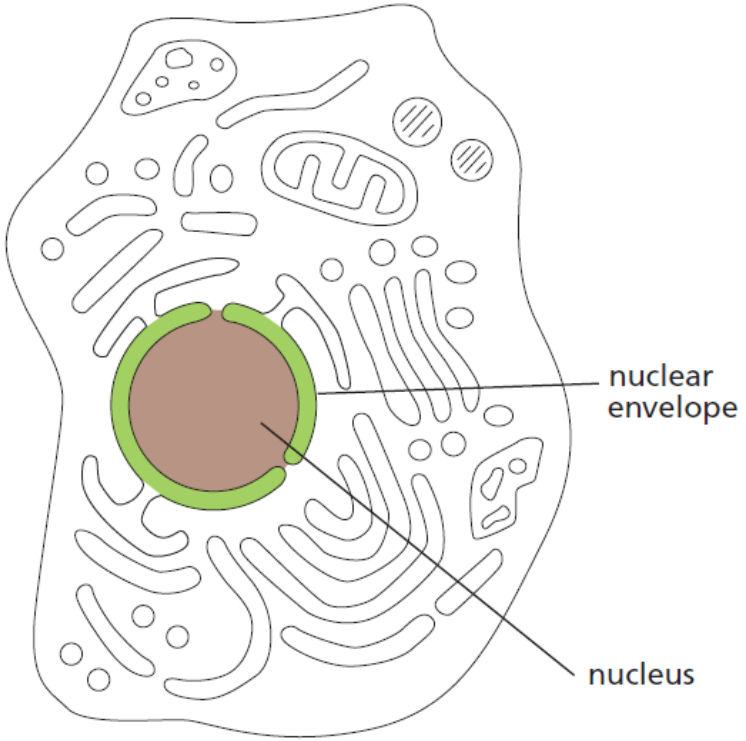
Cell membrane: separates cell interior from outside environment

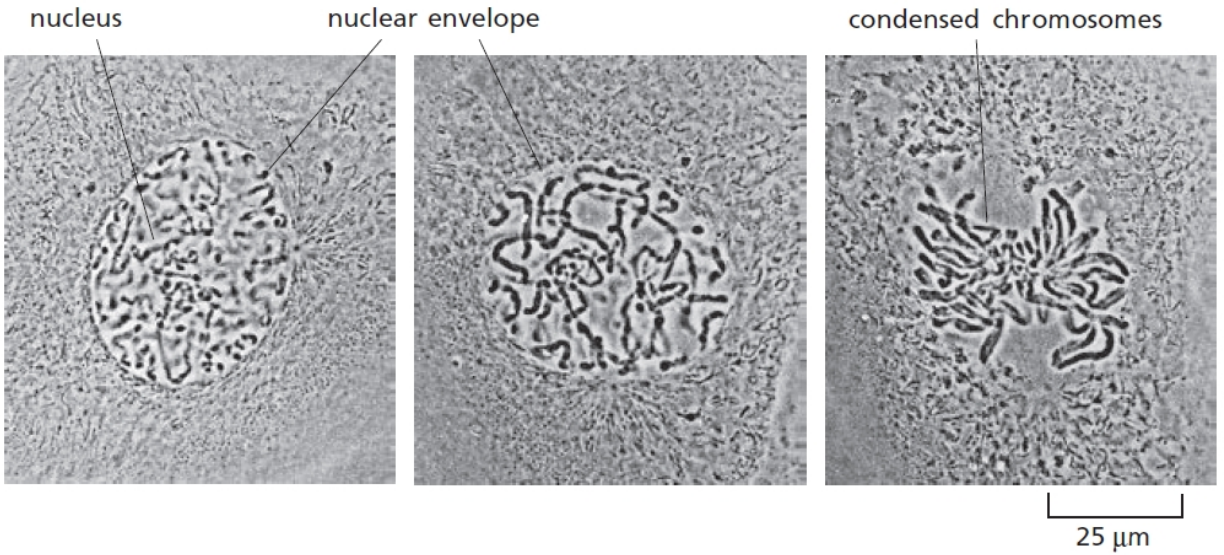


- Lipid bilayer => different types of lipids
- Proteins: transmembrane, peripheral, surface, channels
- Carbohydrates, glycoproteins, glycolipids

NUCLEUS

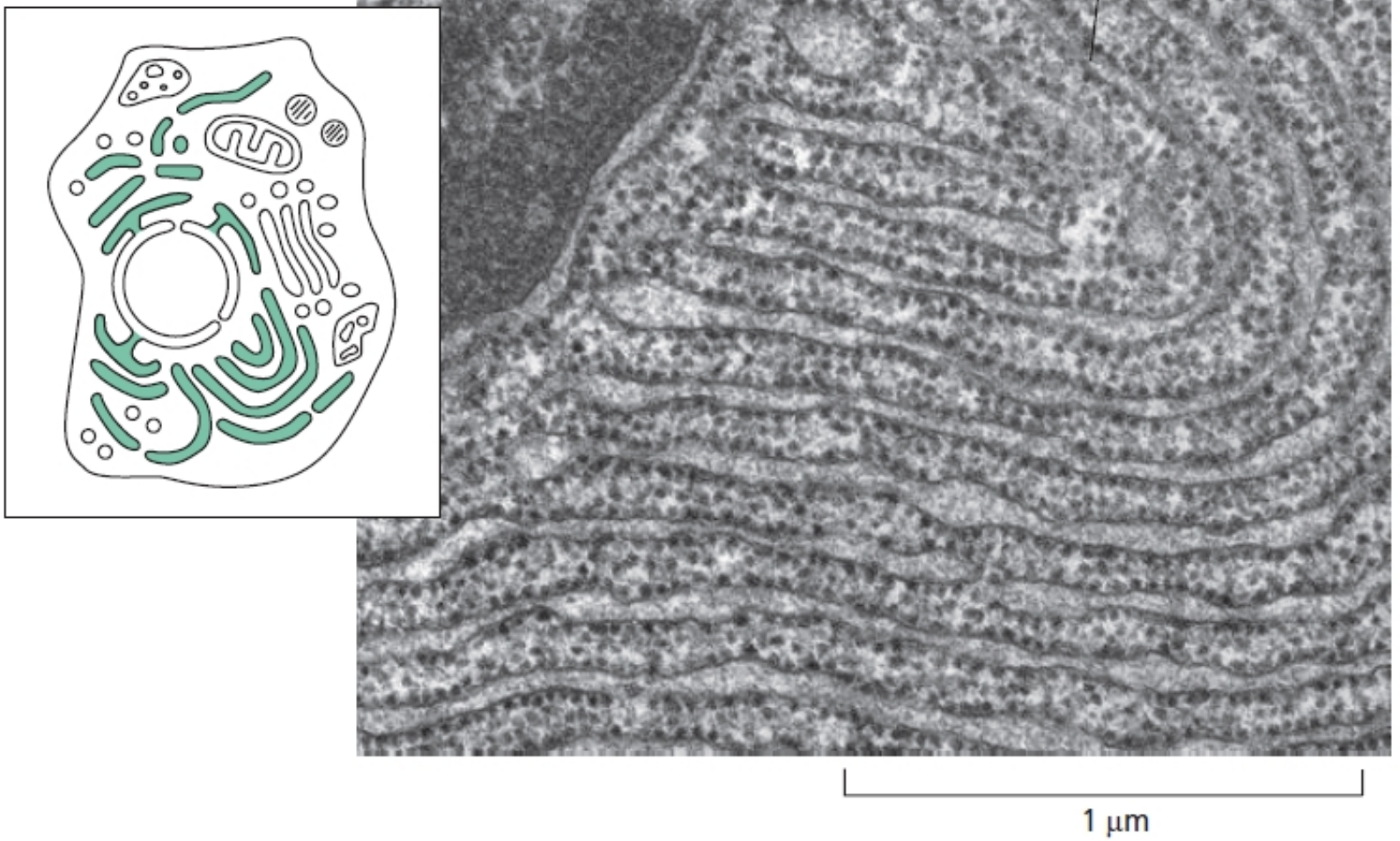
Contains most of the DNA (I14-L15-I16)



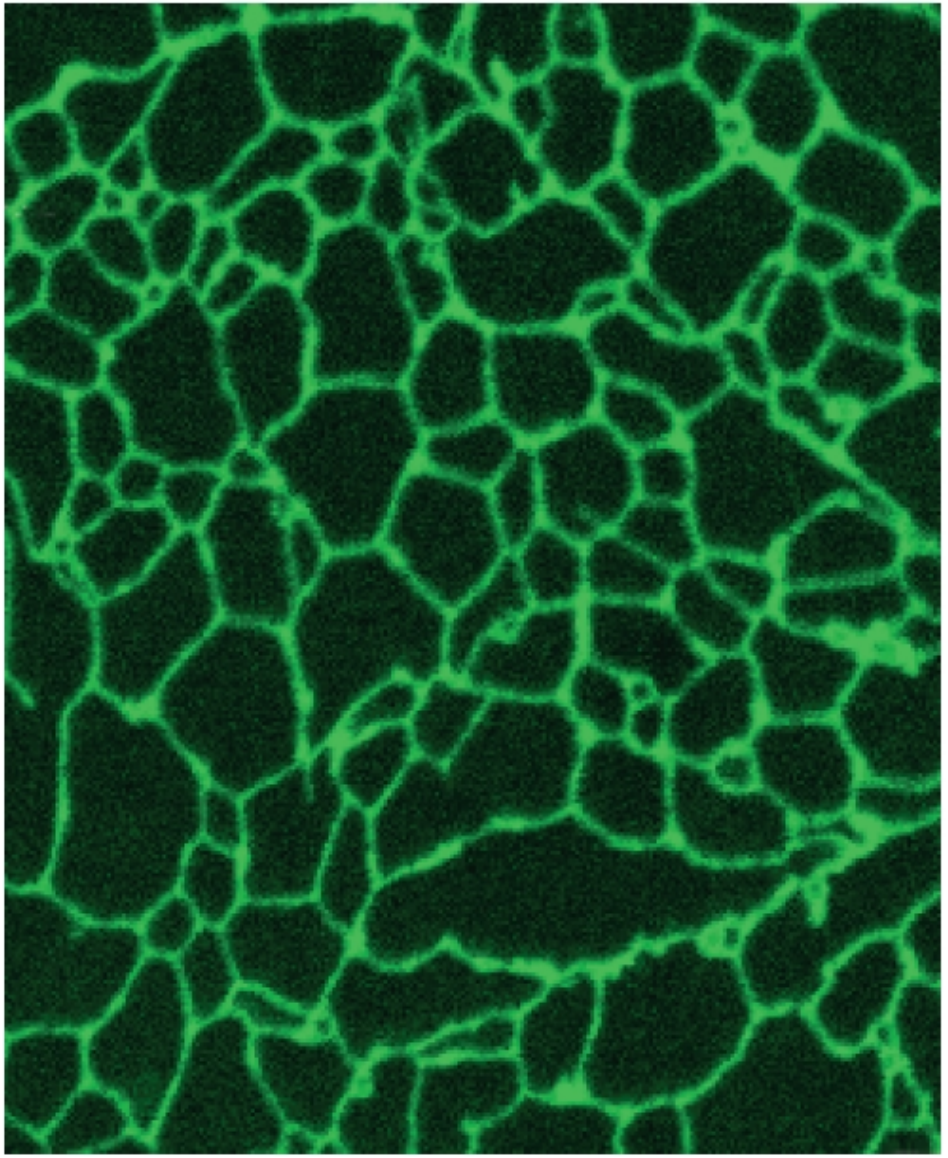


ENDOPLASMATIC RETICULUM

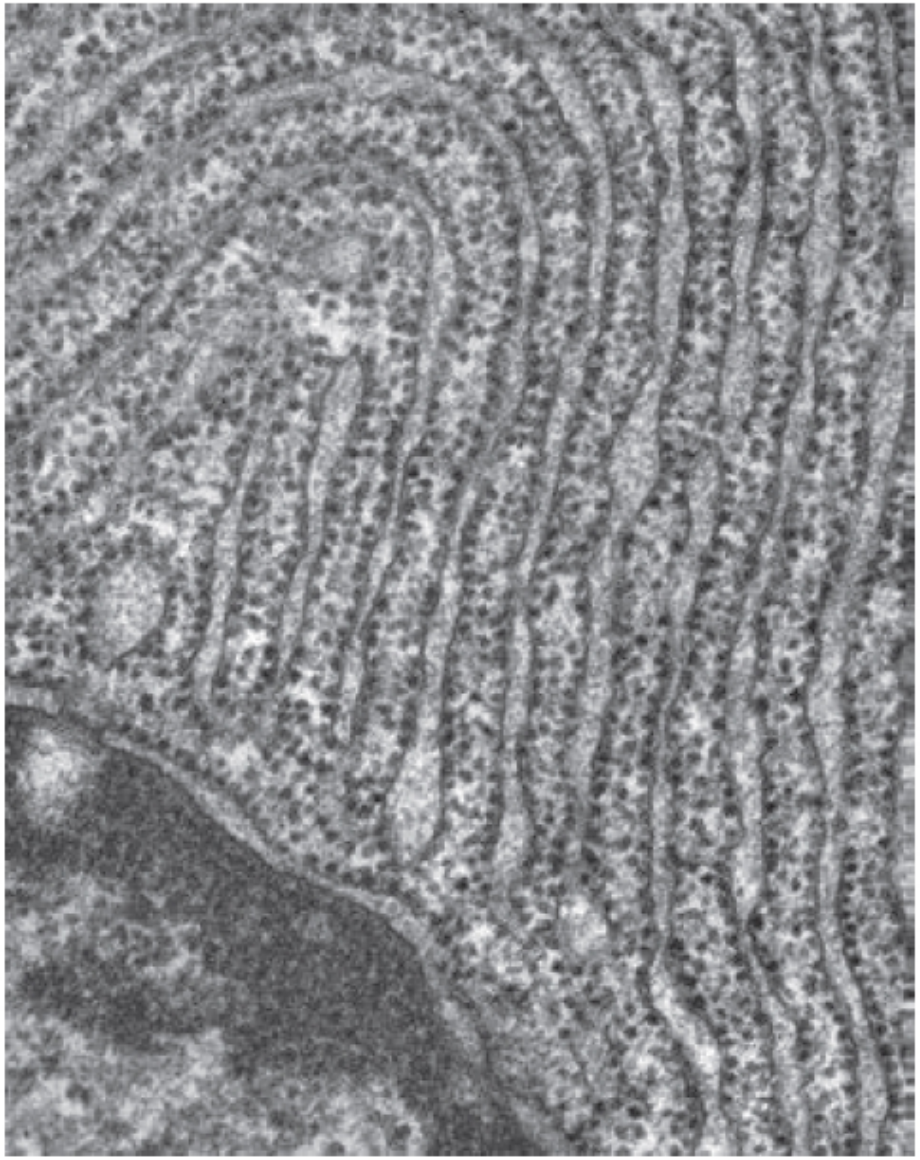
Interconnected membranes, where most of cell membrane components and secreted molecules are made



ENDOPLASMATIC RETICULUM



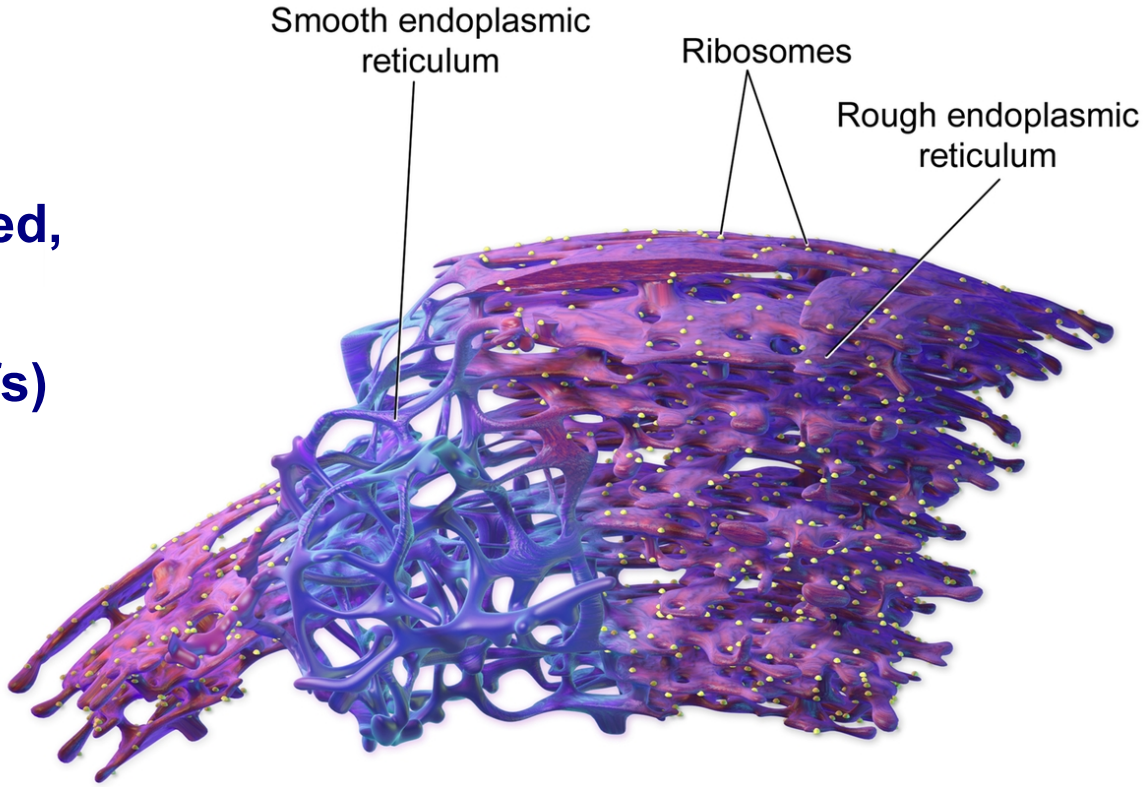
10 µm



200 nm

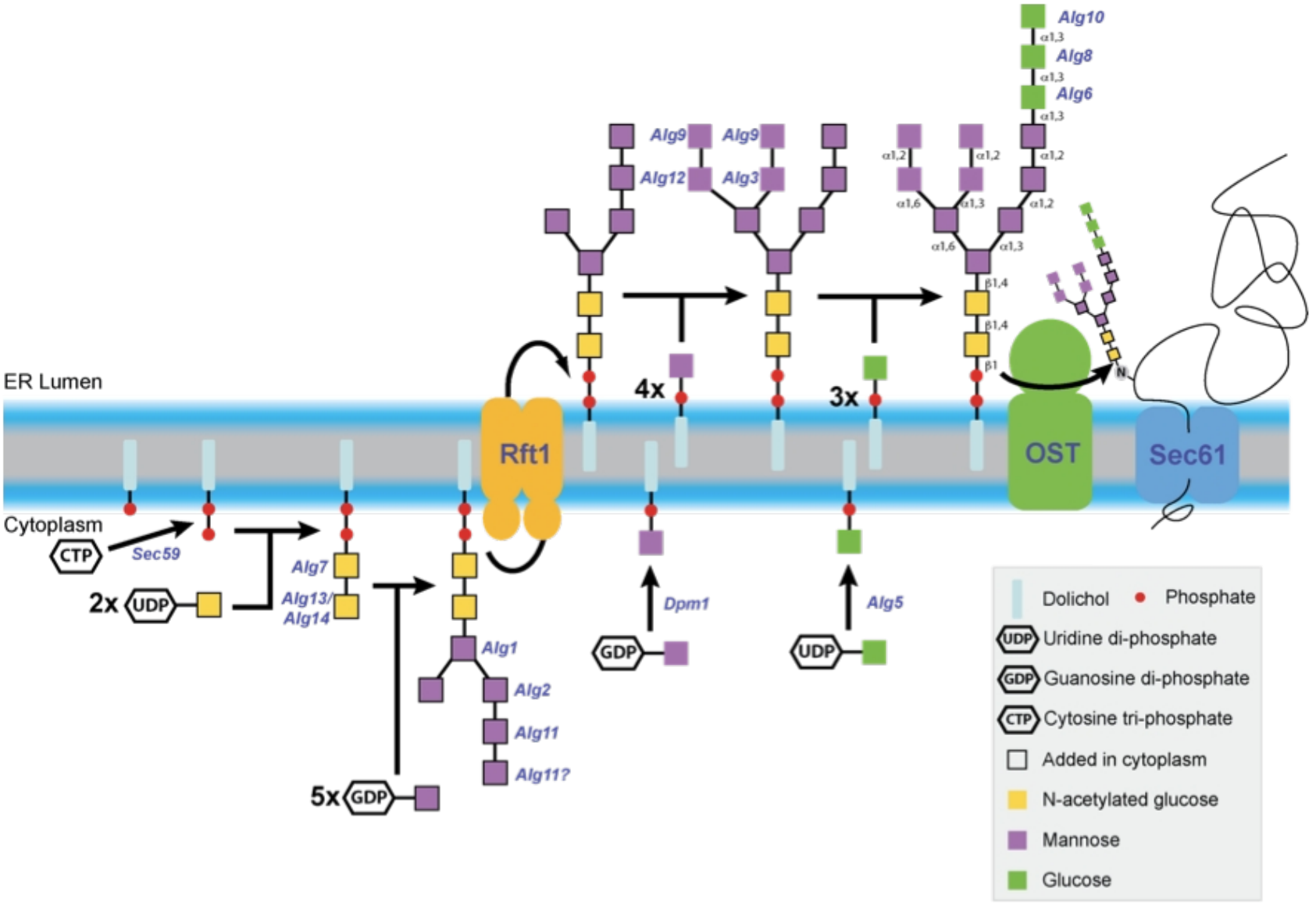
ROUGH AND SMOOTH ER

- **Rough:**
  - **full of ribosomes**
  - **communication with GA**
  - **peptidase, translocase**
  - **production of lysosomal, secreted, membrane proteins**
  - **N-glycosylation (NXS, NXT motifs)**



- **Smooth:**
  - **production of lipids, phospholipids, steroids**
  - **regulation of [Ca^{2+}]**
  - **steroid metabolism**
  - **glucose-6-phosphatase**
  - **sarcoplasmatic reticulum: myocytes, Ca^{2+} storage and release to stimulate muscles contraction**

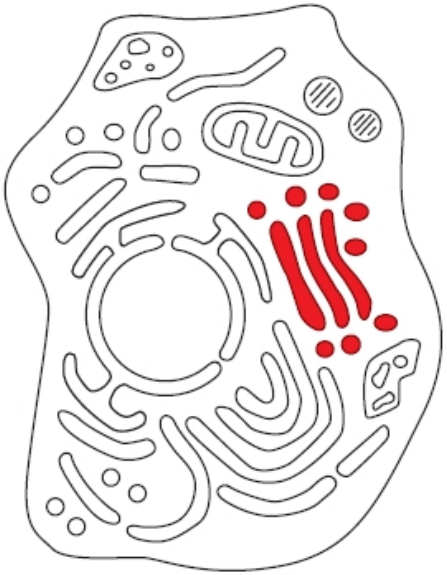
N-GLYCOSYLATION

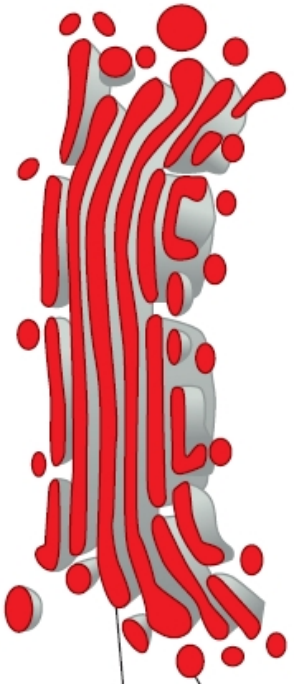


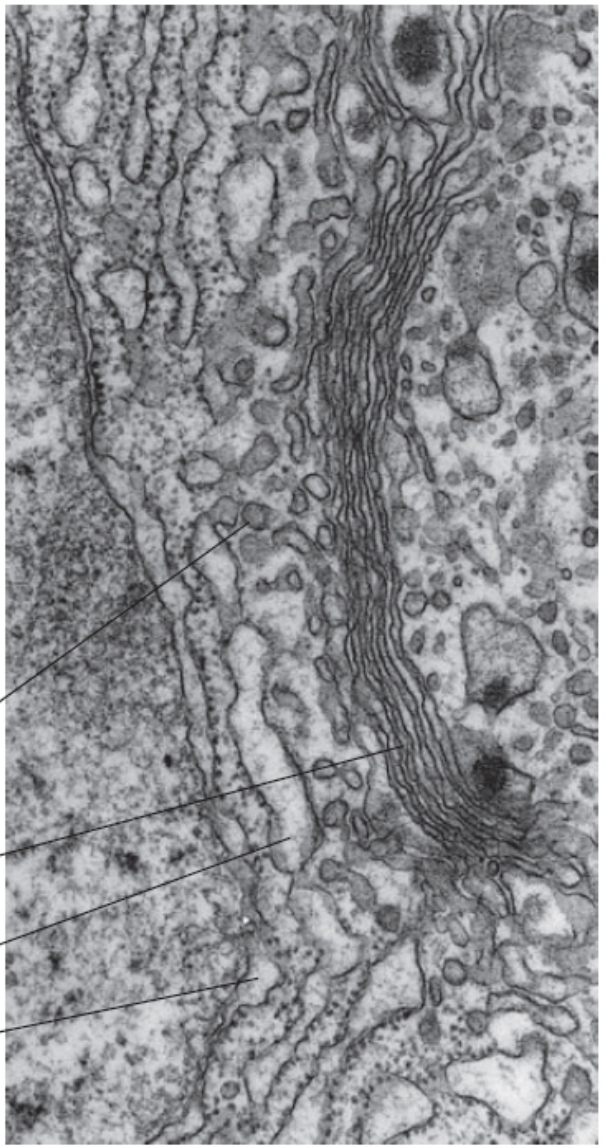
GOLGI APPARATUS

Membranes where the molecules produced in ER are further modified

(f.i. O-glycosylation)







PEROXISOMES

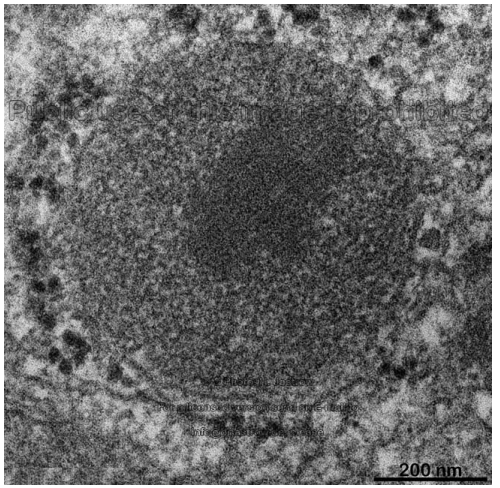
Peroxisomes: small, membrane-enclosed vesicles that provide a contained environment for reactions in which hydrogen peroxide, a dangerously reactive chemical, is generated and degraded.

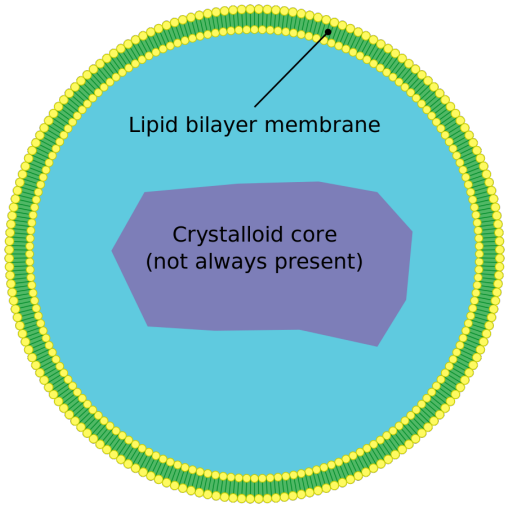
- **Catabolism: long and branched fatty acids, D-amino acids, polyamines**
- **Synthesis: ether phospholipids (plasmalogens => myelin), bile acids**
- **32 known peroxisome proteins: peroxins**
- **Assembly: from ER**
- **Reactions:**

  - oxidation $RH_2 + O_2 => R + H_2O_2$

  - $H_2O_2 + R'H_2 => R' + 2H_2O$

  - $2H_2O_2 => 2H_2O + O_2$





- **Diceases: adrenoleukodystrophy (fatty acids metabolism-associated), peroxisome biogenesis disorders**

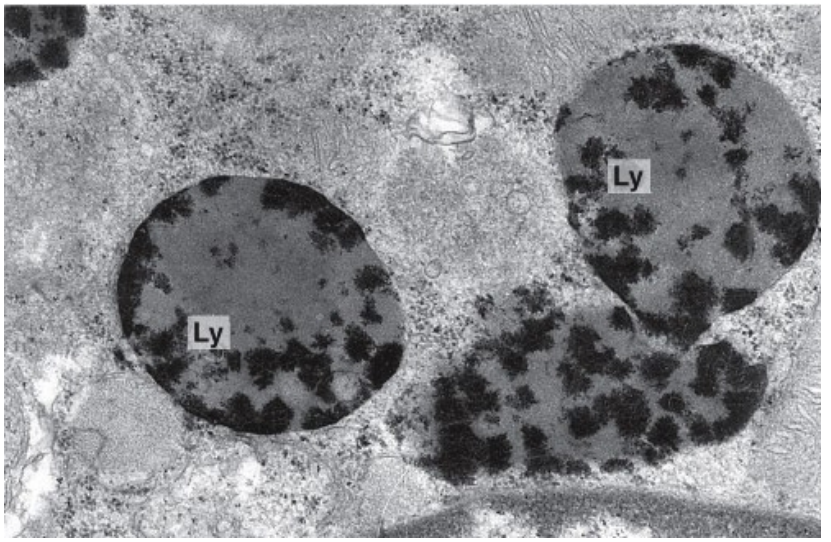
LYSOSOMES

Lysosomes: small, irregularly shaped organelles in which intracellular digestion occurs, releasing nutrients from food particles and breaking down unwanted molecules for recycling or excretion.

- **Degradation of all kinds of biomolecules, other organelles**
- **Optimal pH ~ 4.5**
- **~ 50 proteins (Man-6P signal)**
- **Plasma membrane repair, signalling, energy exchange**
- **Assembly: from ER**
- **Diceases:**

  - lysosomal storage disorders

  - metachromatic leukodystrophy (myelin-associated)



VACUOLE

- **Vacuole:**

  - **Isolating/exporting harmful materials**

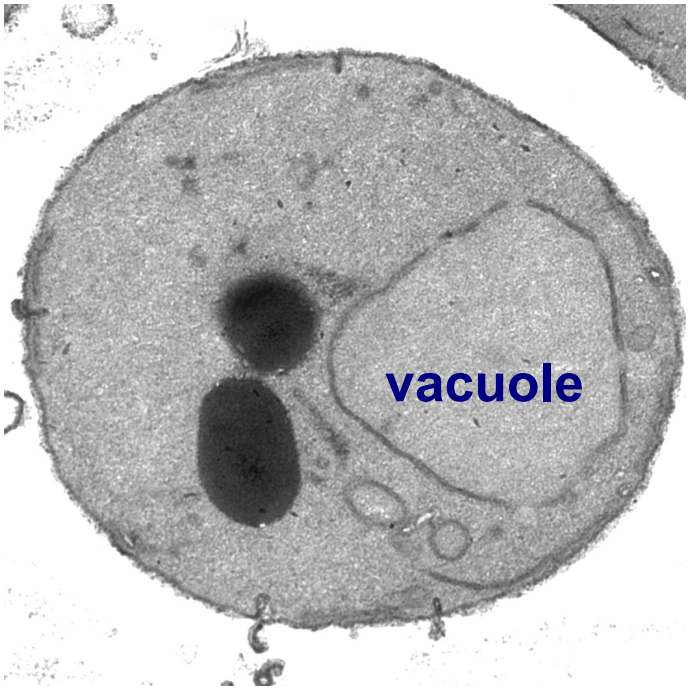
  - **Containing waste products/water in plant cells**

  - **Maintaining hydrostatic pressure and pH**

  - **Depot of small molecules**

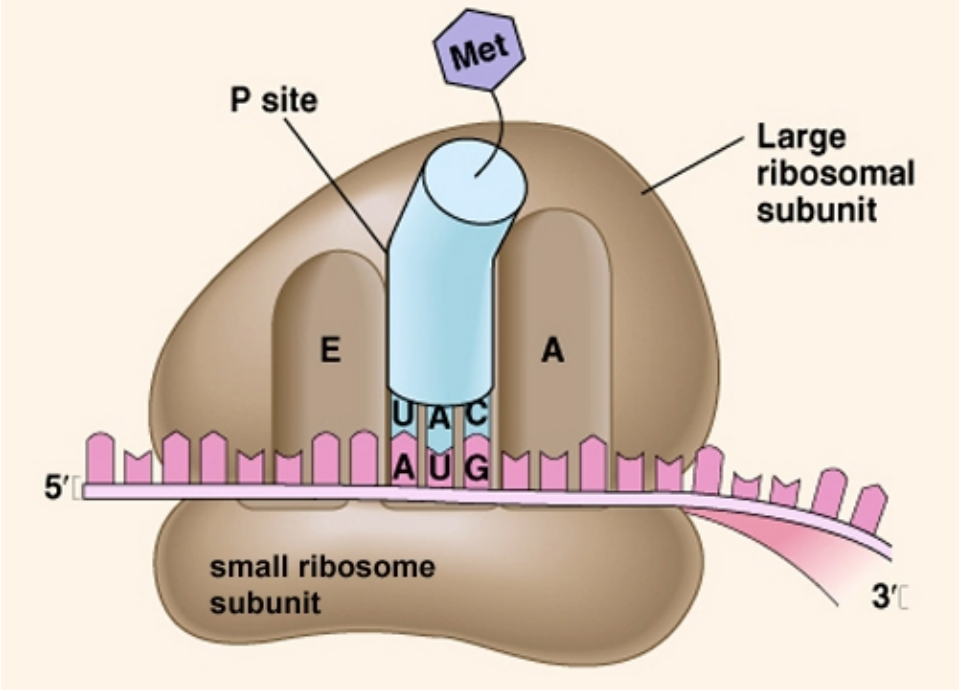
  - **Supporting structures in plants**

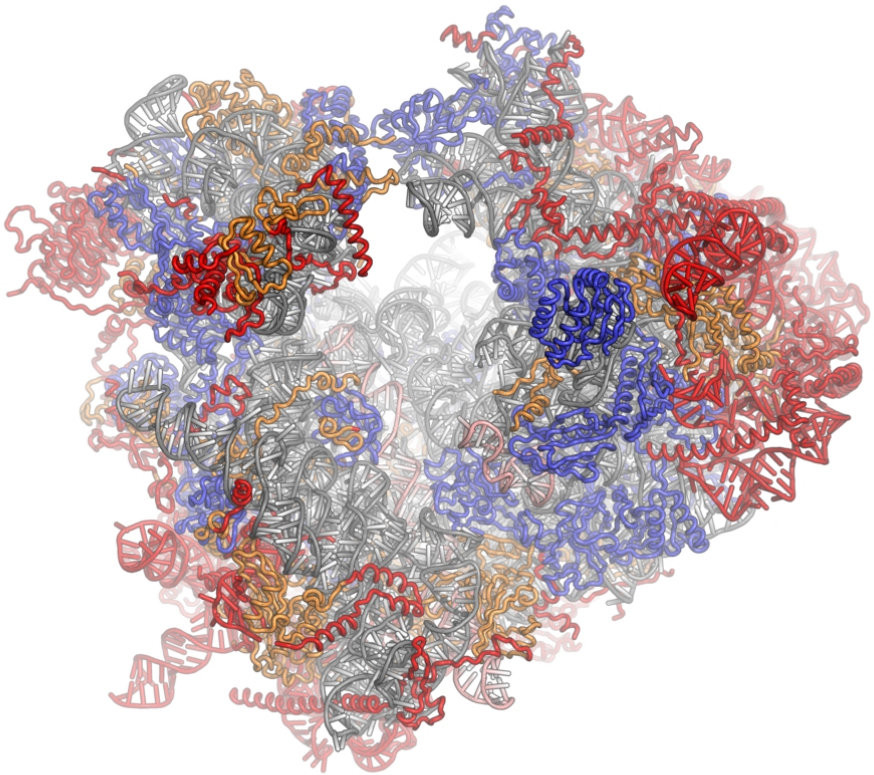
  - **Storing proteins needed for germination**



RIBOSOMES

Multimolecular machines, which carry out translation (RNA=>protein)



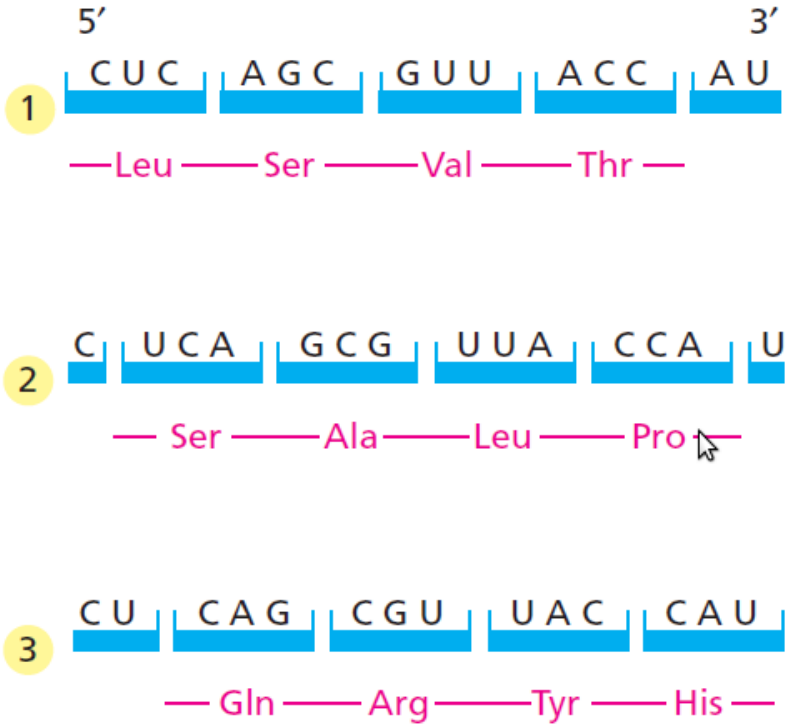


- **Protein + RNA**
- **25-30 nm**
- **Structures: up to 3.5 Å**

mRNA=>PROTEIN: GENETIC CODE

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

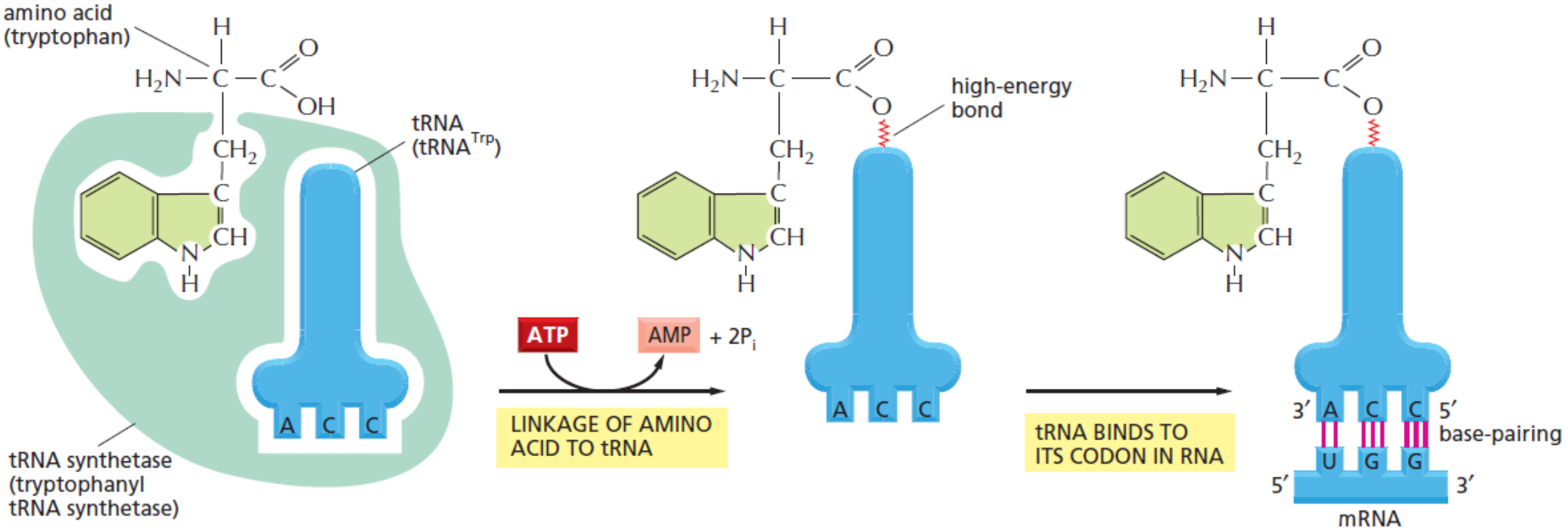
Three frames for reading mRNA



tRNA: BETWEEN mRNA AND PROTEIN

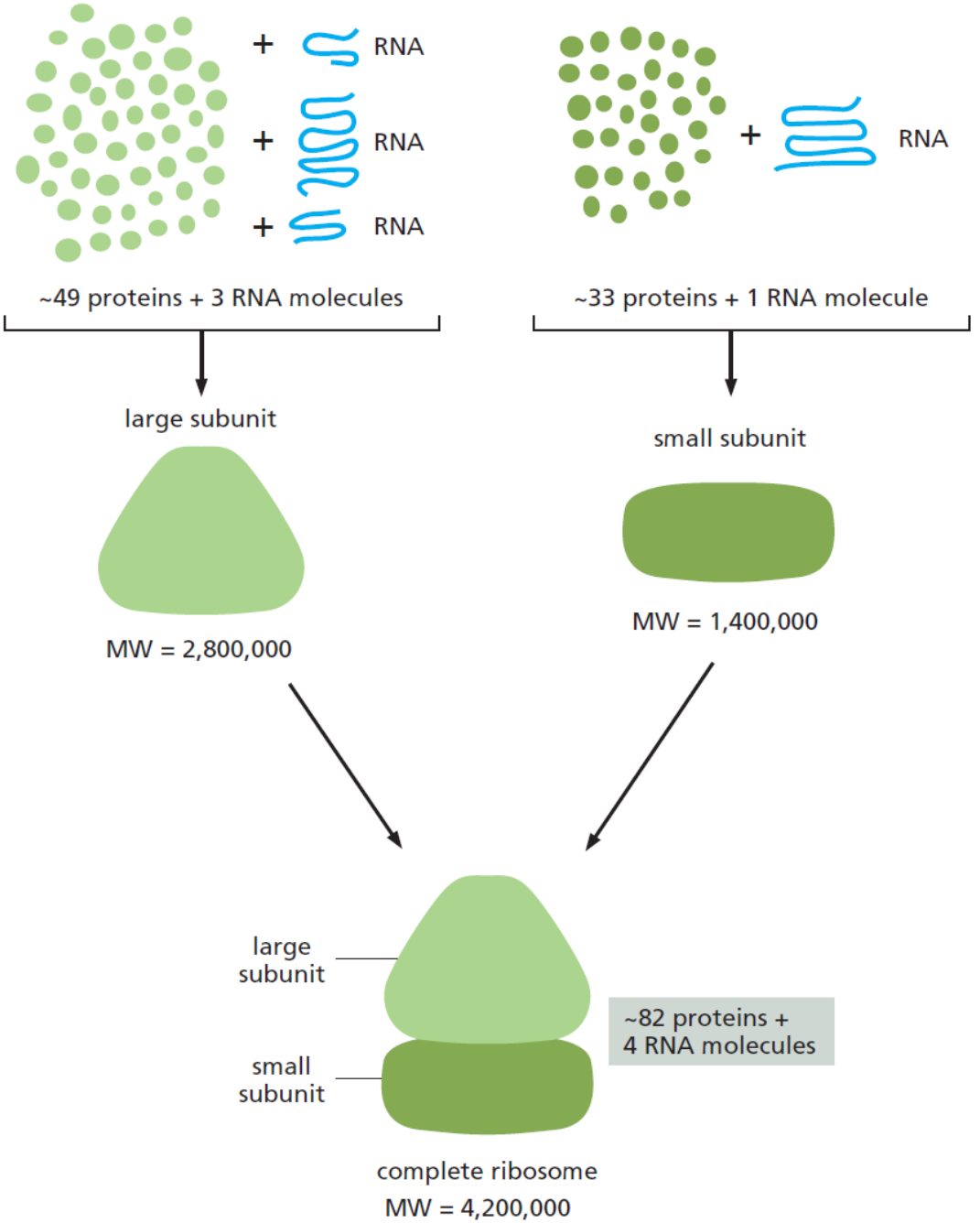
mRNA does not directly recognize an amino acid, so

an adaptor is needed: tRNA

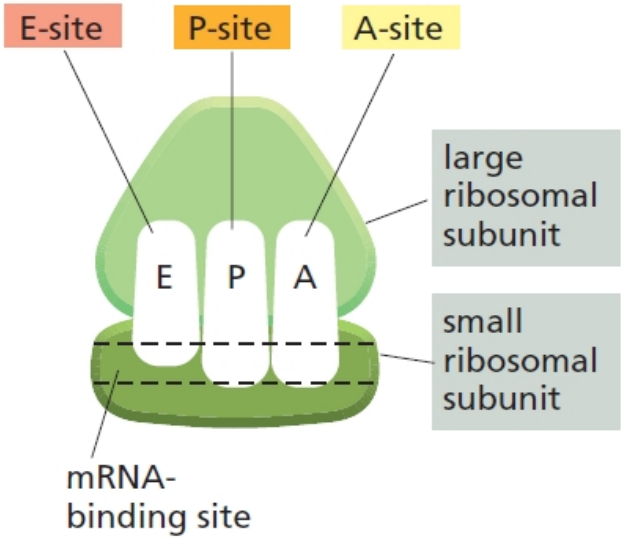


Speed: 2aa/s (eukaryotes), 20aa/s (bacteria)

RIBOSOME COMPOSITION

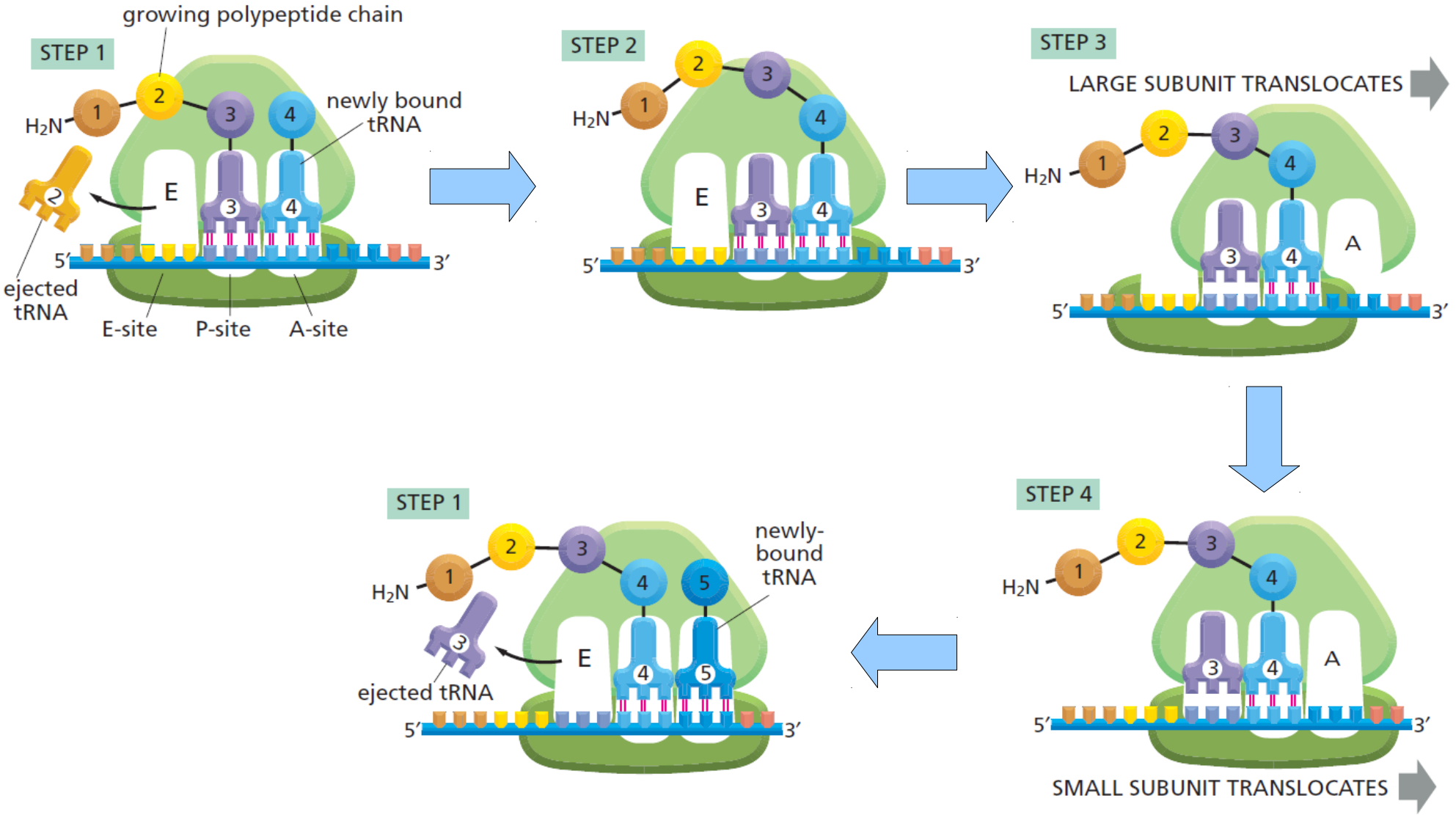


RIBOSOME STRUCTURE



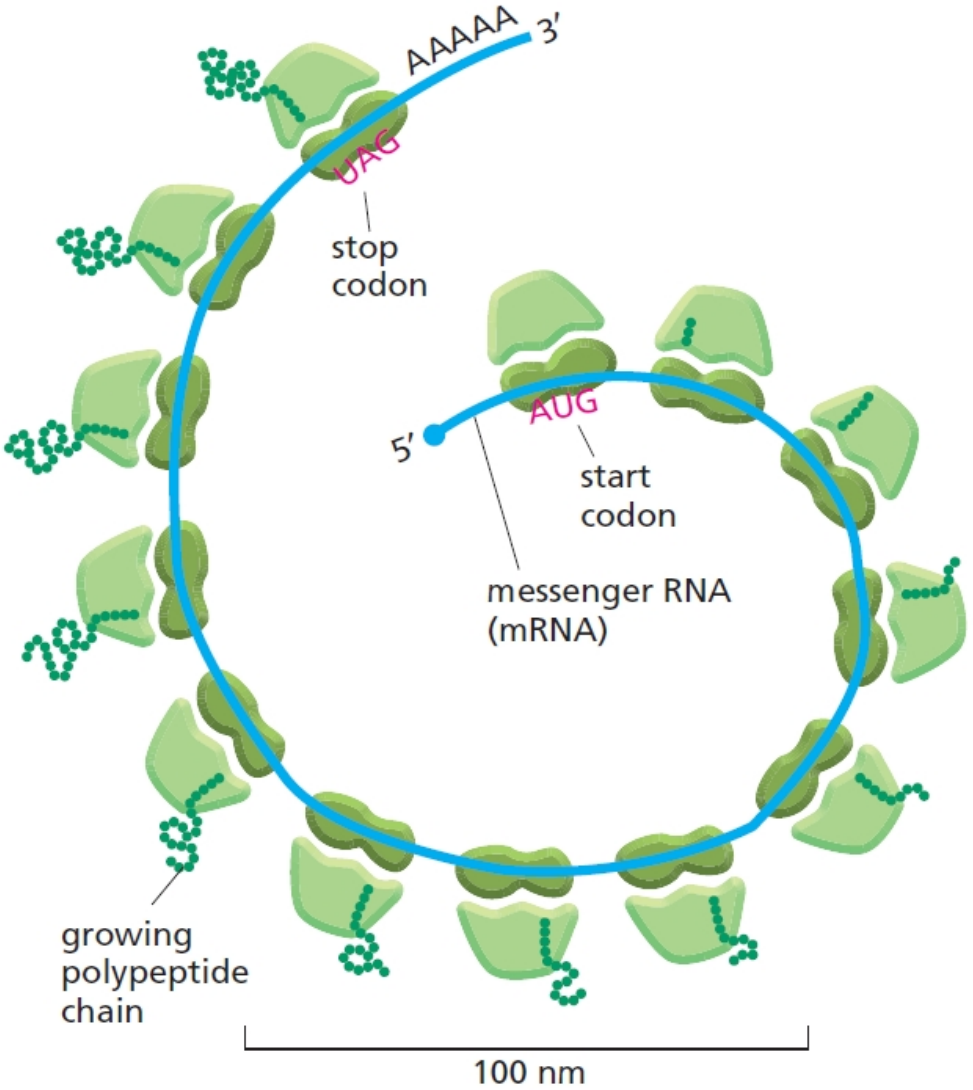
- **A-site: tRNA**
- **P-site: a corresponding amino acid**
- **E-site: used tRNA**

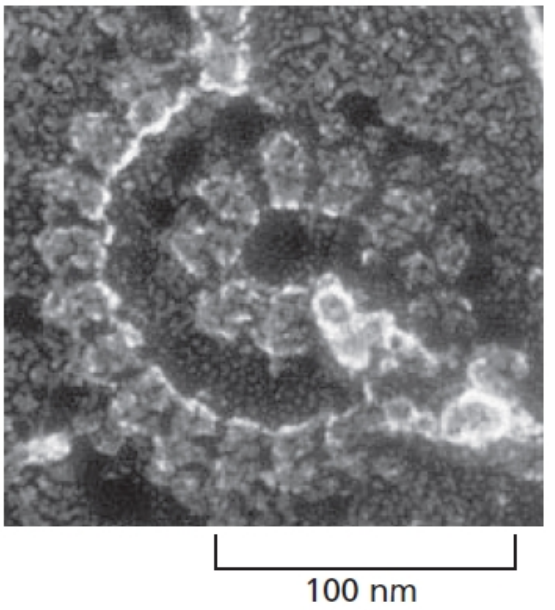
RIBOSOME MECHANISM



RIBOSOME MECHANISM

- Start (Met) and stop codons
- Initiation and termination factors
- Polyribosomes

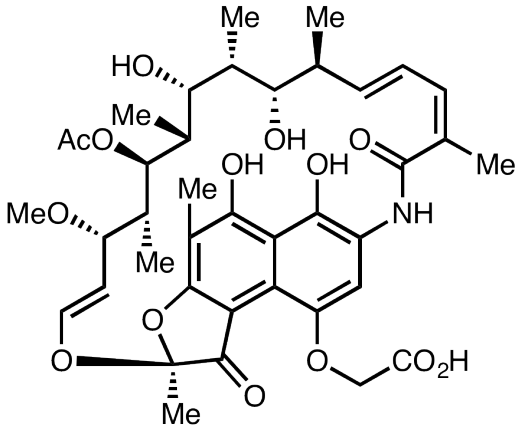




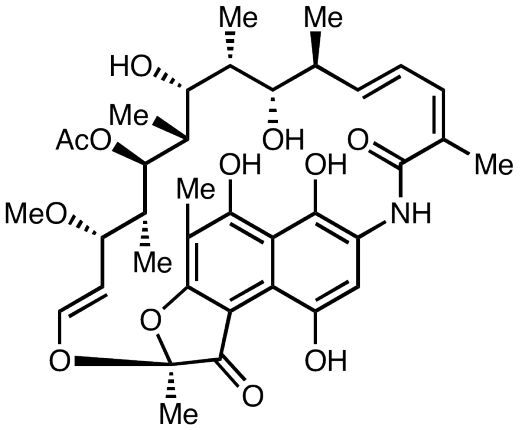
CLASS OF ANTIBIOTICS WITH RIBOSOME-RELATED MECHANISM

Antibiotics: substances which kill or inhibit growth/development of bacteria

ANTIBIOTIC	SPECIFIC EFFECT
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from initiation complex to chain-elongating ribosome ; also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes
Cycloheximide	blocks the translocation reaction on ribosomes
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase



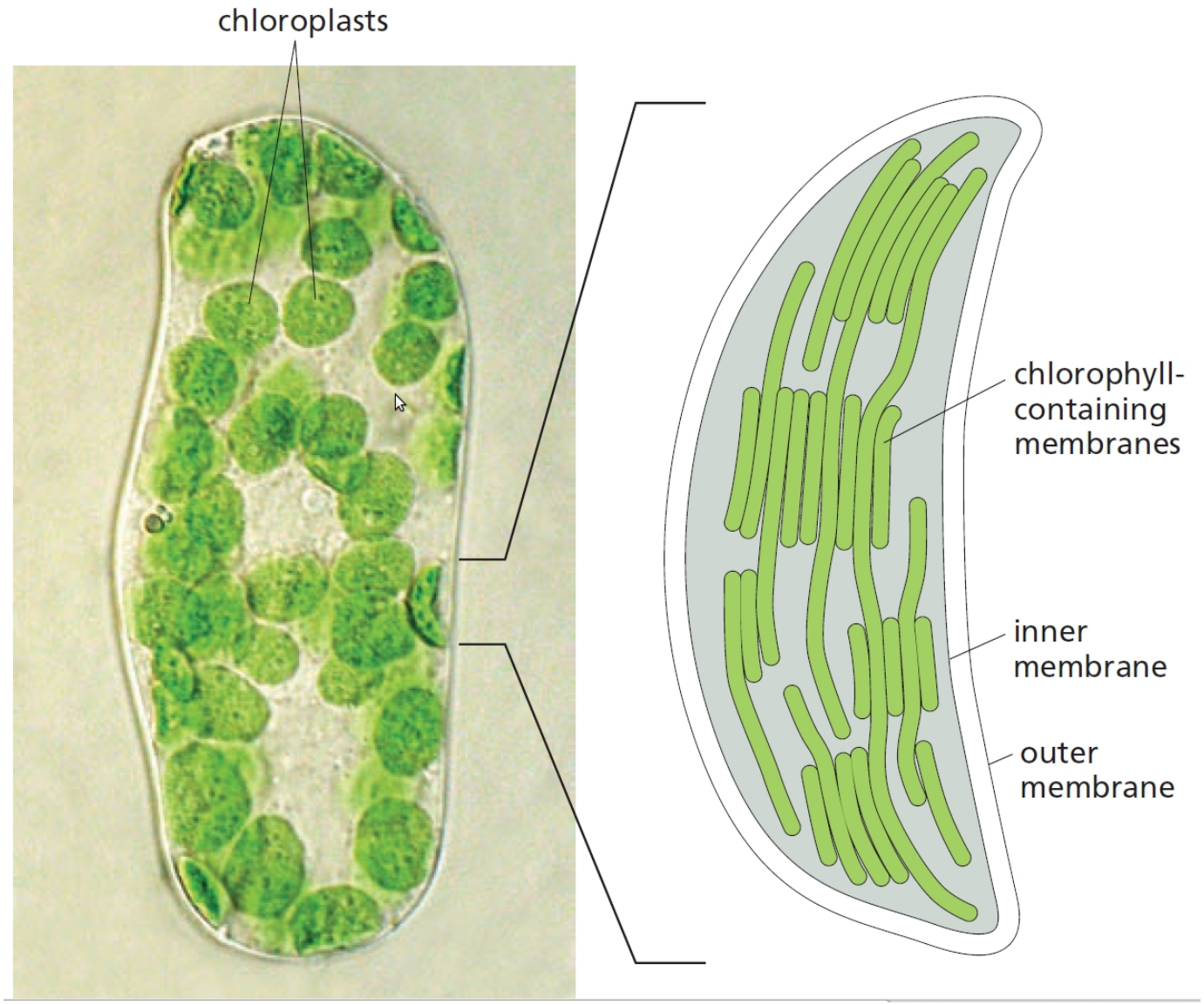
Rifamycin B



Rifamycin SV

CHLOROPLASTS

Organelles which are responsible for photosynthesis (L3)



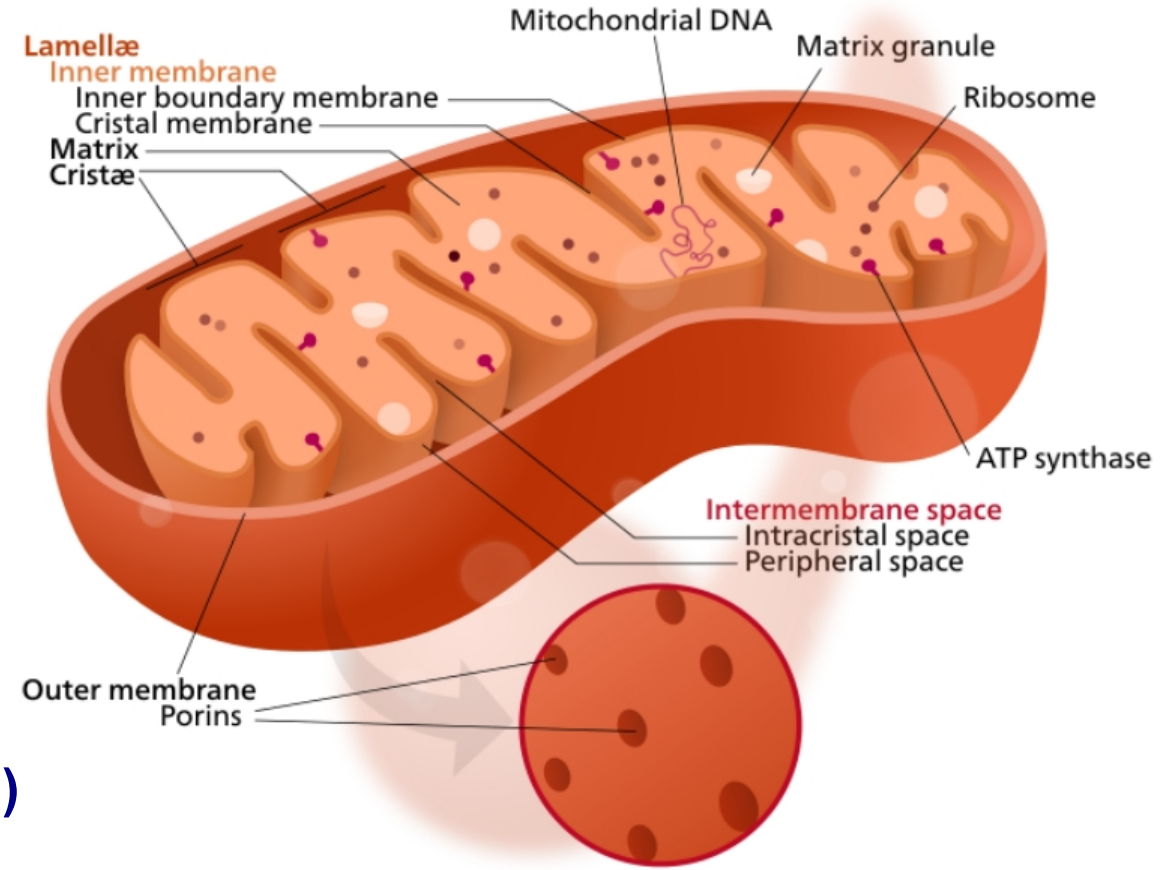
MITOCHONDRION

Mitochondrion: double membrane-bound organelles responsible for respiration/energy production of the cell.

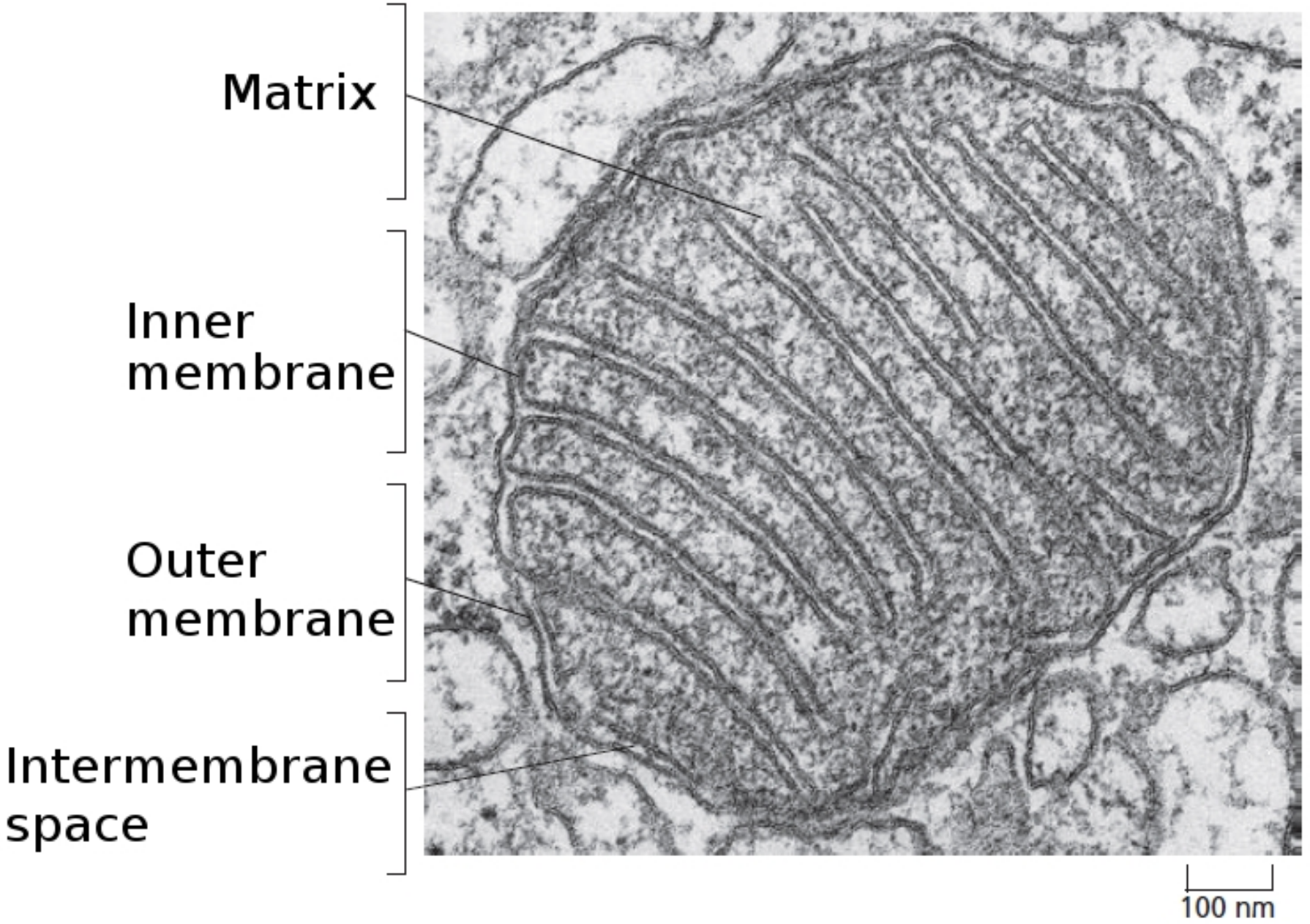
- **Originated from bacteria**
- **Own genome (37 genes in human)**
- **Main source of energy: glucose => 30 ATP (glycolysis 2 ATP): oxidative phosphorylation**
- **Double membrane**
- **Many mitochondrial diseases:**
  - **diabetes**
  - **myopathy**
  - **endocrinopathy**
  - **autism**
  - **...**

MORPHOLOGY OF MITOCHONDRIA

- **Outer membrane:**
  - 60-75 Å thick
  - protein/lipid 1:1
  - Porins/translocase
  - ER <=> Ca^{2+} signaling
- **Intermembrane space:**
  - Homologous to cytosol
  - Cytochrome C
- **Inner membrane:**
  - protein/lipid 3:1 (151 proteins)
  - 1/5 of mitochondria
  - impermeable for most molecules via diffusion
  - Functions: oxidative phosphorylation, ATP synthesis, protein import machinery, mitochondrial fusion/fission proteins, transport regulation
  - Cristae: inner membrane compartmentalization
- **Matrix**



MORPHOLOGY OF MITOCHONDRIA

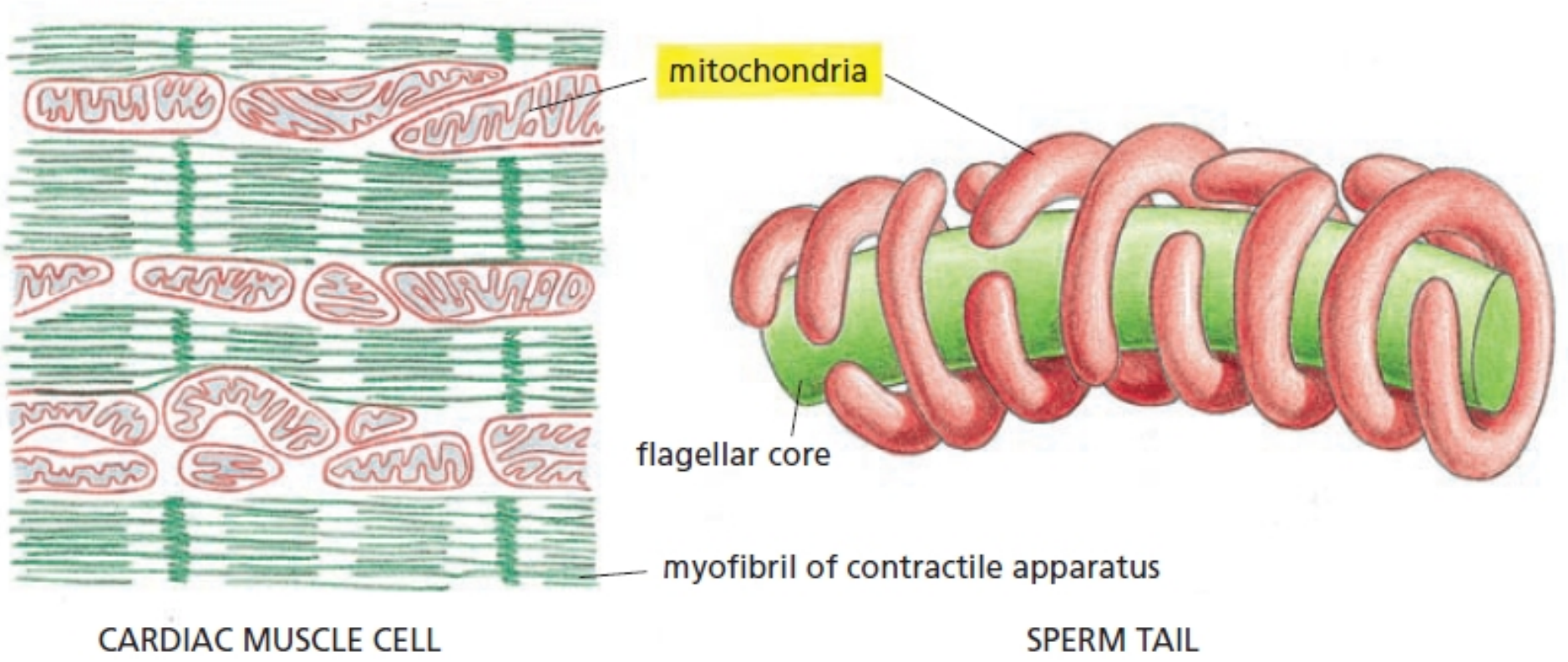


LOCALIZATION IN THE CELL

- **Attachment to:**

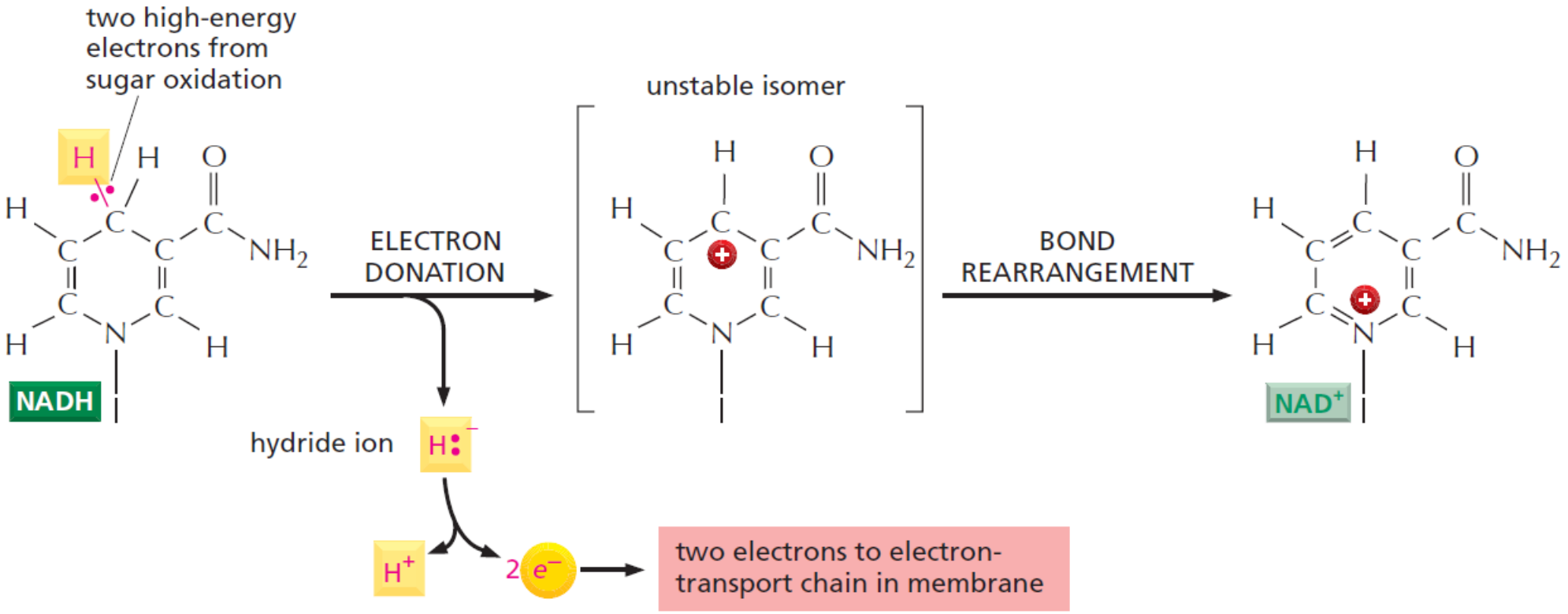
  - ER membranes

  - contractile apparatus in muscles/spermotozoids



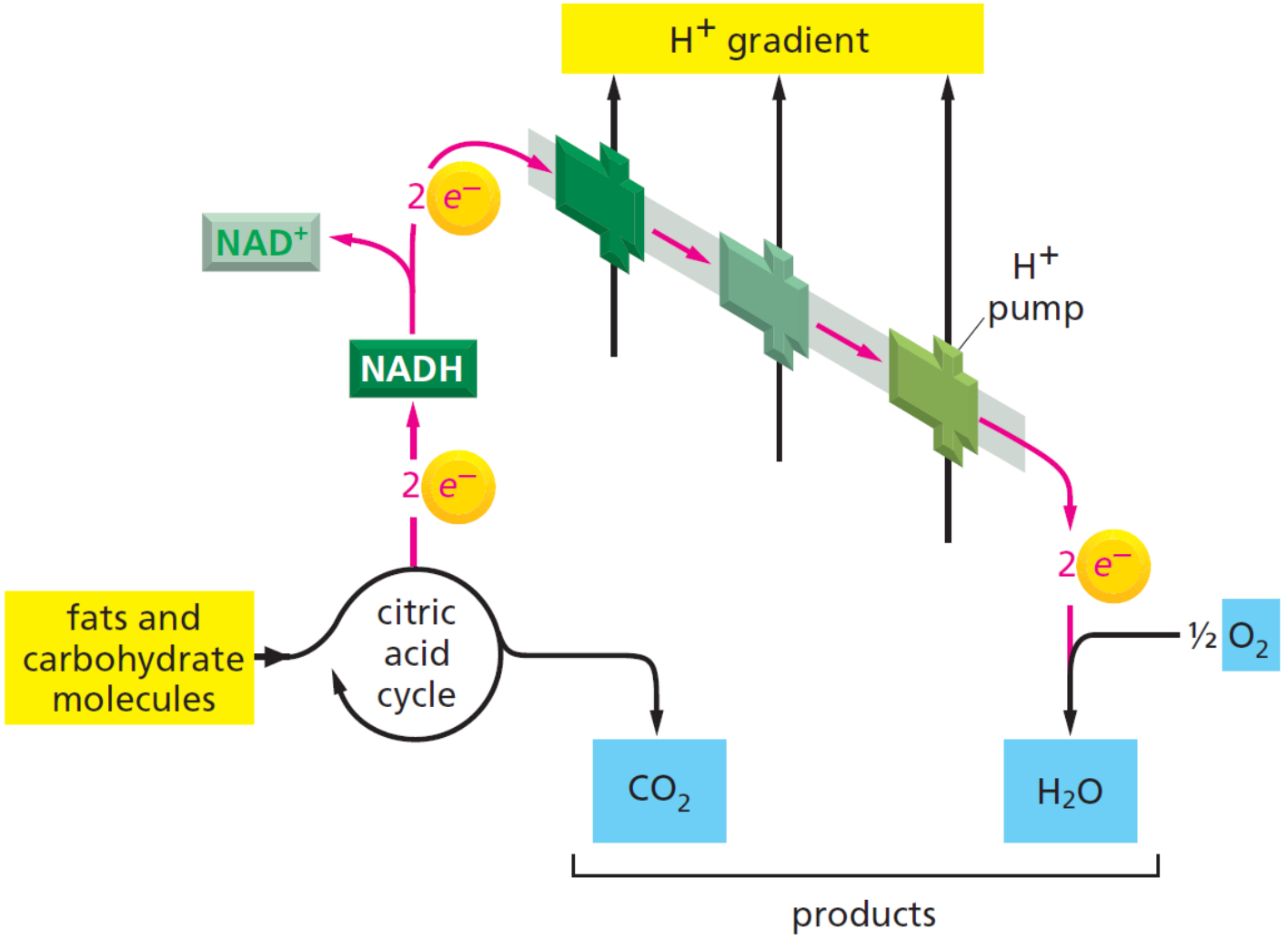
GENERATION OF HIGH-ENERGY ELECTRONS

- Glucose, pyruvate => matrix => AcCoA
- Citric acid cycle => CO_2 + NADH + $FADH_2$

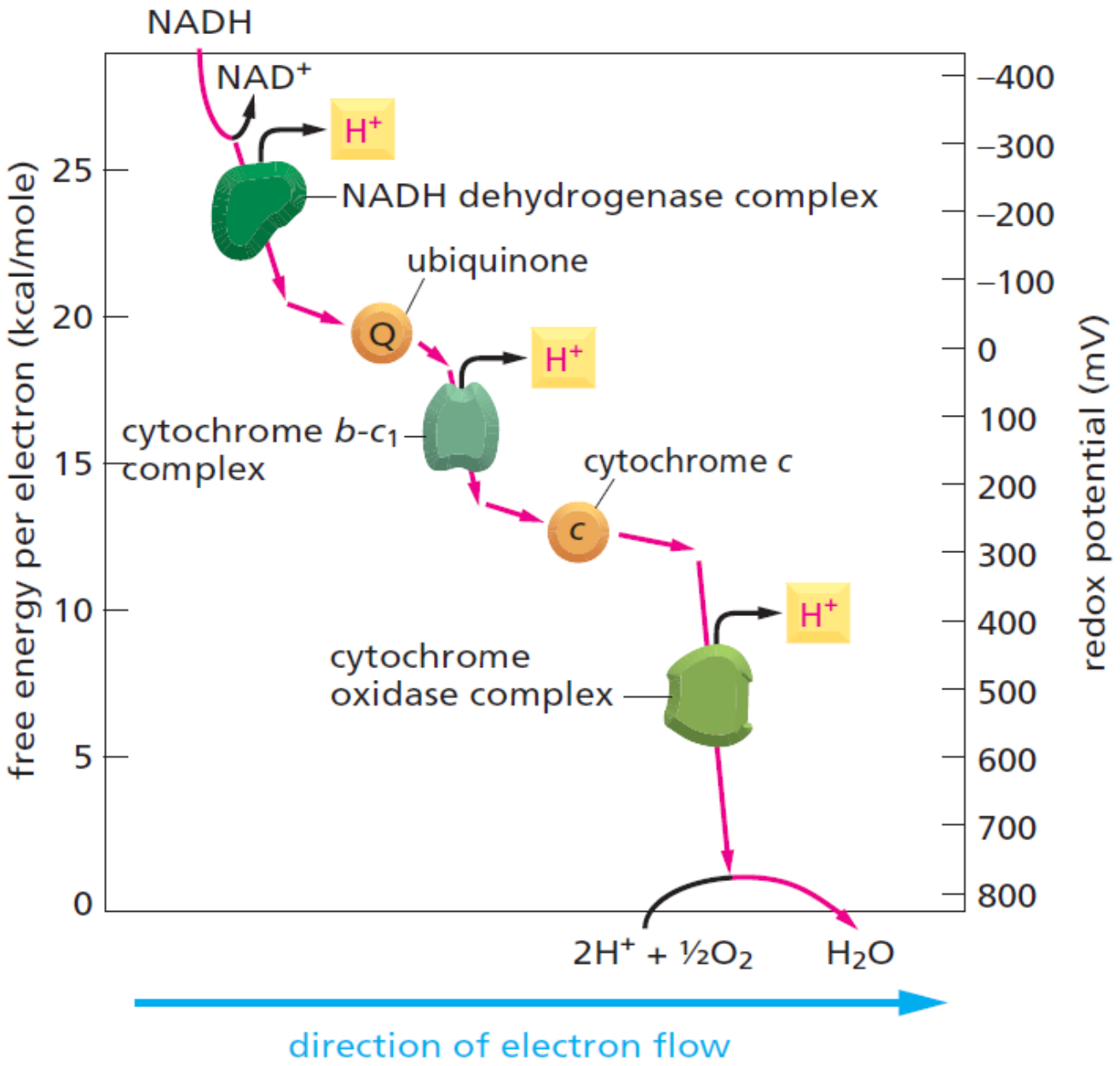


FATE OF HIGH-ENERGY ELECTRONS

Electrons are further transferred to O_2 => H_2O creating proton gradient.

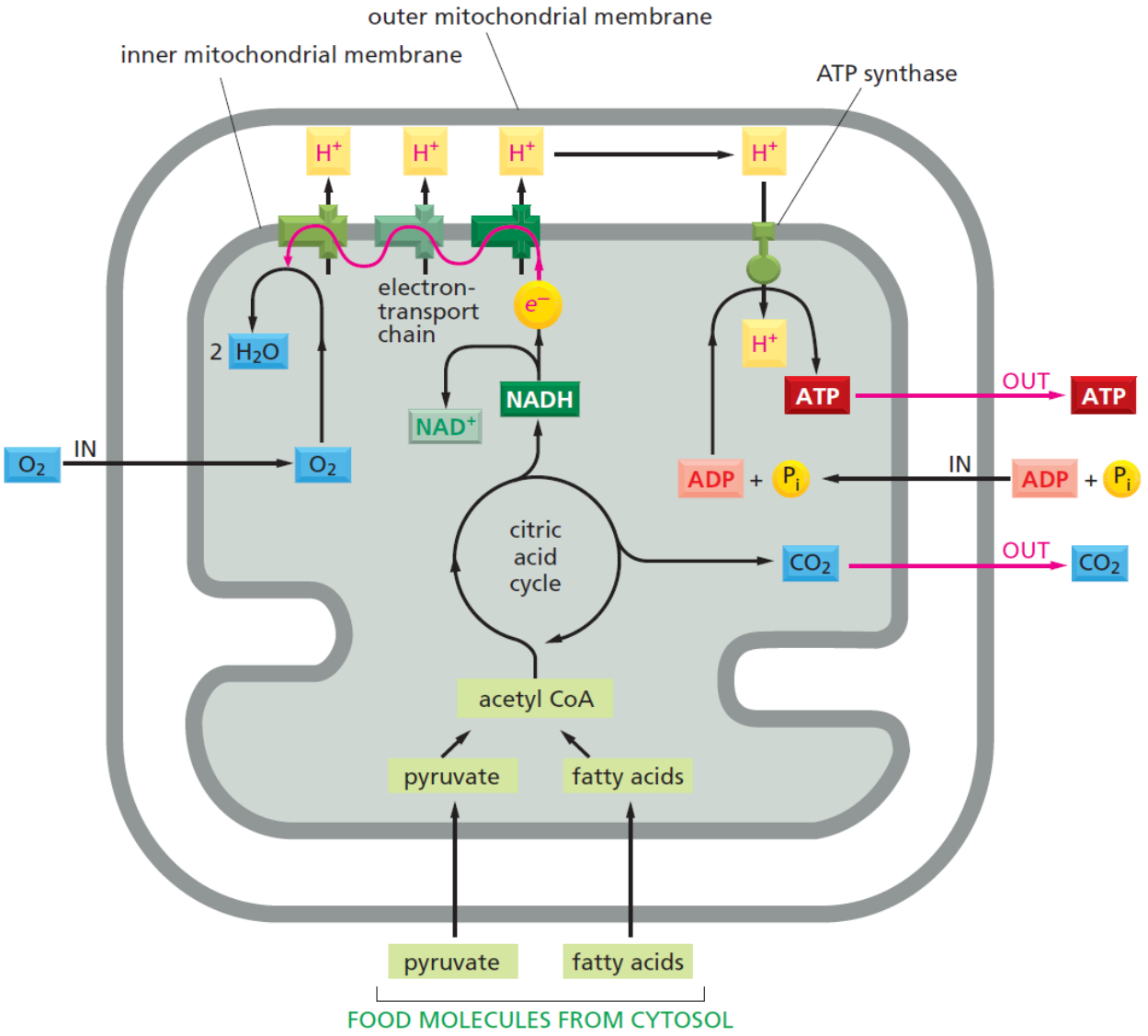


ENERGY DIAGRAM FOR HIGH-ENERGY ELECTRONS

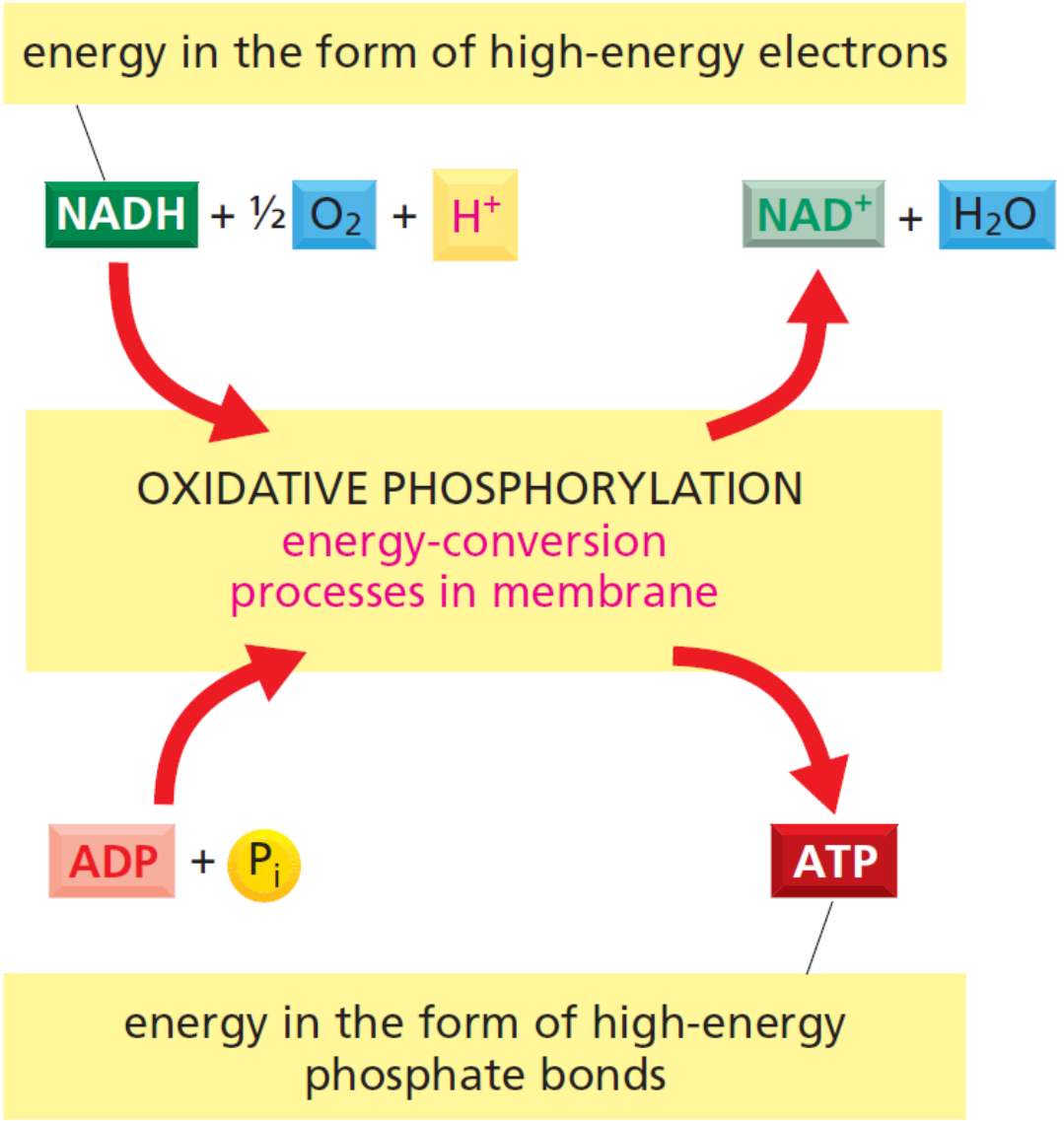


OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation: the process in mitochondria, which uses the energy of nutrients oxidation to synthetize ATP



OXIDATIVE PHOSPHORYLATION: SUMMARY

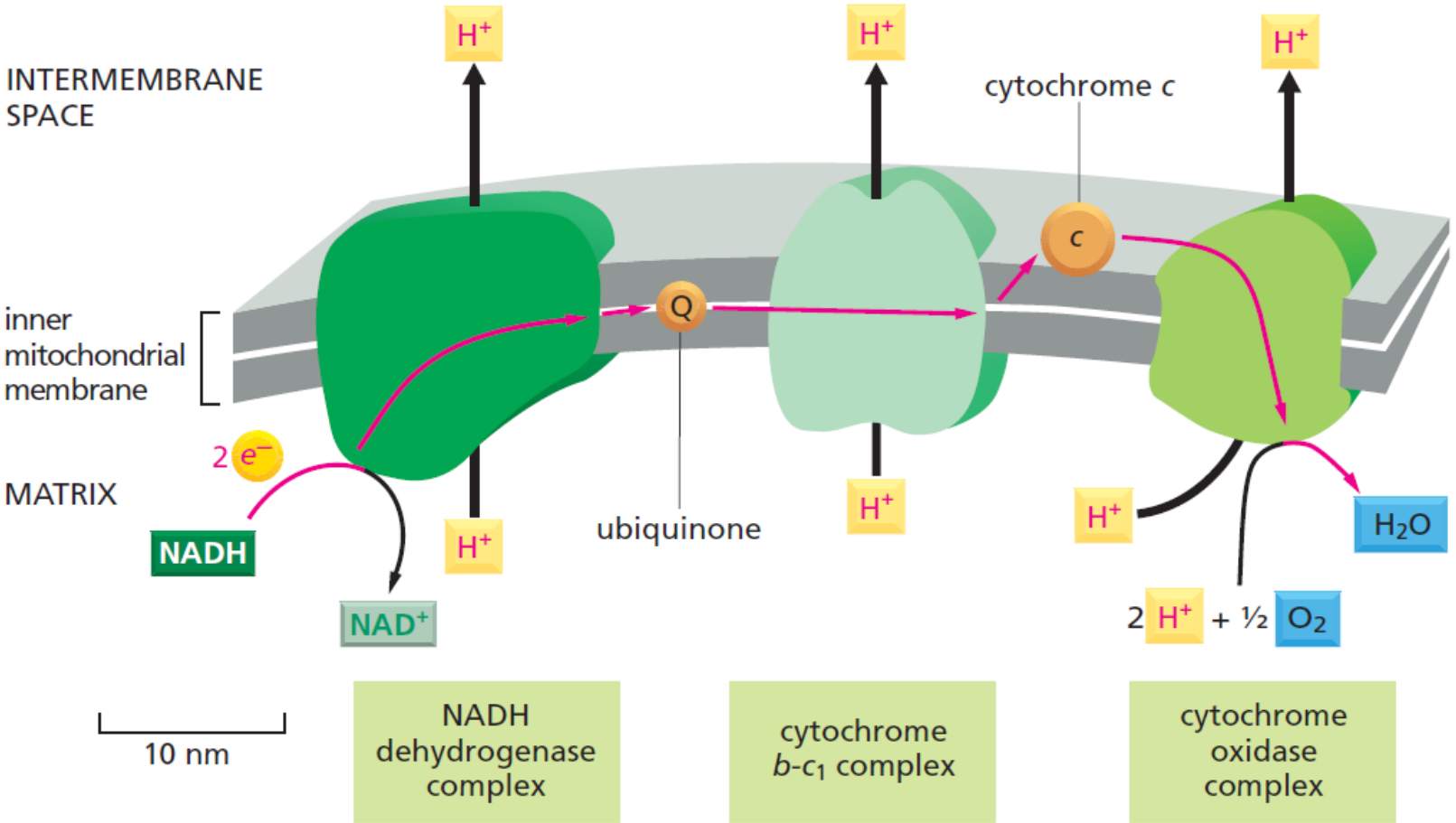


RESPIRATORY CHAIN

Respiratory chain: electron transport chain creating protons gradient

~ 50 proteins (mostly transmembrane):

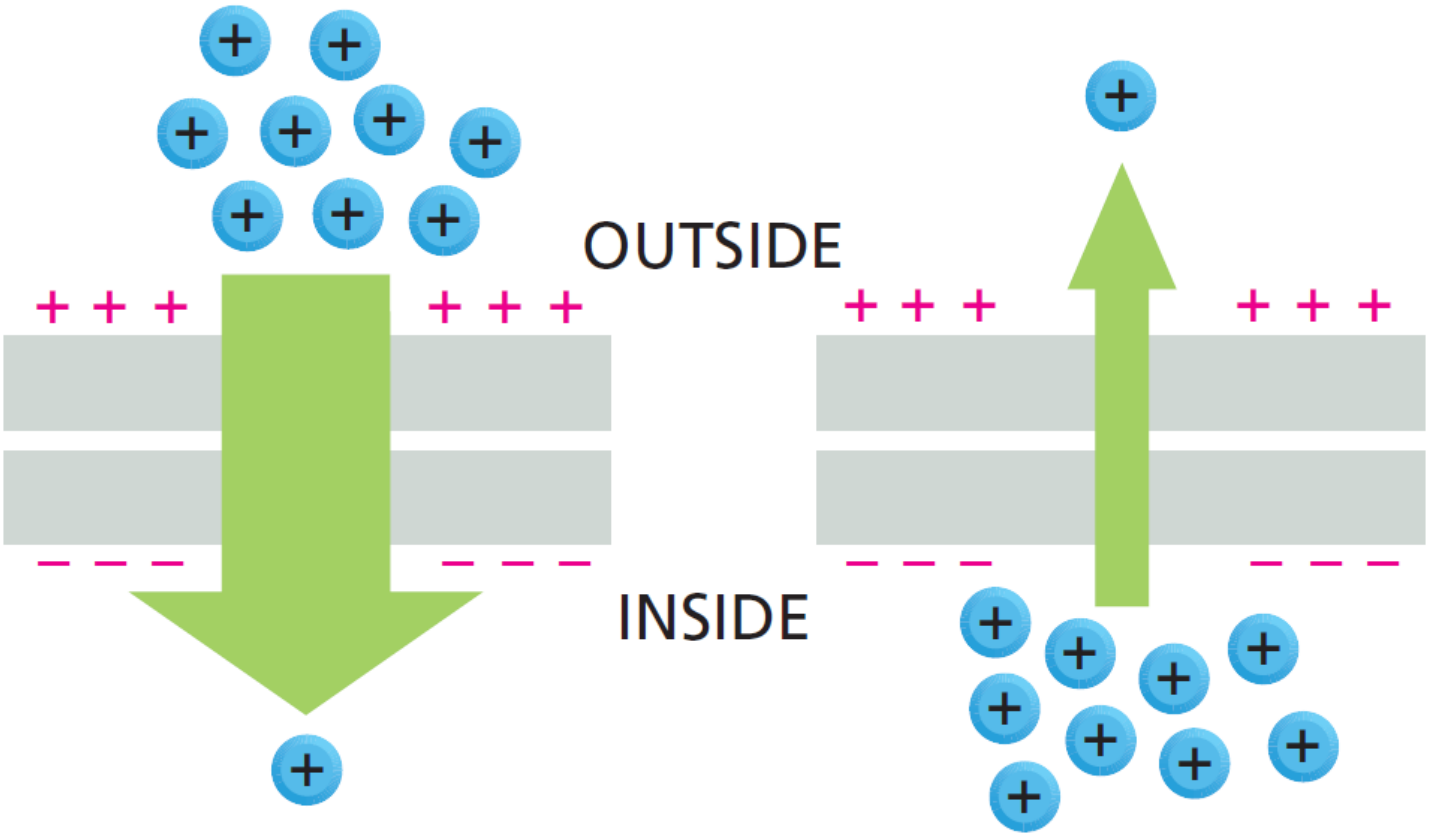
- **NADH dehydrogenase complex**
- **Cytochrome b-c1 complex**
- **Cytochrome oxidase complex**



PRINCIPLE OF ION TRANSFER THROUGH MEMBRANE

The movement of the ions through membrane is dependent on:

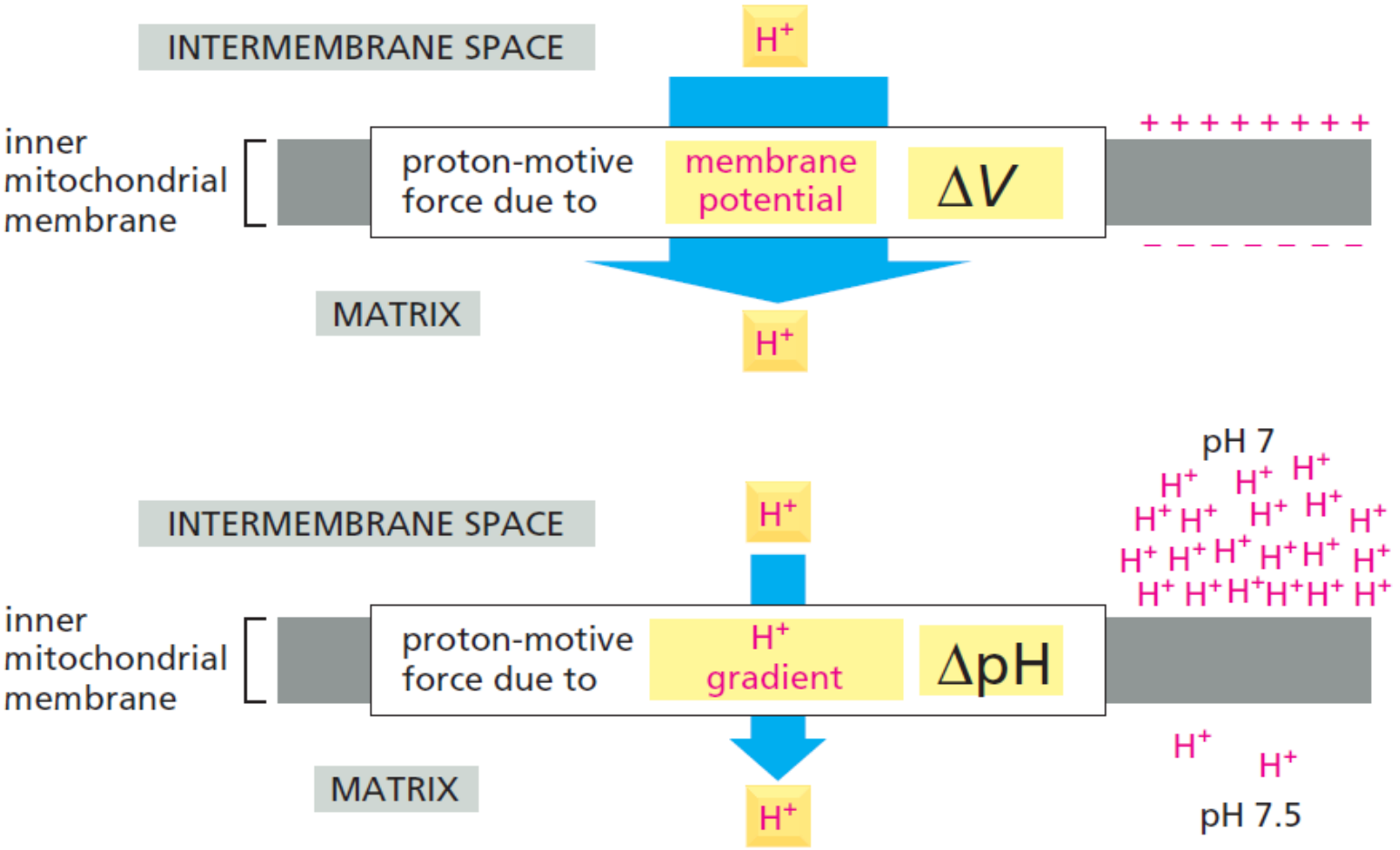
- **membrane voltage**
- **ions gradient**



Membrane voltage and ions gradient work together

Membrane voltage and ions gradient work against each other

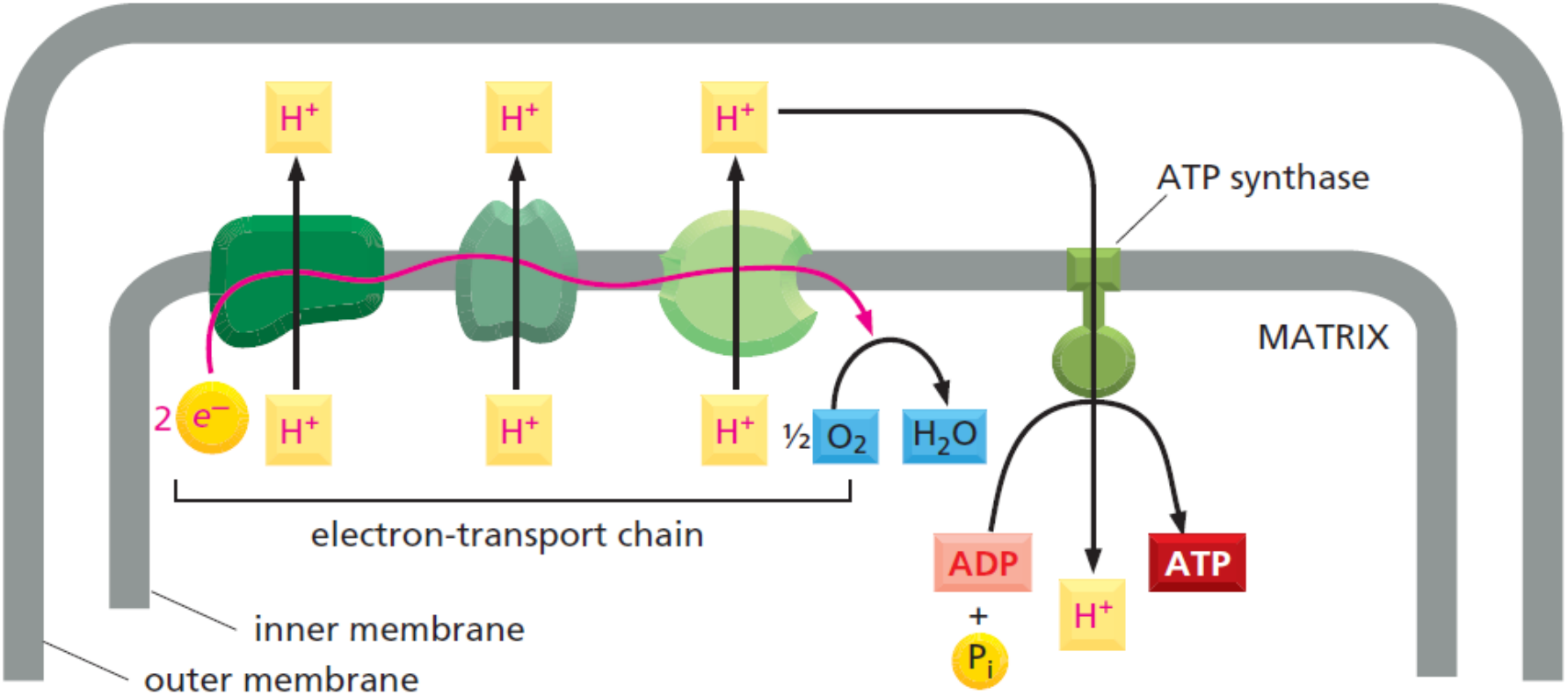
ELECTROCHEMICAL PROTON GRADIENT IN ATP SYNTHESIS



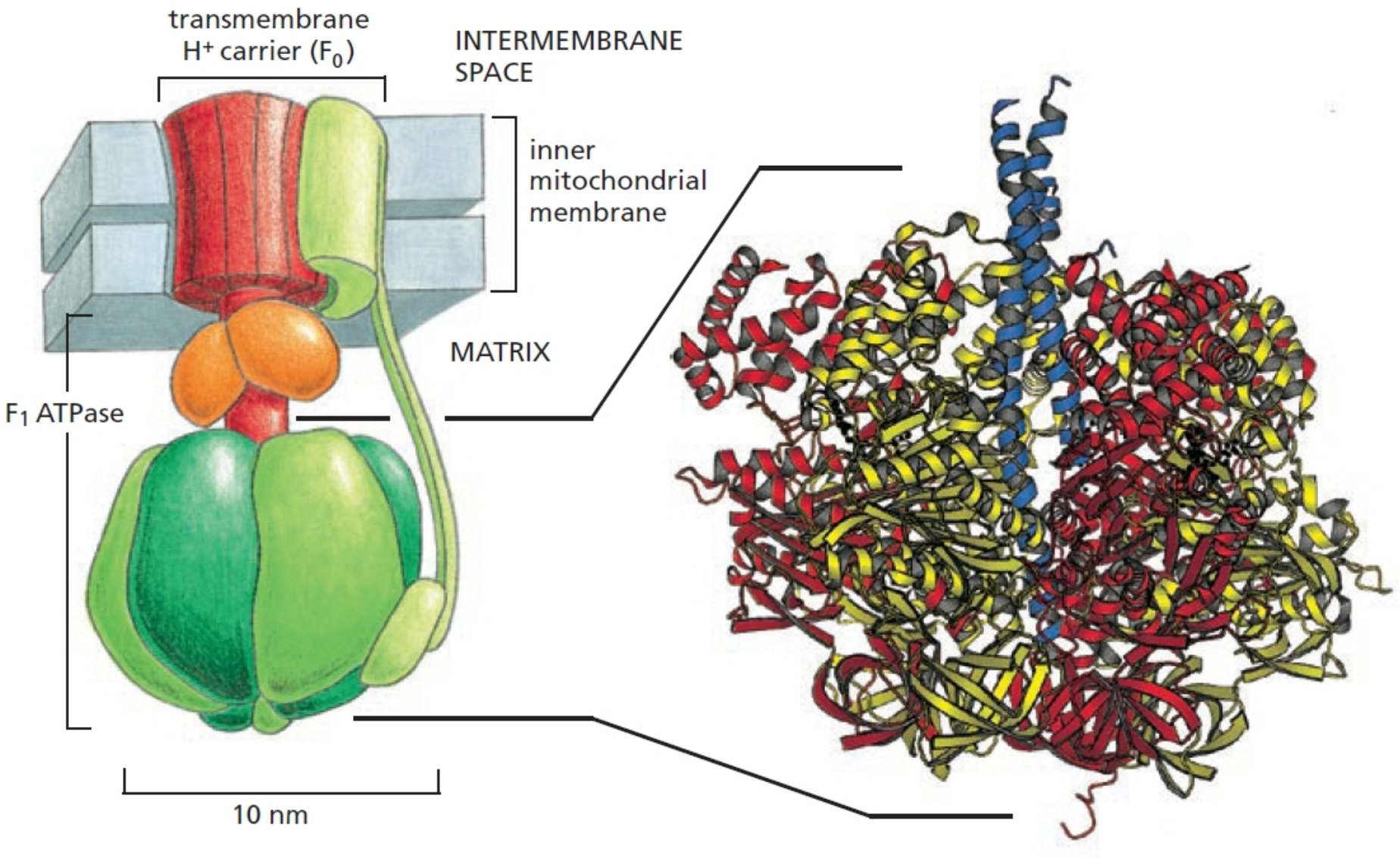
Proton-motive force: driving force for protons through the membrane

ATP SYNTHASE

ATP synthase uses the gradient of protons to synthesize ATP.



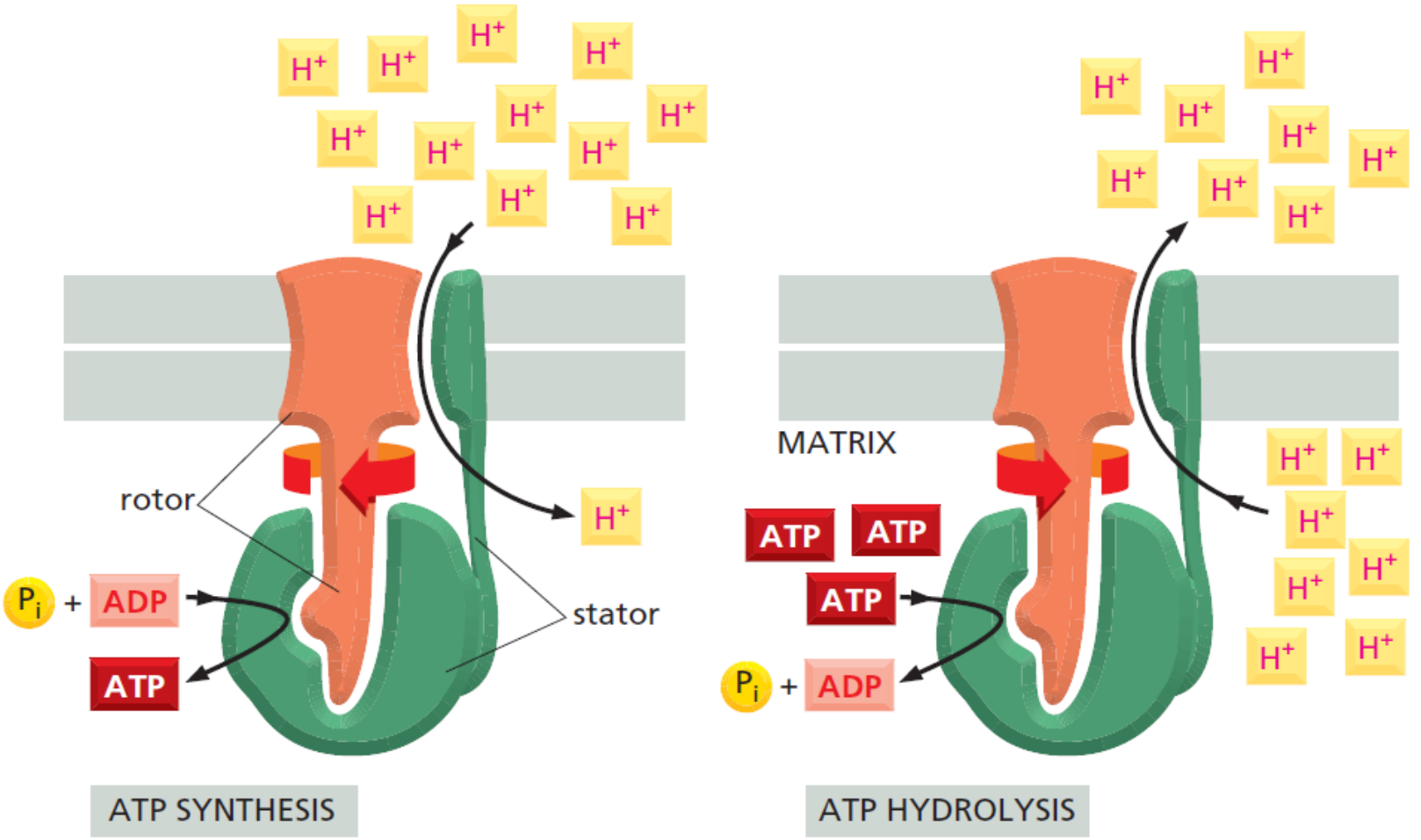
ATP SYNTHASE



One of the most conservative enzymes

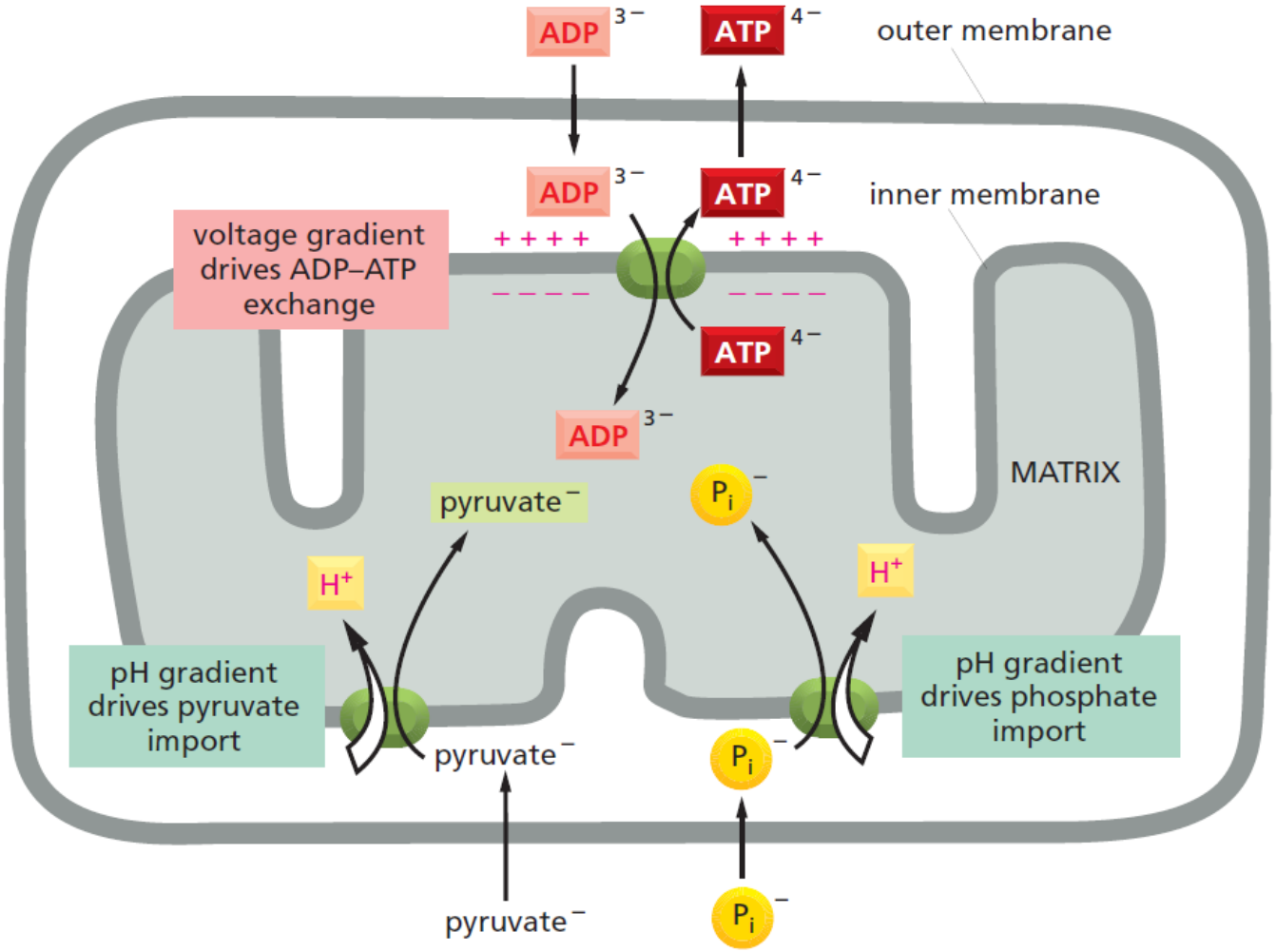
ATP SYNTHASE FUNCTIONS IN BOTH DIRECTIONS

- **ATP synthesis**
- **Proton-pump/ATP-hydrolysis (deficiency of O_2)**



OTHER PROCESSES IN MITACHONDRIA INFLUENCING ELECTROCHEMICAL POTENTIAL

- Pyruvate, ADP, P_i => matrix
- ATP => cytosol
- Cotransport/cosynthesis



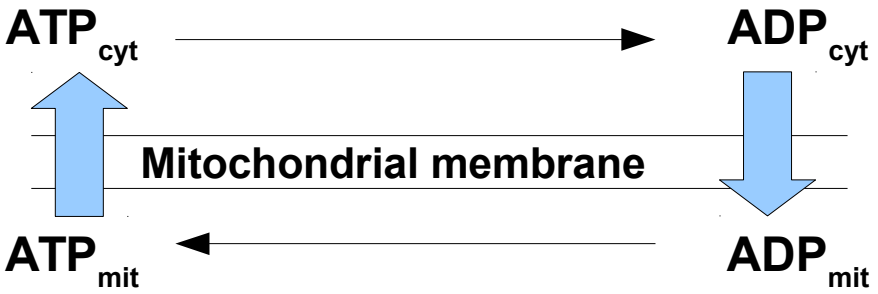
GLUCOSE OXIDATION SUMMARY

PROCESS	DIRECT PRODUCT	FINAL ATP YIELD PER MOLECULE OF GLUCOSE
Glycolysis	2 NADH (cytosolic)	3*
	2 ATP	2
Pyruvate oxidation to acetyl CoA (two per glucose)	2 NADH (mitochondrial matrix)	5
Complete acetyl CoA oxidation (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 GTP	2
	TOTAL	30

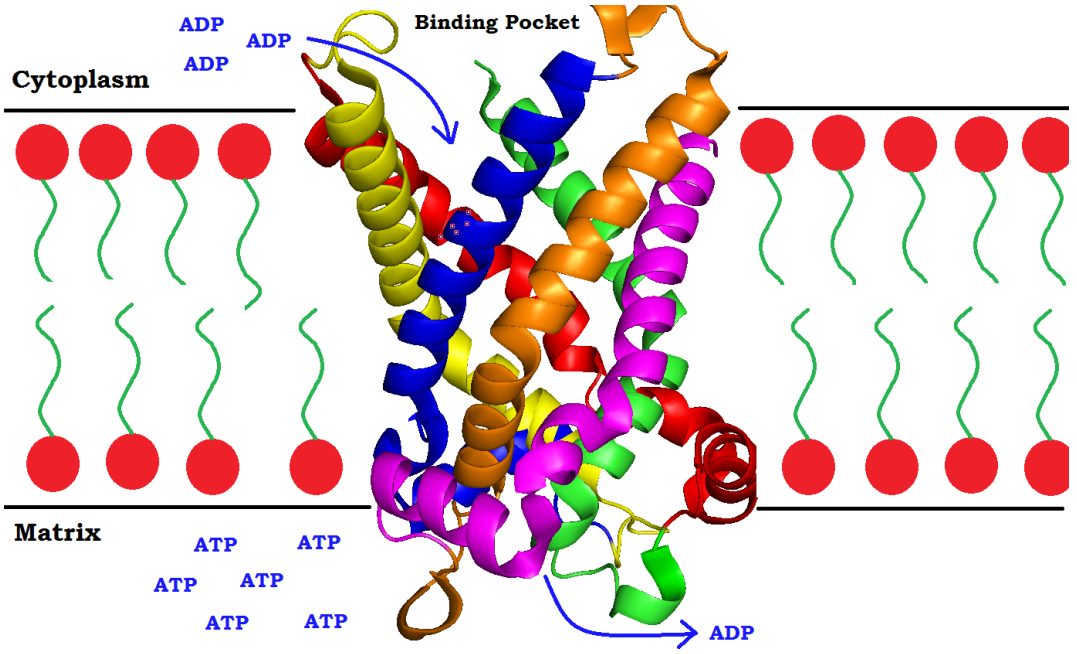
- **NADH is transported from cytosol to mitochondria (energy-dependent)**
- **$FADH_2$: does not require NADH dehydrogenase**

ATP/ADP balance in the cell

- One cycle ~ 1 minute
- $[ATP]_{cell} \sim 10[ADP]_{cell}$



- ATP is needed for energetically unfavourable reactions



- Cyanide: CN^- is a inhibitor of cytochrome c oxidase => respiration defect => neural system and heart

PROTEIN SORTING

Protein sorting: mechanism by which proteins are transported to their appropriate destination within or outside the cell

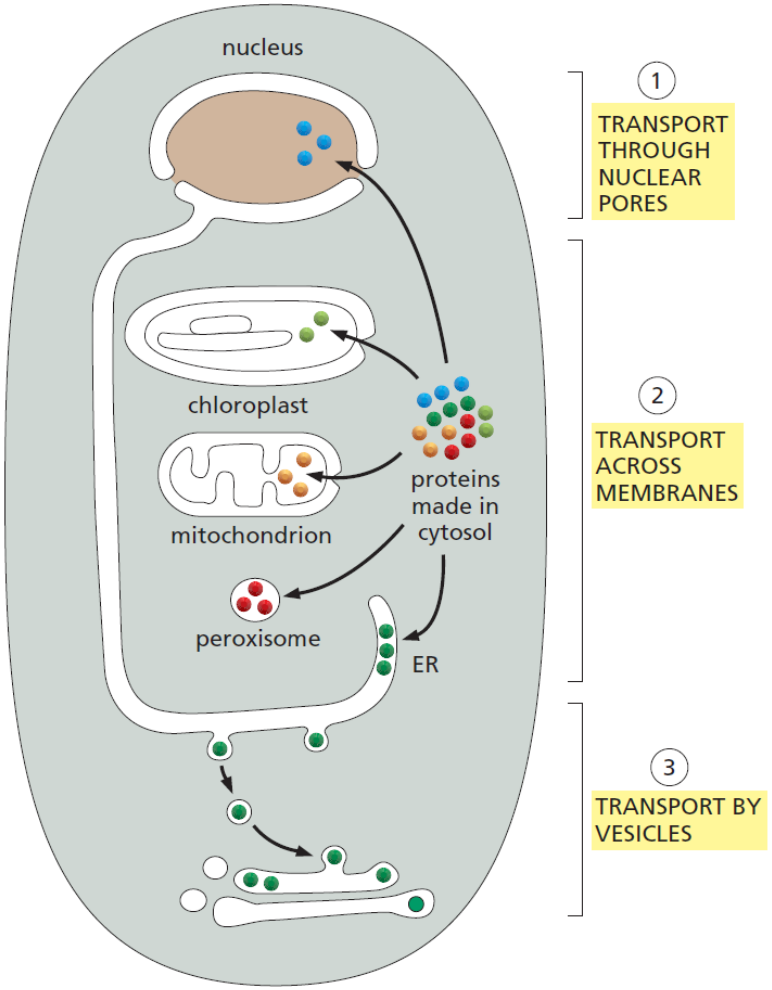
- **Most protein synthesis starts in the cytosole**
- **Depending on a sorting signal (sequence) the destination is chosen**
- **Three main pathes:**

  - to the nucleus though nuclear pores (L12-13)

  - to the organelles using trnslocators

  - from ER to endomembrane systems:

  vesicular transport (L17-18)



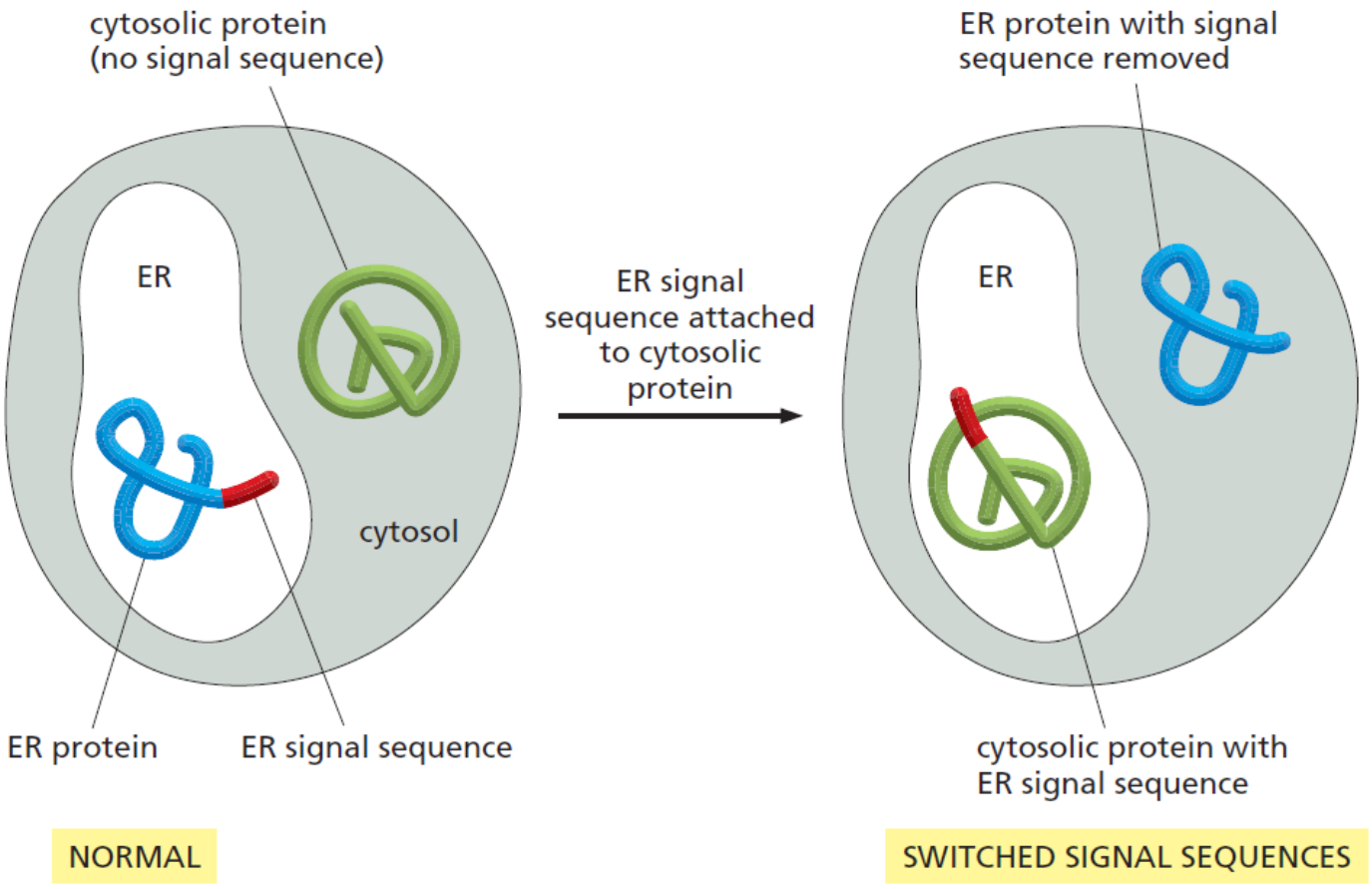
SIGNAL

- **Stretch of amino acids**
- **15-60 aa long**
- **Often is removed after the transport event**
- **Highly specific**
- **Physico-chemical basis of interactions with membranes**

FUNCTION OF SIGNAL	EXAMPLE OF SIGNAL SEQUENCE
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

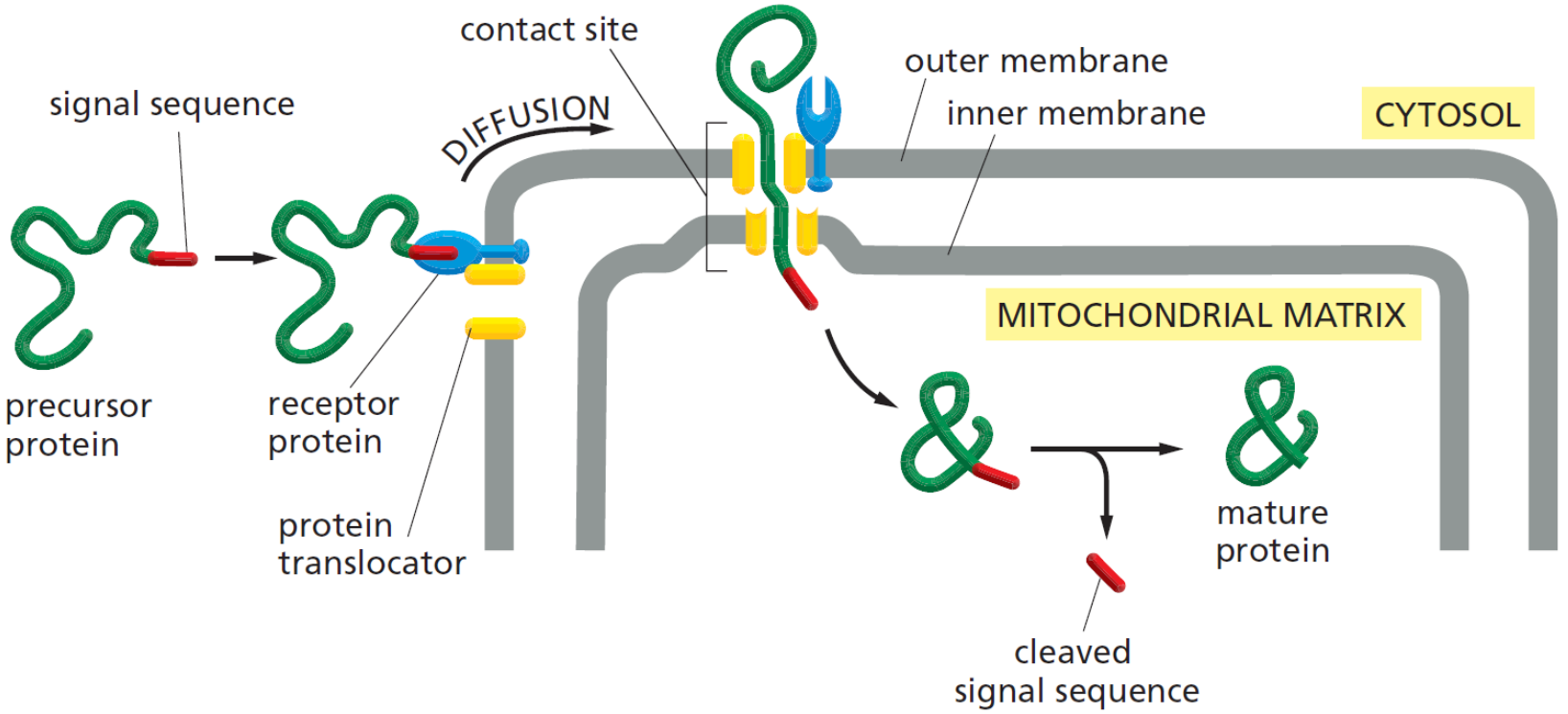
SIGNALS

Exchanging signals => exchanging destinations



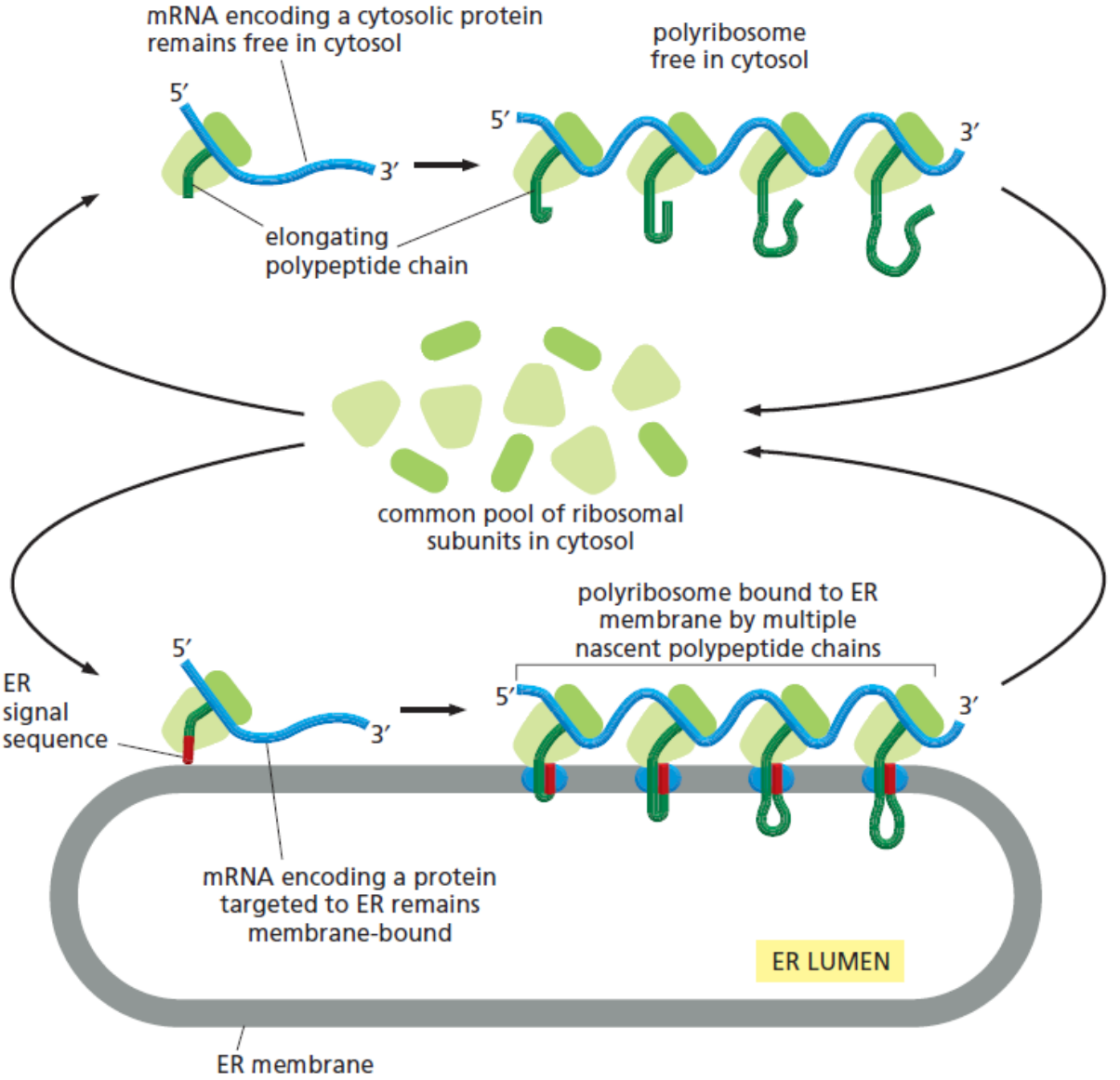
TRANSPORT TO MITACHONDRION AND CHLOROPLASTS

- Chloroplasts and mitochondria: proteins from both genomes
- Transported through both membranes
- N-terminal signal
- Unfolded while transported
- To be transported further an another signal is needed



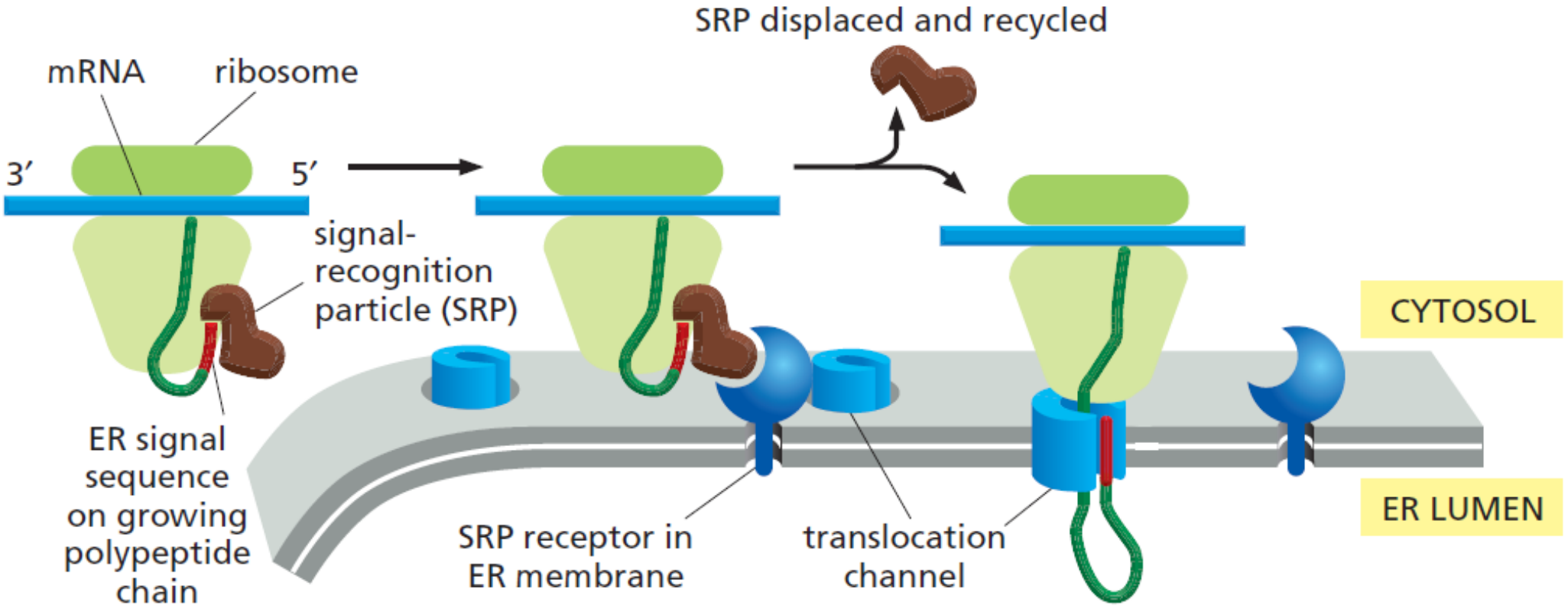
PROTEINS ENTER ER CONTRANSLATIONALLY

- Final destination: ER, GA, endosomes, lysosomes, cell surface, secretion
- Water soluble and transmembrane proteins
- No reentering cytosol
- Ribosomes bind to ER



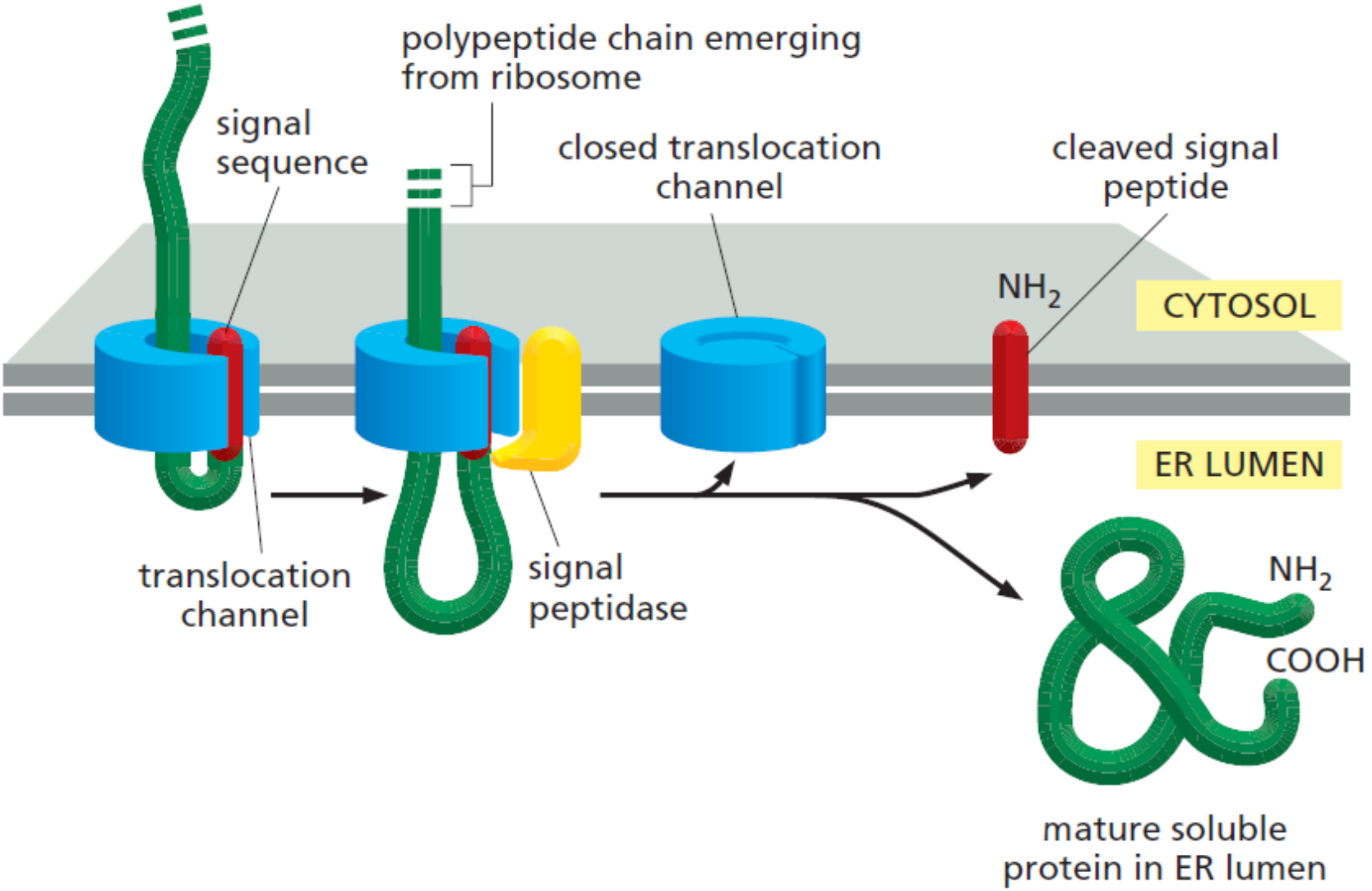
PRINCIPLE OF ER-SIGNALING

- **Signal-recognition particle (SRP)**
- **SRP-receptor**
- **Signal sequence open translocating channel**



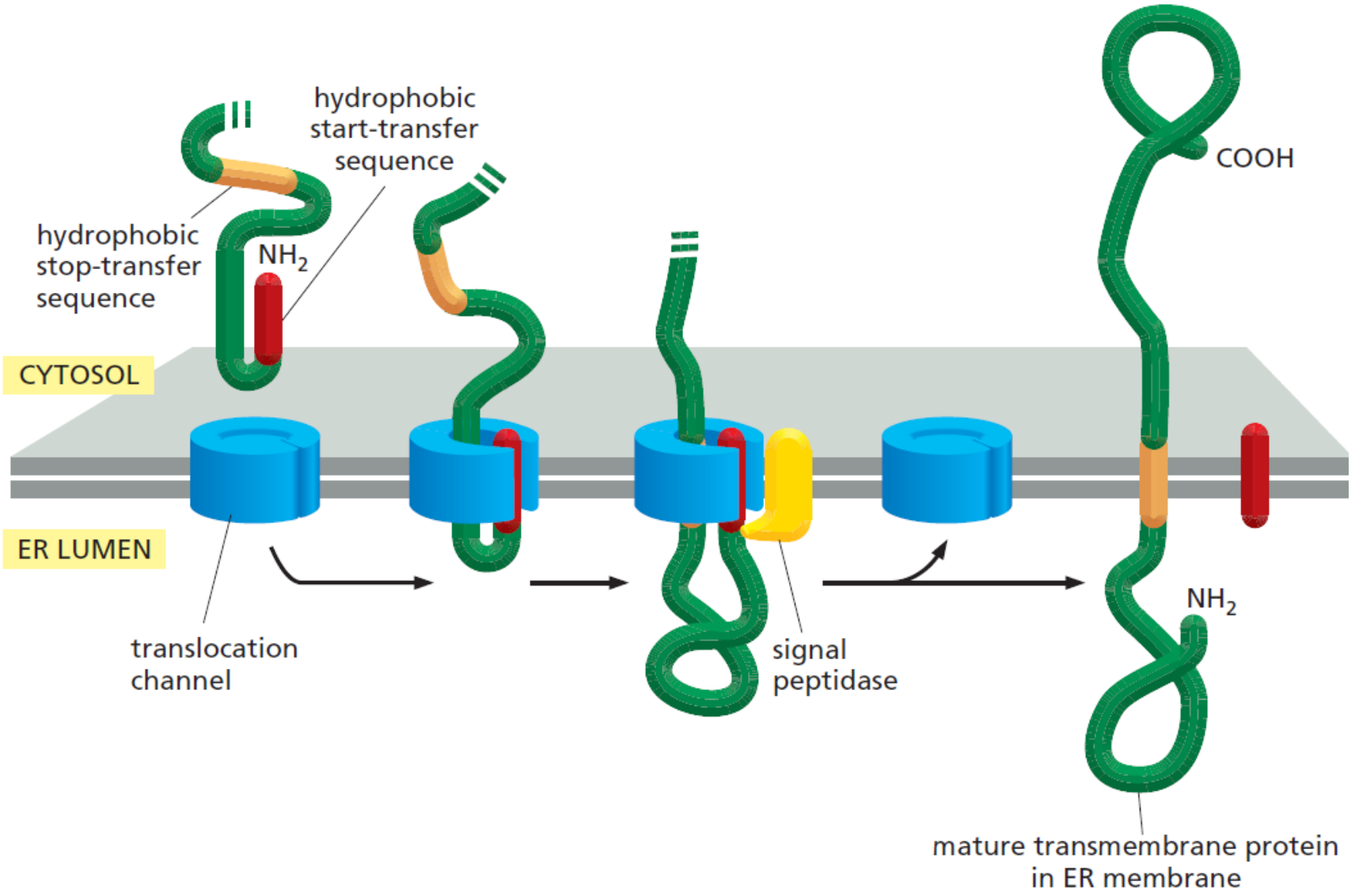
PRINCIPLE OF ER-SIGNALING

- **Signal peptide is cleaved and degraded in the cytosol**

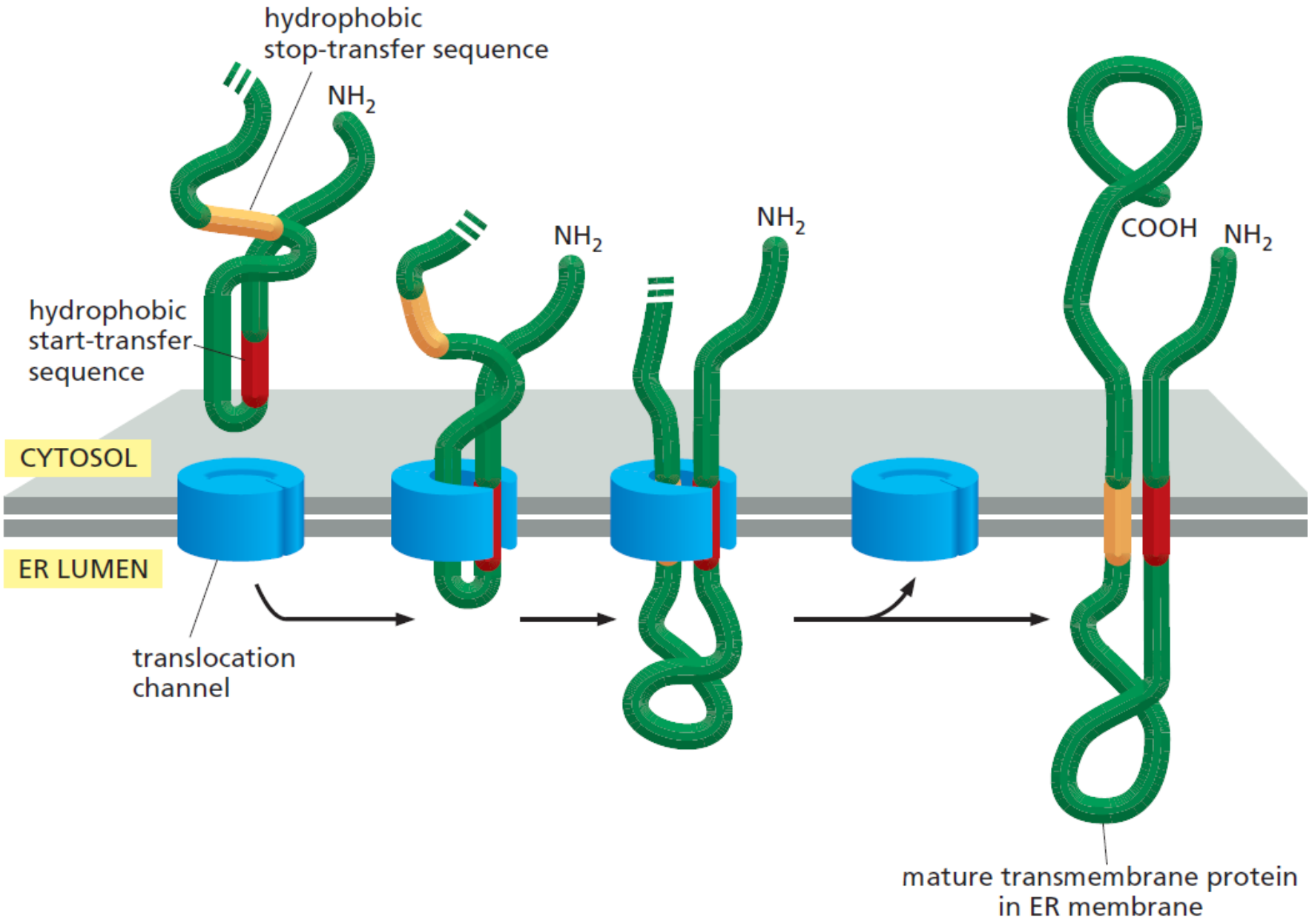


STOP-SIGNALS IN TRANSMEMBRANE PROTEINS

Stop-transfer sequence, usually hydrophobic, stops the translocation



STOP-SIGNALS IN TRANSMEMBRANE PROTEINS SPANNING THE MEMBRANE SEVERAL TIMES



LECTURES 7-8: CELL ORGANIZATION I

- Type of cells: eukaryotic (animal/plant) and prokaryotic
- Cellular compartmentalization
- Organelles and their functions:
  - cell wall
  - cytosol, cell mebrane, nucleus, ER, GA, peroxisomes, lysosomes, vacuole, ribosomes
  - chloroplasts, mitochondrion
  - oxidative phosphorylation
- Protein sorting