Bioenergetics and metabolism

- Mitochondria
- Chloroplasts
- Peroxisomes

B. Balen

Chemiosmosis

✓common pathway of mitochondria, chloroplasts and prokaryotes to harness energy for biological purposes → **chemiosmotic coupling –**

ATP synthesis (chemi) + membrane transport (osmosis)

✓ Prokaryotes

– plasma membrane → ATP production

✓ Eukaryotes

– plasma membrane → transport processes

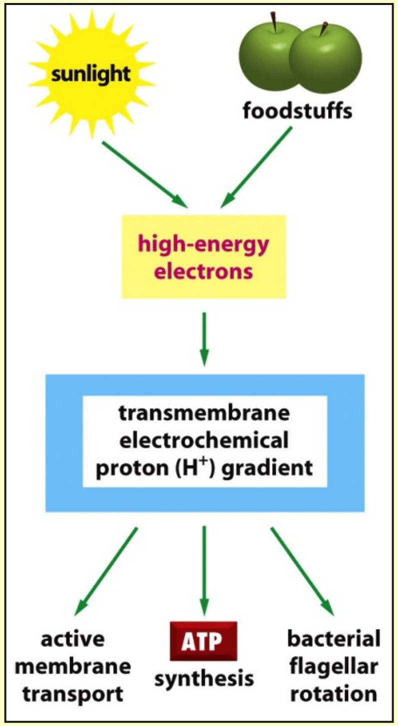
– membranes of cell compartments – *energy-converting organelles* → production of ATP

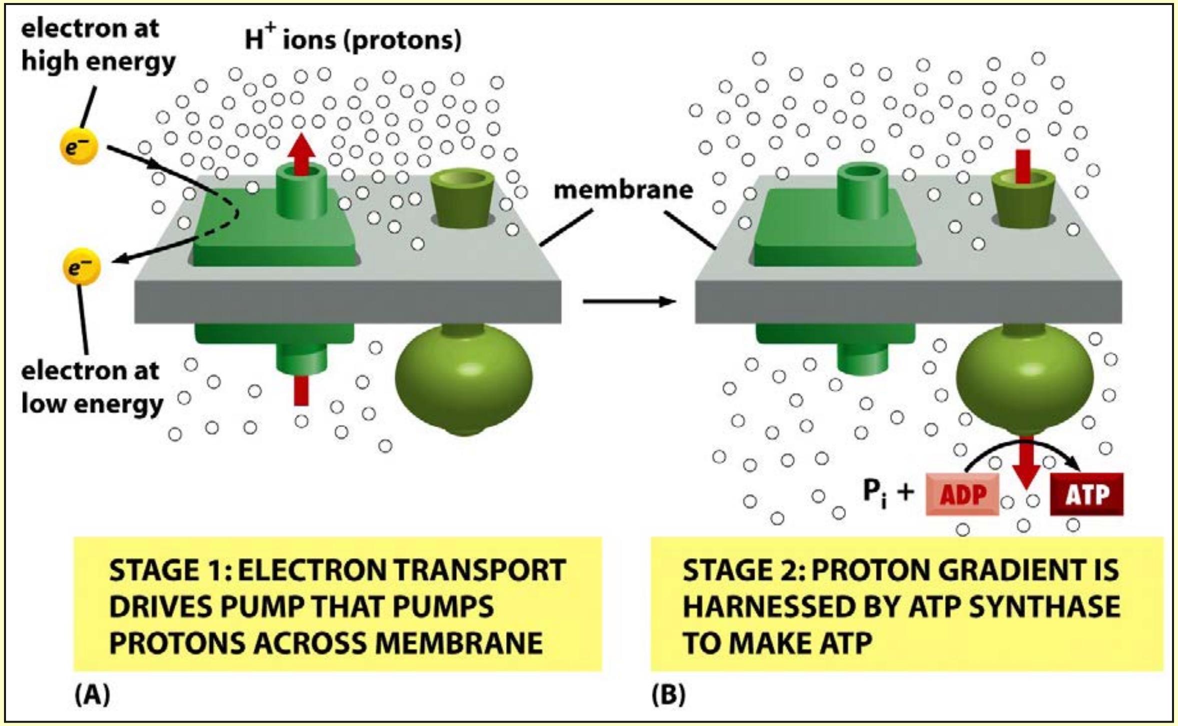
- Mitochondria – fungi, animals, plants
- Plastids (chloroplasts) – plants

The essential requirements for chemiosmosis

✓ **source of high-energy e^-**

✓ membrane with embedded **proton pump** and **ATP synthase**





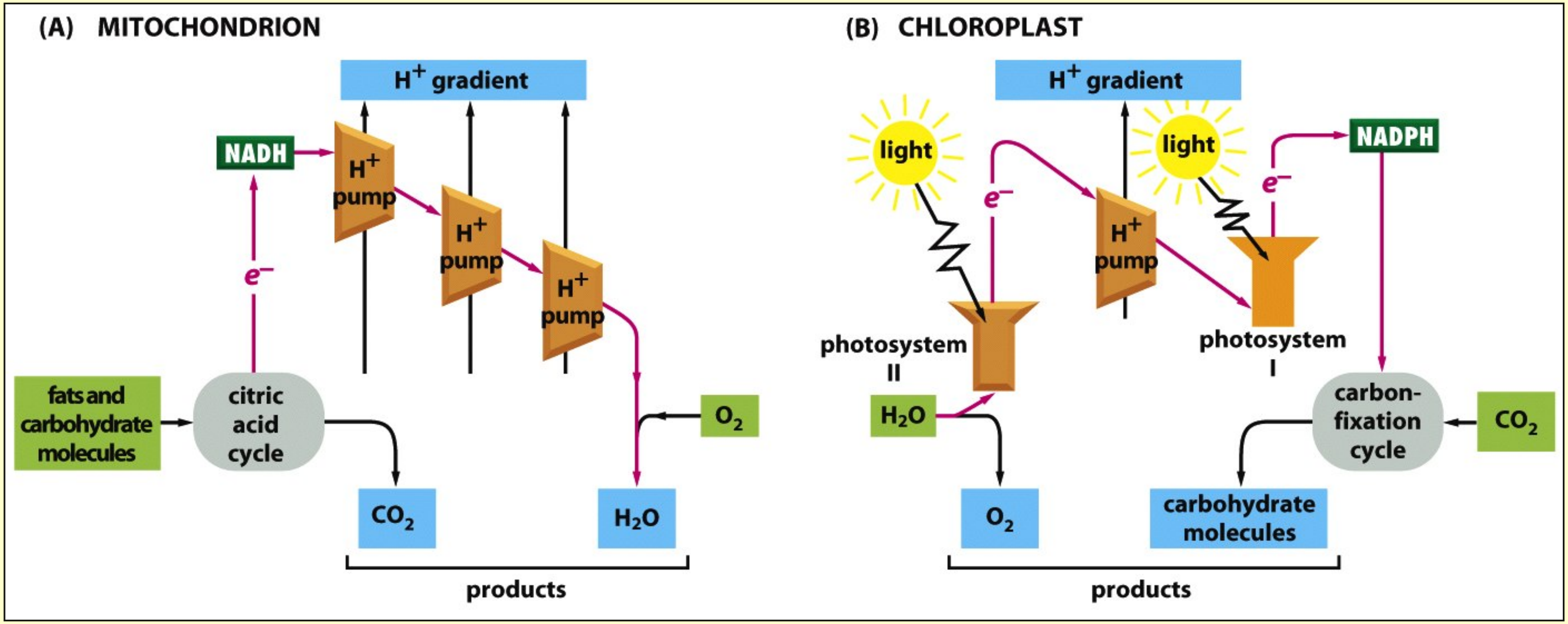
✓energy from sunlight or the oxidation of foodstuffs is used to create H^+ gradient across a membrane

✓pump harnesses the energy of e^- transfer to pump $H^+ \rightarrow$ proton gradient across the membrane

✓H^+ gradient serves as an energy store that can be used to drive ATP synthesis

Figures 14-1; 14-2 *Molecular Biology of the Cell* (© Garland Science 2008)

Electron transport processes



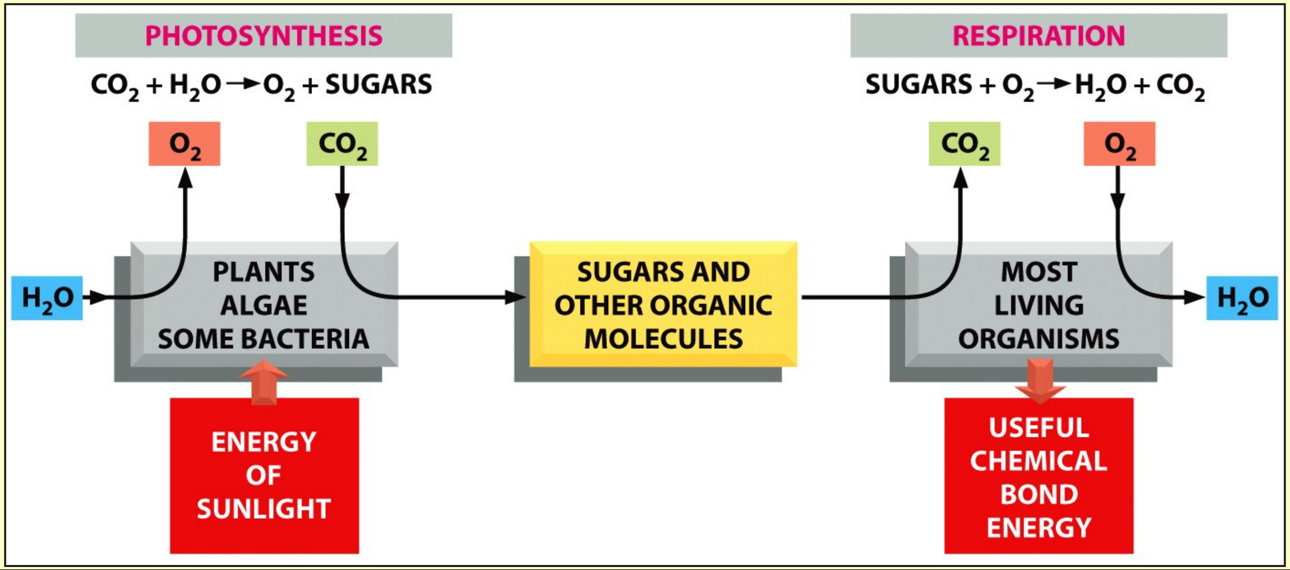
(A) mitochondrion converts energy from chemical fuels

(B) chloroplast converts energy from sunlight

→ electron-motive force generated by the 2 photosystems enables the chloroplast to drive electron transfer from H_2O to carbohydrate
→ chloroplast electron transfer is *opposite* of electron transfer in a mitochondrion

Figure 14-3 *Molecular Biology of the Cell* (© Garland Science 2008)

Carbohydrate molecules and O_2 are products of the chloroplast and inputs for the mitochondrion



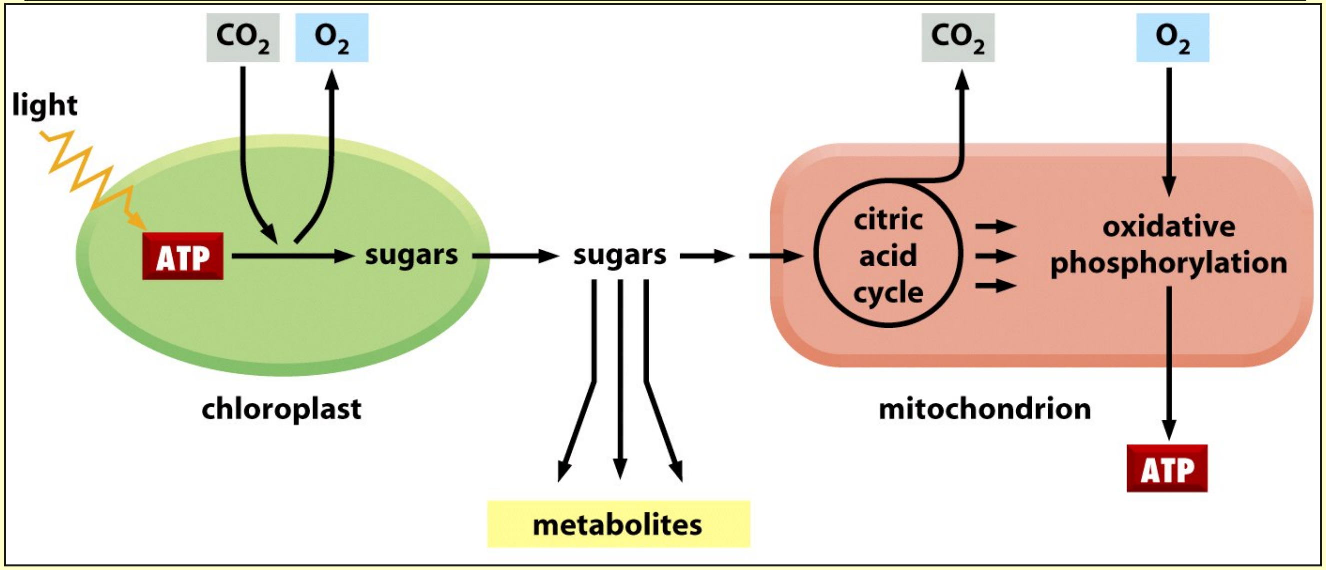
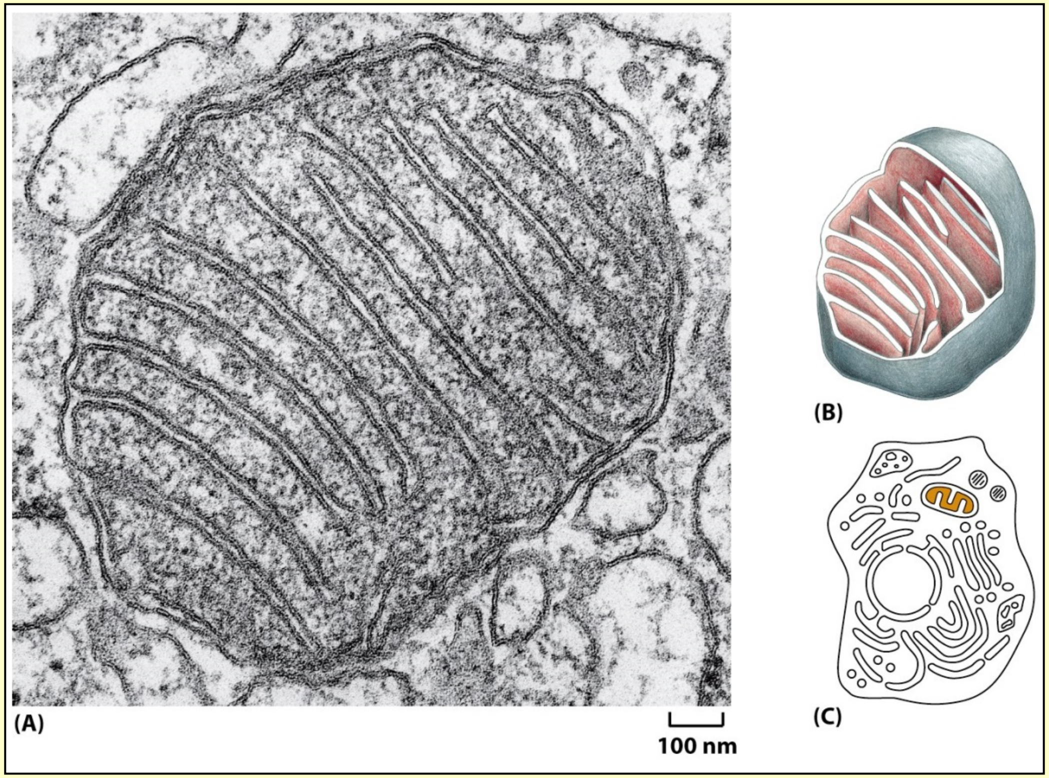


Figure 2-41; 2-76 *Molecular Biology of the Cell* (© Garland Science 2008)

Mitochondria

Structure and ultrastructure



✓ all eukaryotic cells
→ diameter 0,5 -1µm

✓ discovered in 19th century
→ light microscopy

✓ 1948. isolated from liver cells

✓ key function in metabolic energy production

✓ double membrane

- outer membrane
- intermembrane space
- inner membrane

Figure 1-33 *Molecular Biology of the Cell* (© Garland Science 2008)

Mitochondria mobility

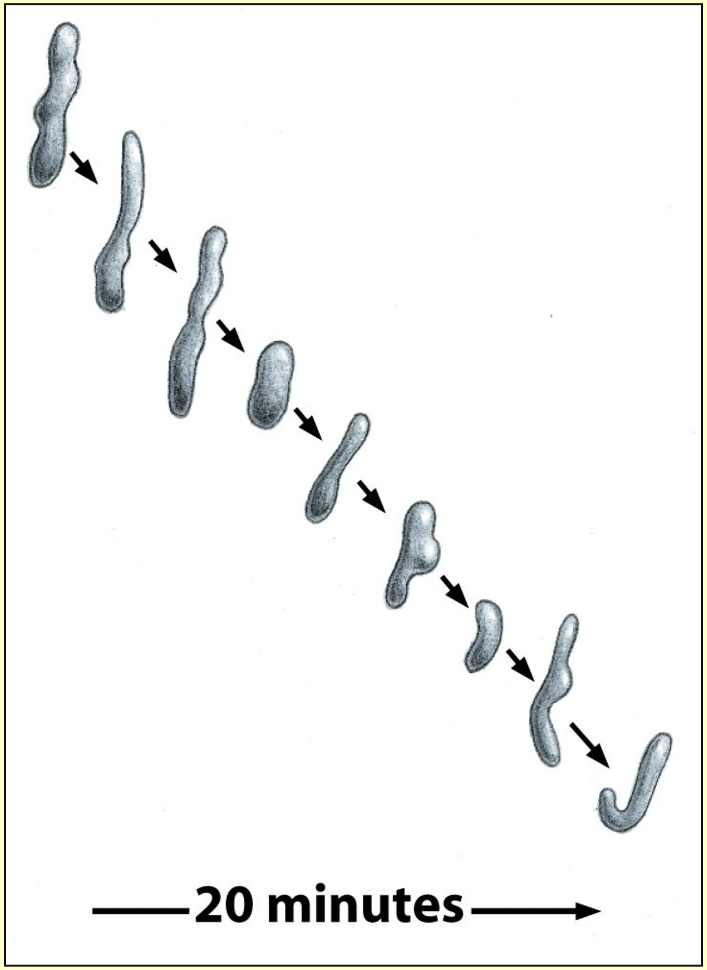


Figure 14-4 *Molecular Biology of the Cell* (© Garland Science 2008)

- ✓ extremely mobile and plastic organelles
- ✓ constant change of shape
- ✓ fisions and fusions

Animation

http://il.youtube.com/watch?v=7Jqal35vqD4

Ultrastructure

✓ **Outer membrane**
- porins (water channels)
- < 5000 Da free passage

✓ **Inner membrane**
- christae
- cardiolipin

→ impermeability for ions

✓ **Matrix**
- enzymes for citric acid cycle
- DNA
- ribosomes (70 S)

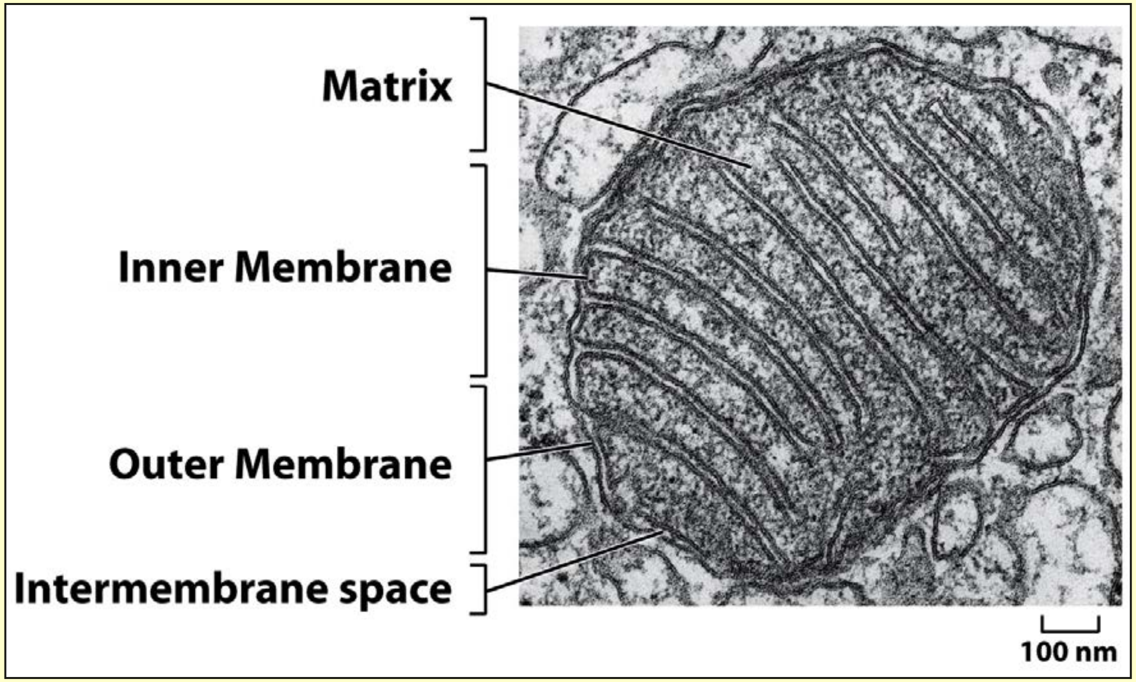


Figure 14-8 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

Cardiolipin

→ impermeability of the inner membrane for ions

→ phospholipid with 4 fatty acid chains

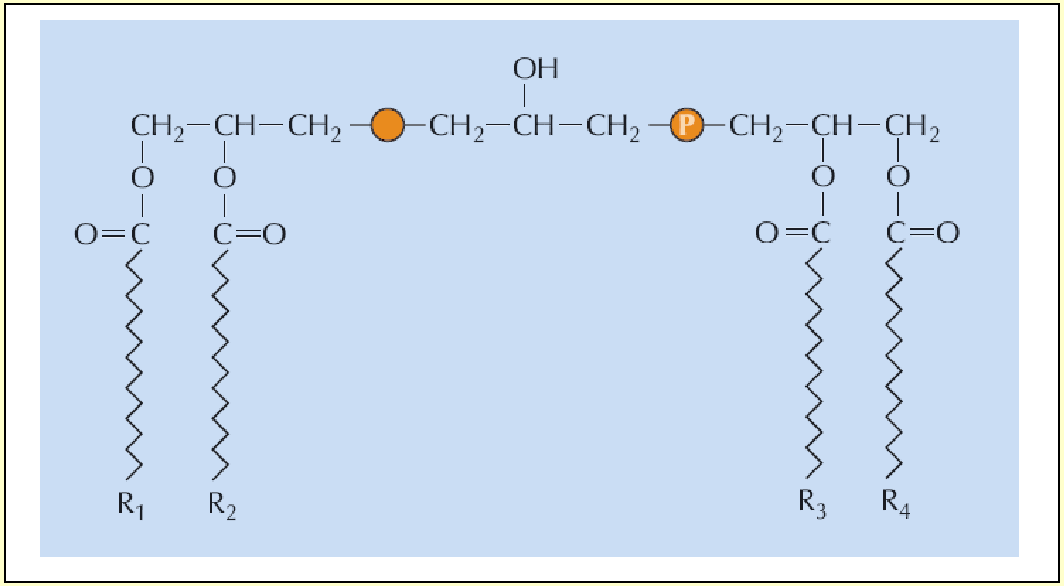


Figure 10-7. 2004. Cooper and Hausman

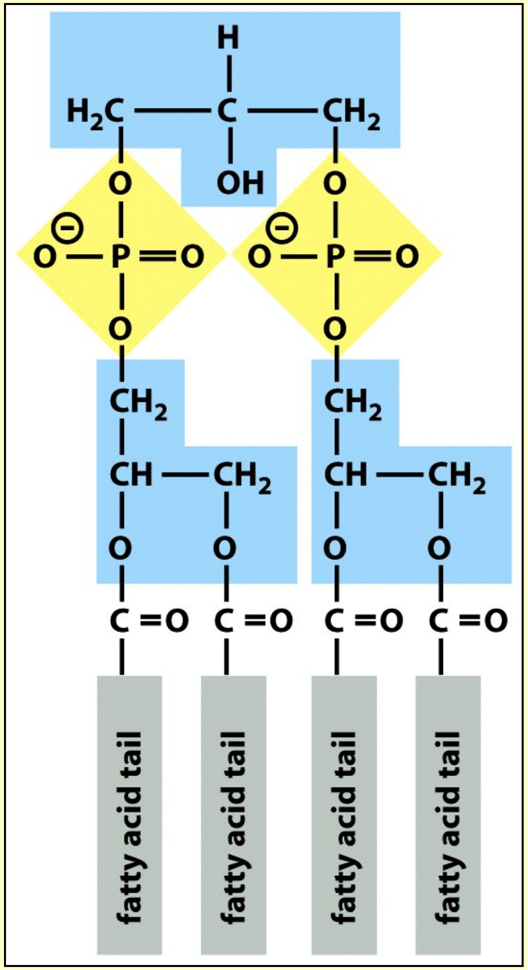


Figure 14-65 *Molecular Biology of the Cell* (© Garland Science 2008)

Biochemical activity of mitochondria

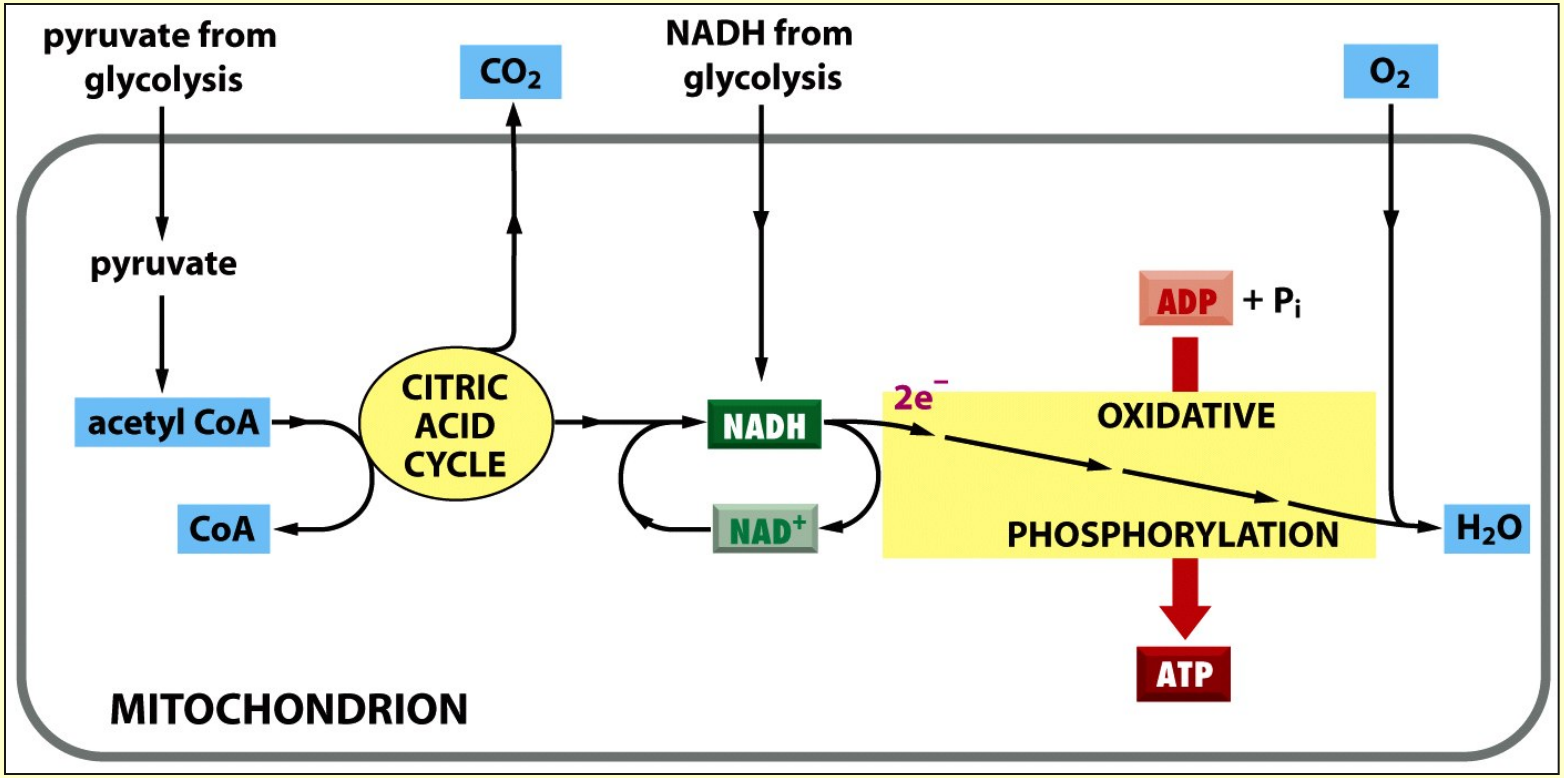


Figure 2-86 *Molecular Biology of the Cell* (© Garland Science 2008)

✓ Oxidative degradation of glucose and fatty acids → the main source of metabolic energy in animal cells

✓ First step of glucose metabolism
→ glycolysis (glucose → pyruvate)
→ cytosol
→ 2 ATP molecules

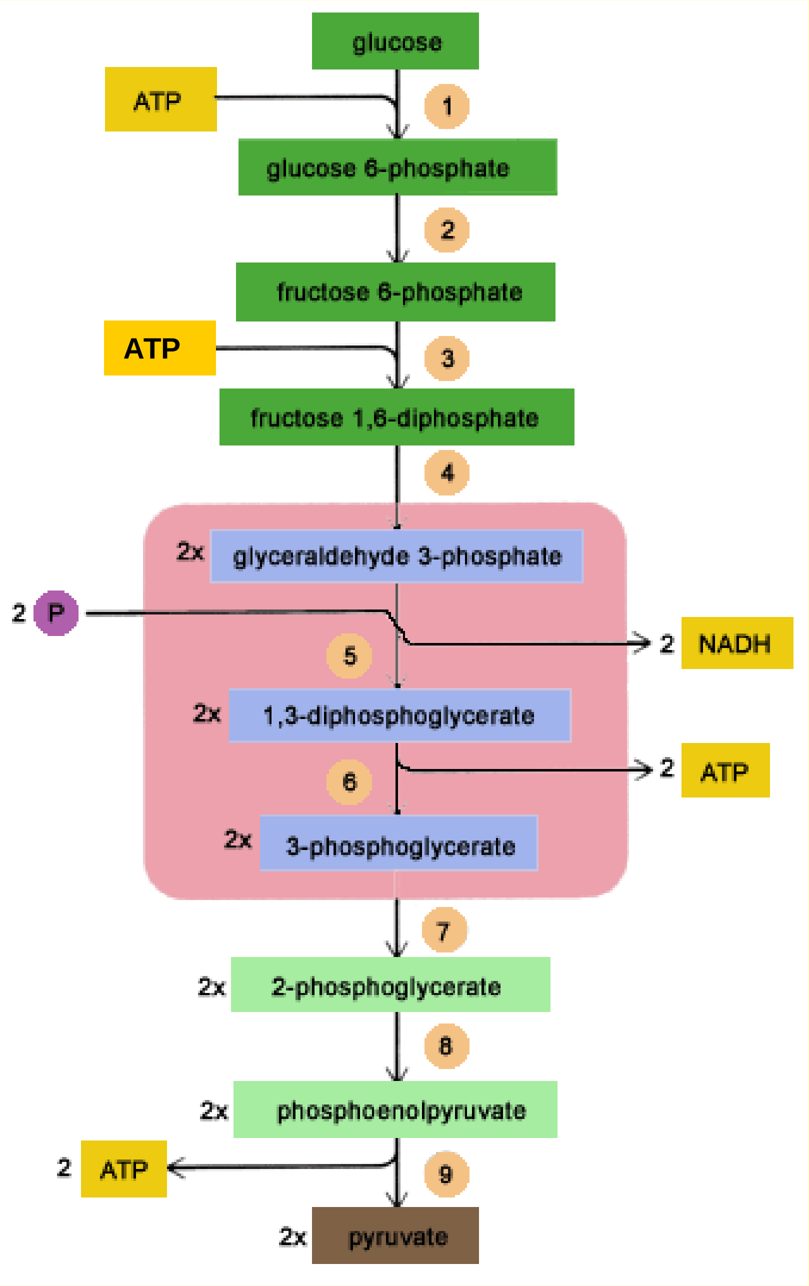
✓ Pyruvate is transported to mitochondria
→ complete oxidation to CO_2
→ gain in energy is **15 x** higher than in glycolysis

❖ Glycolysis - cytosol

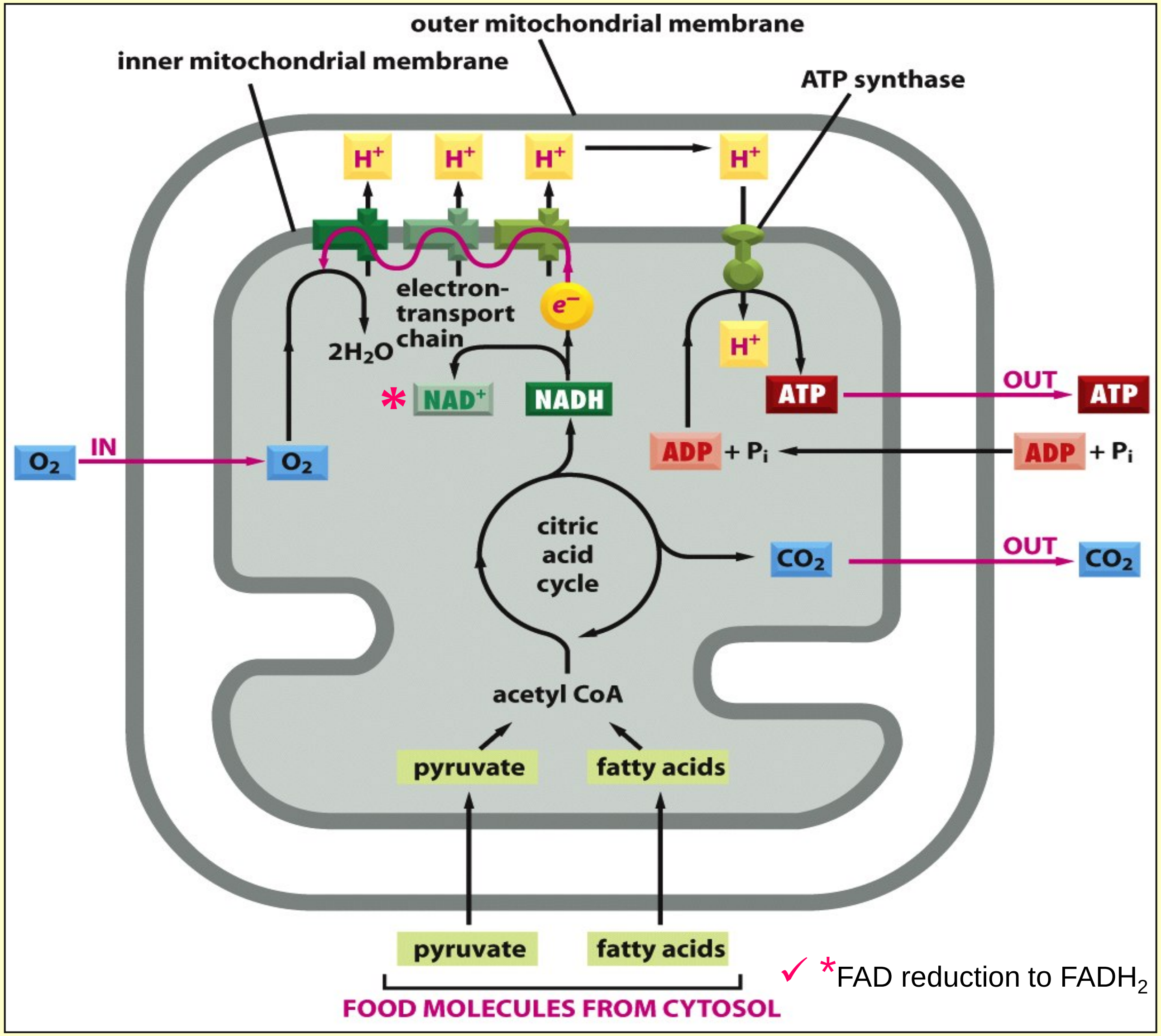
glucose → 2 pyruvate + 2 ATP + 4 H^+ (2 NADH)

mitochondria matrix
citric acid cycle

mitochondria inner membrane
oxidative phosphorylation



Reactions that take place in mitochondrion



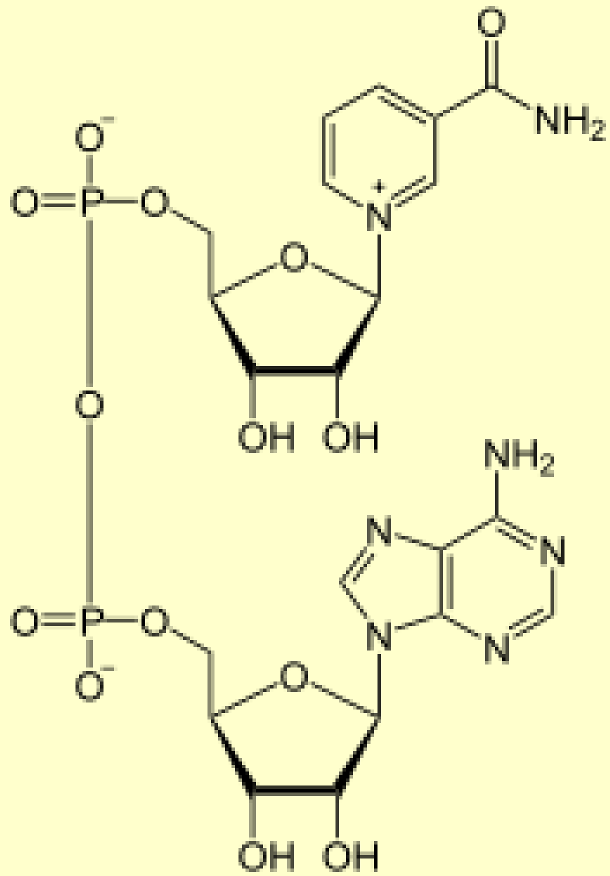
✓ *FAD reduction to $FADH_2$

Figure 14-10 *Molecular Biology of the Cell* (© Garland Science 2008)

❖ NADH - nicotinamide adenine dinucleotide

✓ coenzyme found in all living cells

✓dinucleotide
→ consists of two nucleotides joined through their phosphate groups
→ one nucleotide contains an adenine base and the other nicotinamide



✓ involved in redox reactions

✓ found in two forms in cells:

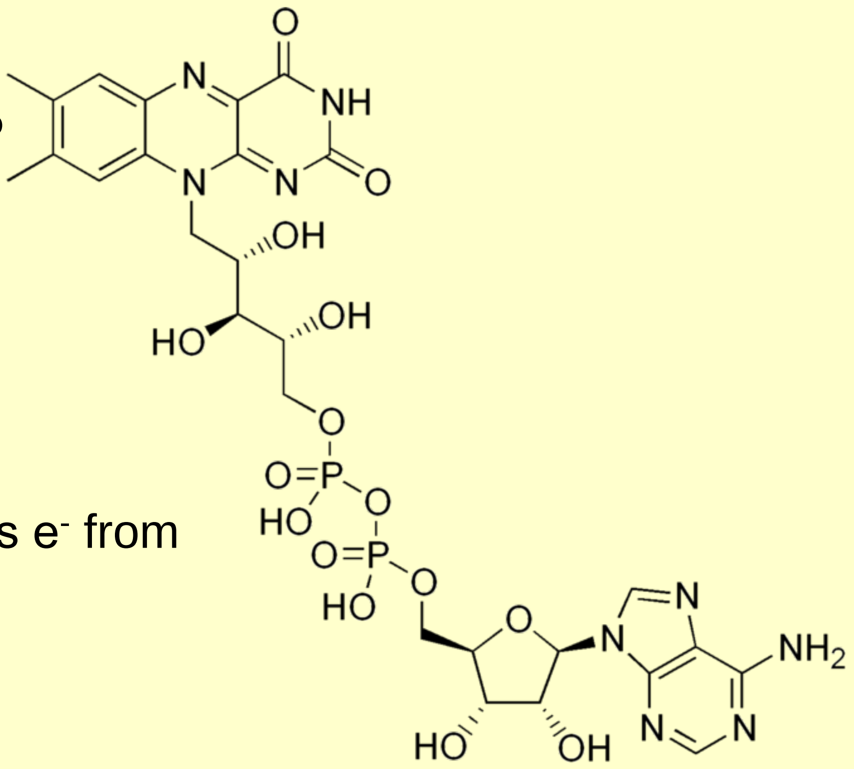
- **NAD⁺** - is an oxidizing agent → it accepts e^- from other molecules and becomes reduced
- **NADH** - reducing agent → donates e^-

✓ the main function of NAD^+ - e^- transfer reactions

❖ $FADH_2$ - flavin adenine dinucleotide

✓ coenzyme found in all living cells

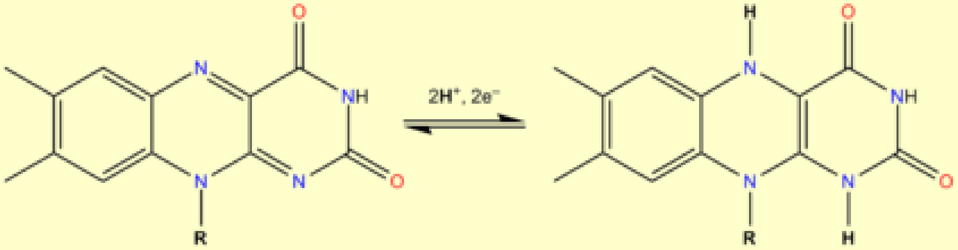
✓ consists of riboflavine (vitamin B2) bound to the phosphate group of an ADP molecule



✓ involved in redox reactions

✓ found in two forms in cells:

- **FAD** - is an oxidizing agent → it accepts e^- from other molecules and becomes reduced
- **$FADH_2$** - reducing agent → donates e^-

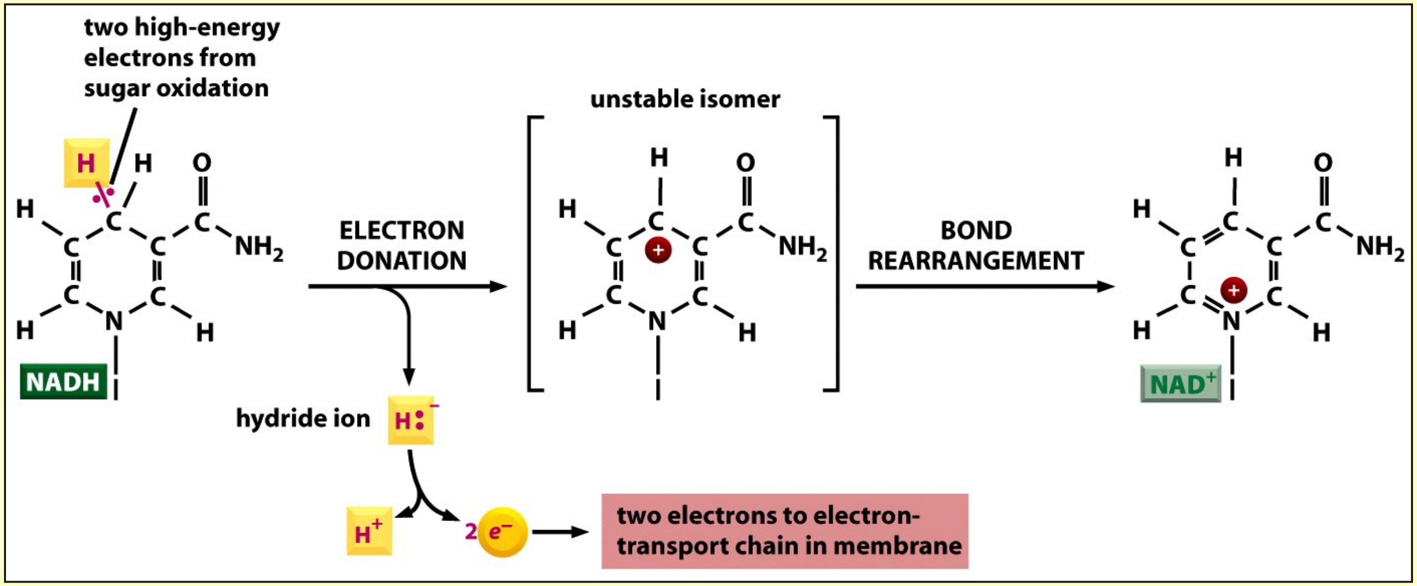


✓ mitochondria use both pyruvate and fatty acids → acetyl CoA

✓ acetyl groups of acetyl CoA are being oxidized in **citric acid cycle**

→ C-atoms from acetyl CoA converted to CO_2

→ cycle forms high-energy e^- transferred by NADH and $FADH_2$

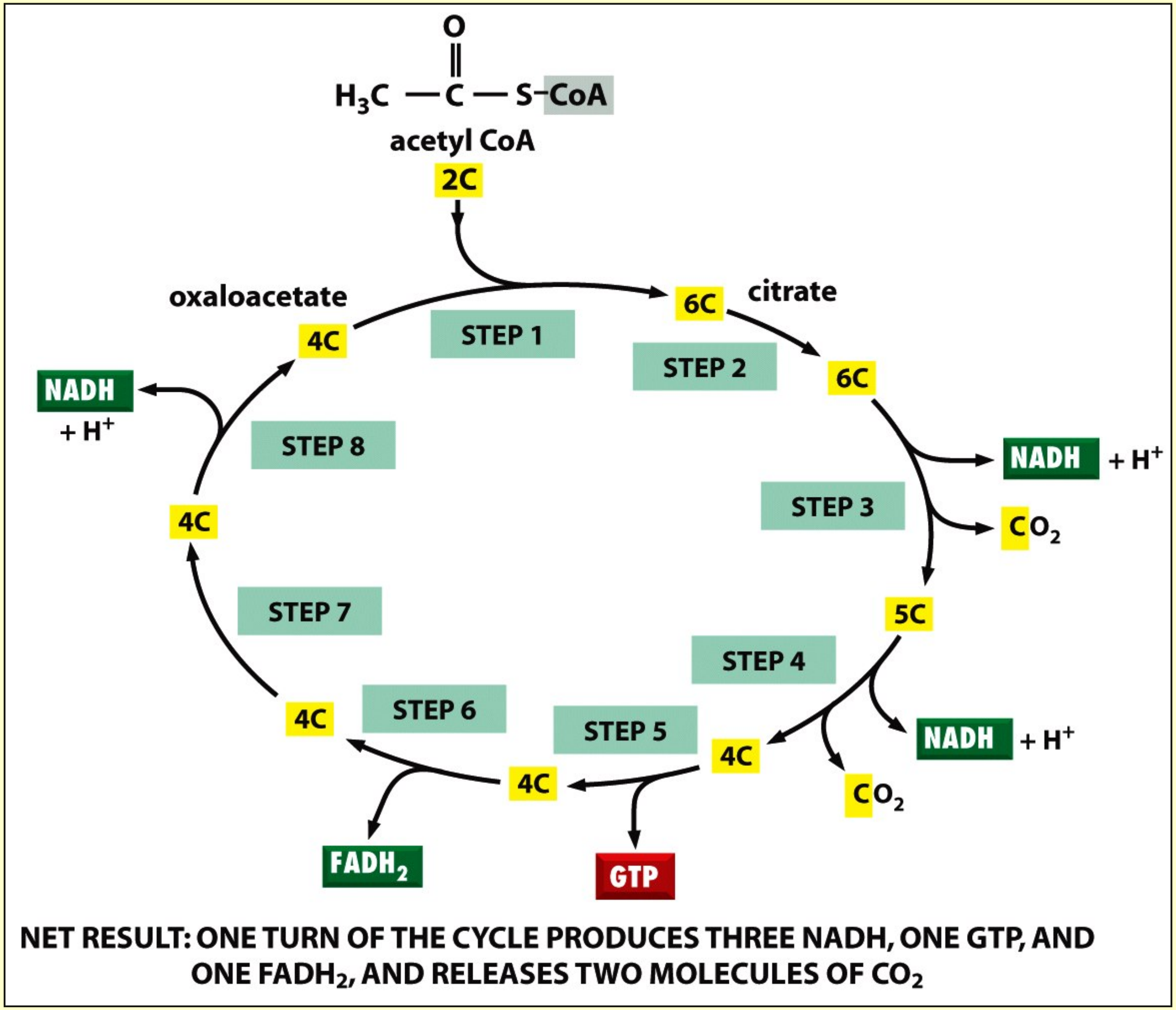


✓ hydride ion is removed from NADH and is converted into H^+ and 2 high-energy e^-

$$H^- \rightarrow H^+ + 2e^-$$

Figure 14-9 *Molecular Biology of the Cell* (© Garland Science 2008)

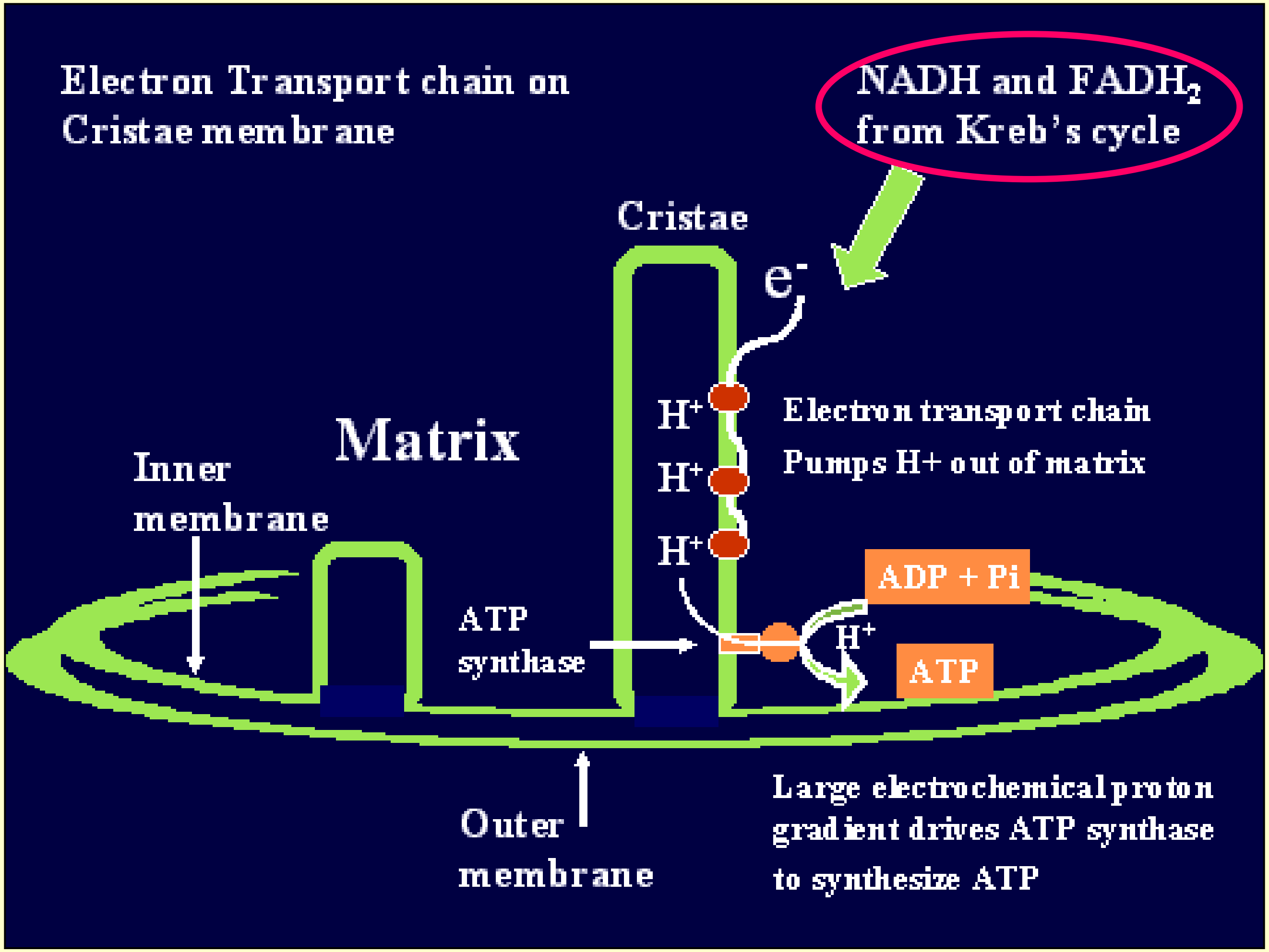
Citric acid cycle (Kreb's cycle)



- ✓ **Matrix**
- ✓ **Yield:**
- **3 NADH molecules**
- **1 $FADH_2$ molecule**
- **1 GTP**

Figure 2-82 *Molecular Biology of the Cell* (© Garland Science 2008)

Mitochondrium inner membrane



e^- transport in respiratory chain

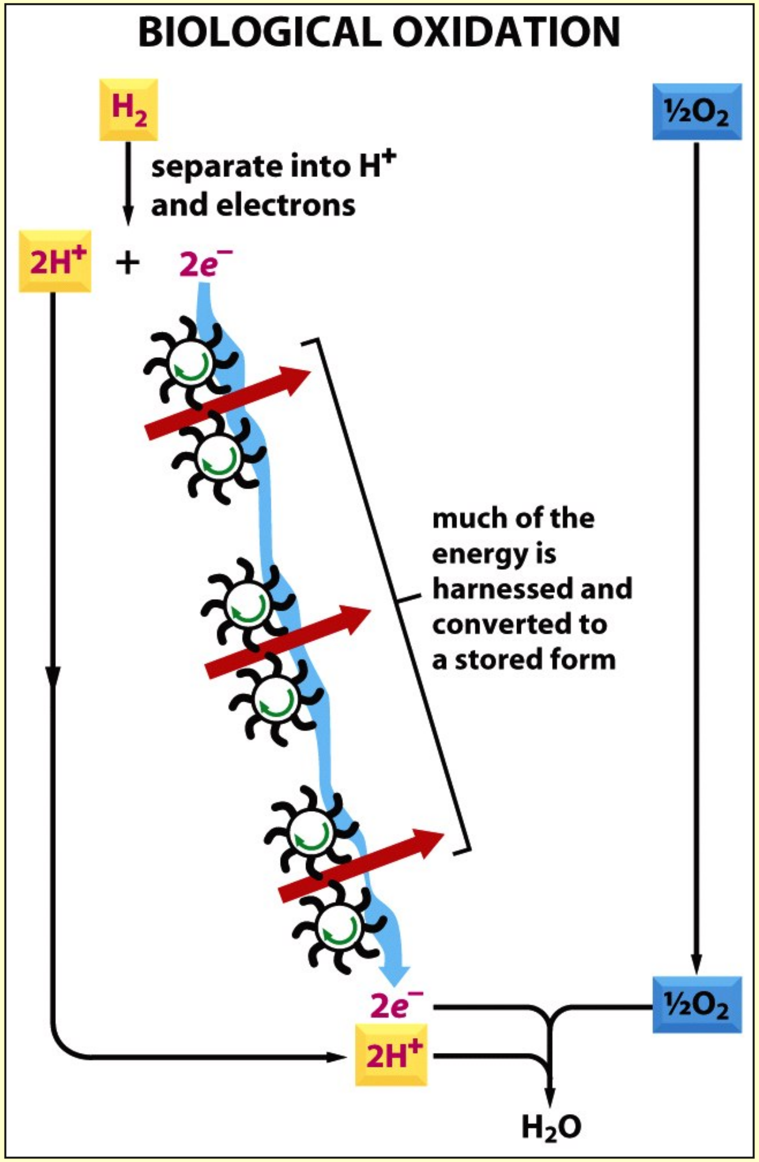


Figure 14-12b *Molecular Biology of the Cell* (© Garland Science 2008)

Oxidative phosphorylation

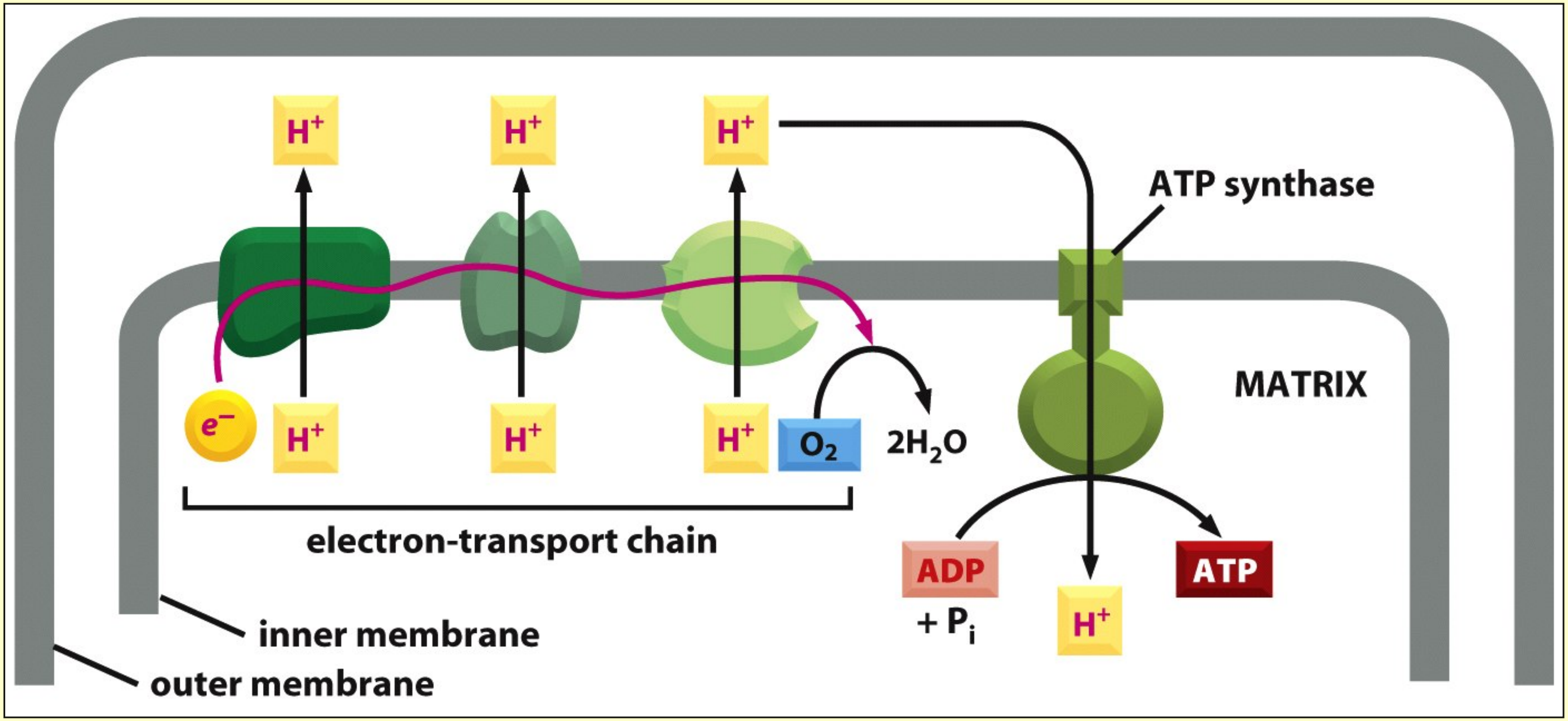
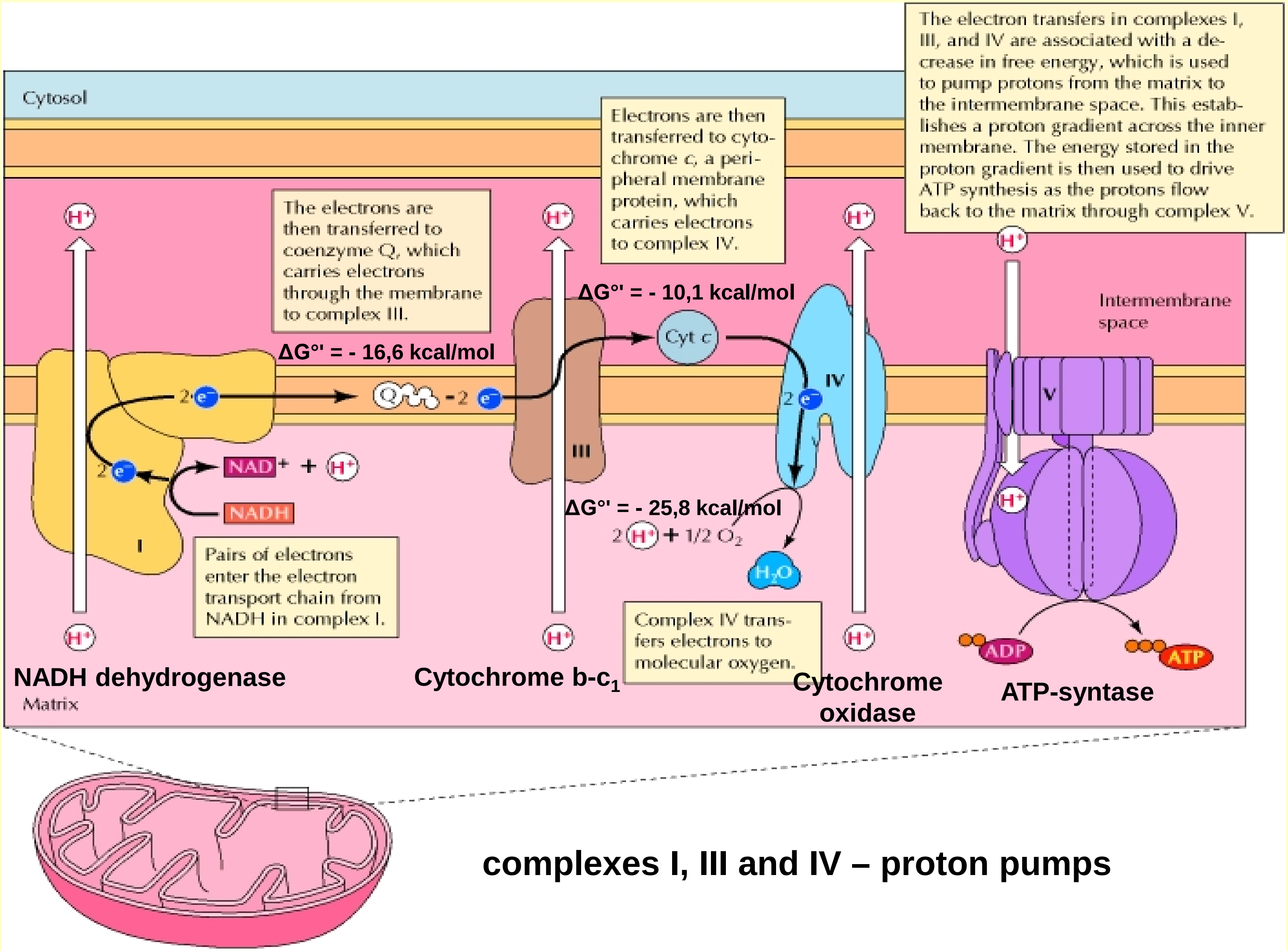


Figure 14-14 *Molecular Biology of the Cell* (© Garland Science 2008)

Transport of electrons from NADH

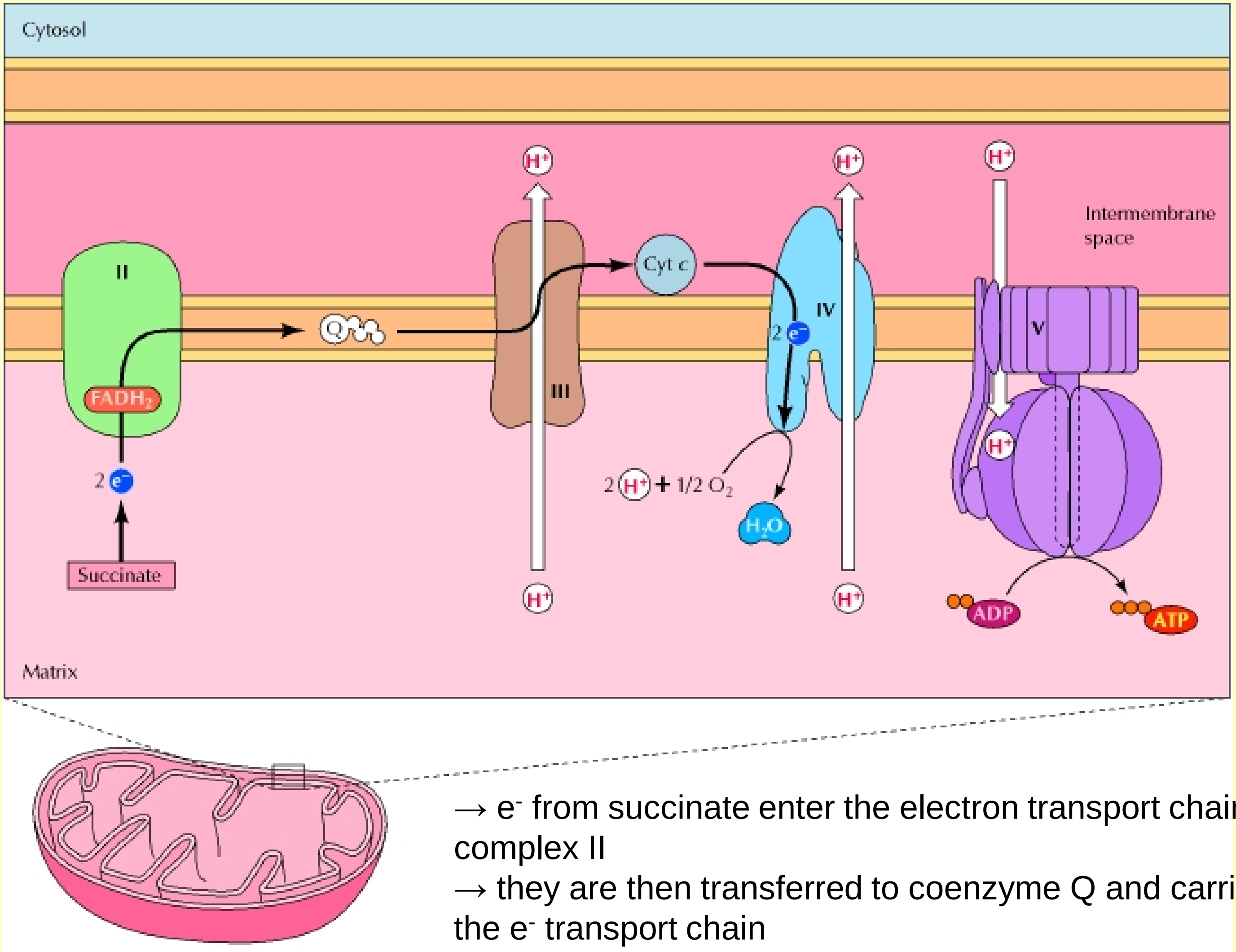


complexes I, III and IV – proton pumps

2000. Cooper Figure 10.8

Animation - http://il.youtube.com/watch?v=xbJ0nbzt5Kw

Transport of electrons from $FADH_2$



→ e^- from succinate enter the electron transport chain via $FADH_2$ in complex II
→ they are then transferred to coenzyme Q and carried through the rest of the e^- transport chain
→ e^- transfer from $FADH_2$ to coenzyme Q is not associated with a significant decrease in free energy, so protons are not pumped across the membrane at complex II

Figure 10-9. 2000. Cooper

The mitochondrial ATP synthase (complex V)

✓ consists of two multisubunit components, F_o and F_1, which are linked by a slender stalk

✓ **F_o** spans the lipid bilayer, forming a channel through which H^+ cross the membrane

✓ **F_1** harvests the free energy derived from H^+ movement down the electrochemical gradient by catalyzing the synthesis of ATP

✓return of protons through F_o induces rotation of F_1 → ATP synthesis

✓ flow of 4 H^+ back across the membrane is required to drive the synthesis of 1 ATP

→ oxidation of **1 NADH** leads to the synthesis of **3 ATP**

→ oxidation of **1 $FADH_2$**, which enters the electron transport chain at complex II, yields only **2 ATP**

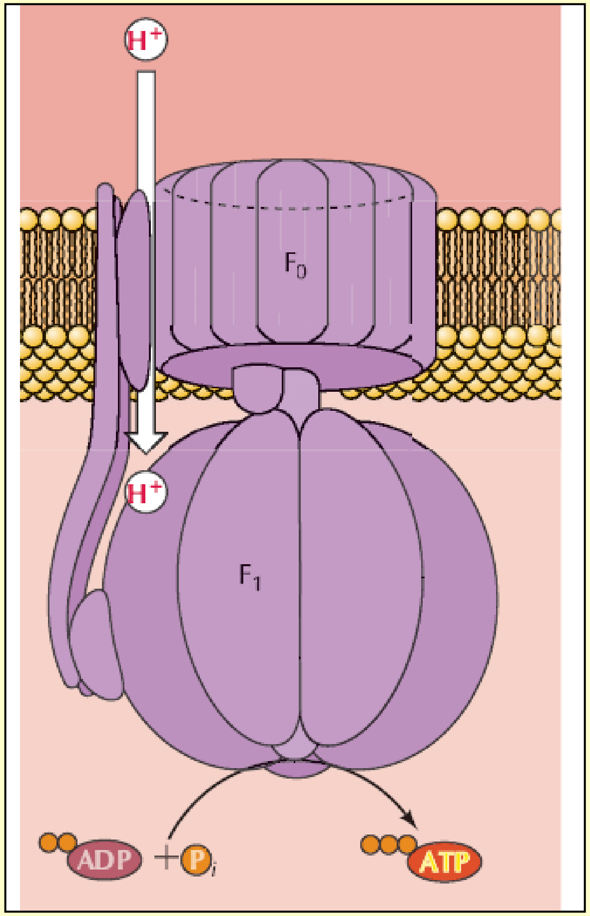
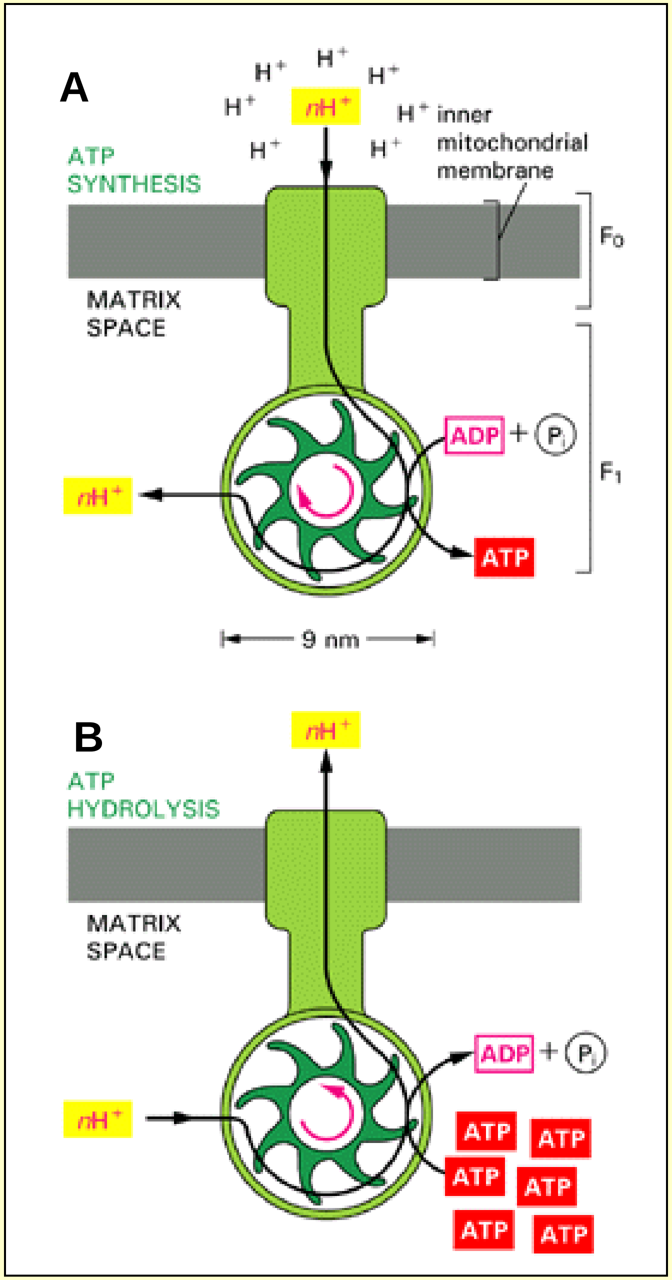


Figure 10-11. 2000. Cooper

Animation
http://il.youtube.com/watch?v=3y1dO4nNaKY



ATP synthase can work in both ways

A – ATP synthesis

→ energetically favorable return of protons to the matrix is coupled to ATP synthesis

B – ATP hydrolysis

→ ATP-ase pumps protons against their electrochemical gradient

2002. Alberts et al.

❖ Net yield in energy

During oxidative phosphorylation:

- each e^- pair from NADH (citric acid cycle) → 2.5 ATP

- each e^- pair from $FADH_2$ (citric acid cycle) → 1.5 ATP

- each e^- pair from NADH (glycolysis) → 1.5 - 2.5 ATP

A – 38 ATP

2 ATP (glycolysis) + 2 ATP (citric acid cycle) + 34 ATP (e^- transport)

B – 36 ATP

- **in some cells → 2 NADH (glycolysis) can not enter mitochondria directly**

- **their enter through "*shuttle*" system → their e^- might enter the chain at complex II**

2002. Alberts et al.

Mitochondrial genome

✓ contain their own genetic system, which is separate and distinct from the nuclear genome of the cell

✓autoreduplicative and semiautonomous organelle

✓ circular DNA molecules (like those of bacteria); present in multiple copies per organelle

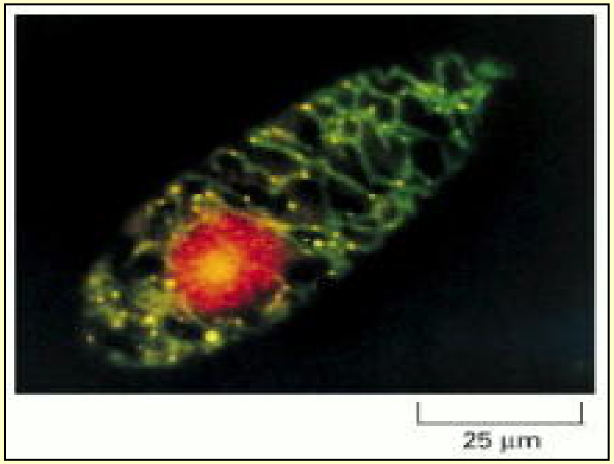


Figure 14-50. 2002. Alberts et al.

Mitochondrial and nuclear DNA in ***Euglena gracilis.***

- ✓ genome – red (ethydium bromide)
- ✓ multiple small $_m$genomes – yellow
- ✓ mitochondrial matrix – green fluorescent dye

Mitochondrial genomes

✓ variations in size:

- human $_m$genome cca 16 kb
- yeast $_m$genom cca 80 kb
- plant $_m$genom cca 200 kb

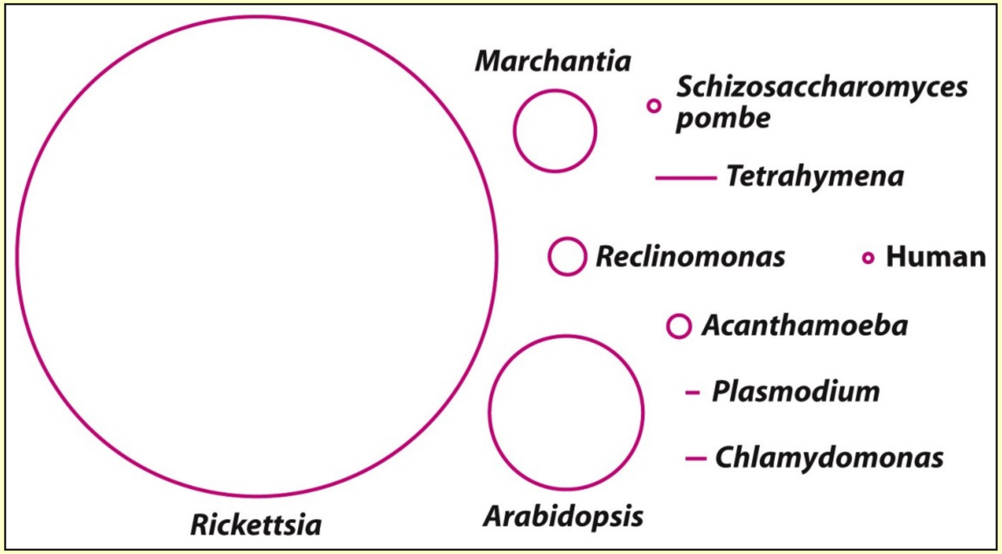


Figure 14-57 *Molecular Biology of the Cell* (© Garland Science 2008)

✓ largest sequenced *A. thaliana* $_m$genom
- 367 kb (32 proteins)

✓ greatest number of genes in mDNA protozoa *Recclinomonas americana*
- 69 kb → 97 genes
- more like bacterial genomes

Human $_m$genome

- ✓ 13 proteins involved in e^- transport chain and oxidative phosphorylation
- ✓ 16S and 12S rRNA
- ✓ 22 tRNA

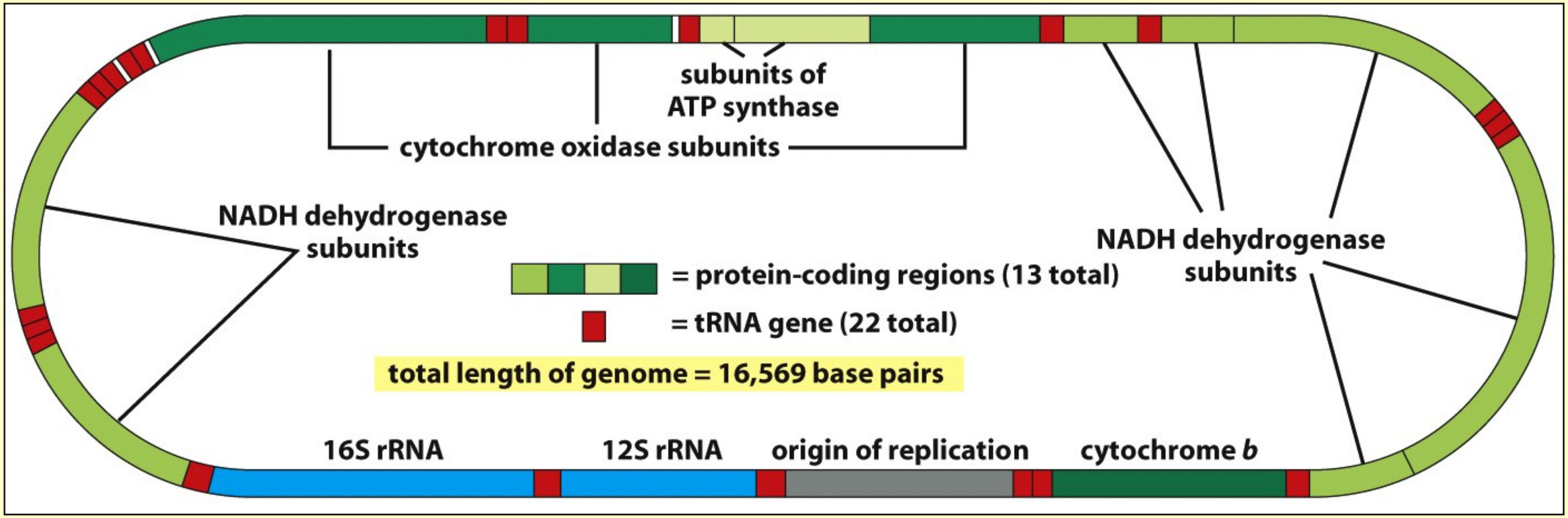


Figure 14-60 *Molecular Biology of the Cell* (© Garland Science 2008)

$_m$genomes

✓ distributed in several clusters → nucleoids

✓ DNA without histones (like bacteria and chloroplast)

✓ dense gene packing → very little room for regulatory sequences

✓ relaxed codon usage → many tRNAs recognize any of the 4 nucleotides in the third position → 22 tRNA

✓ variant genetic code → 4 of the 64 codons have different meanings form those in other genomes

✓ $_m$protein synthesis starts with N-formyl methionin (like bacteria and chloroplast)

Differences between universal and mitochondrial genetic code

Table 14–3 Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

CODON	"UNIVERSAL" CODE	MITOCHONDRIAL CODES			
		MAMMALS	INVERTEBRATES	YEASTS	PLANTS
UGA	STOP	*Trp*	*Trp*	*Trp*	STOP
AUA	Ile	*Met*	*Met*	*Met*	Ile
CUA	Leu	Leu	Leu	*Thr*	Leu
AGA, AGG	Arg	*STOP*	*Ser*	Arg	Arg

Red* italics indicate that the code differs from the "Universal" code.

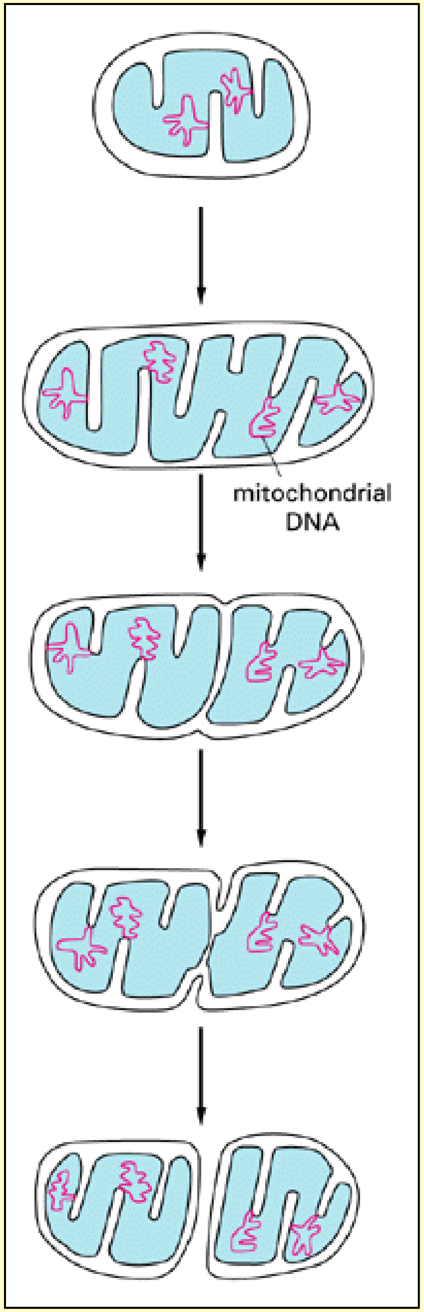
✓ prokaryota and eukaryota – at least 30 tRNA

✓ mitochondria – 22 tRNA

→ **U** can be paired with any of the 4 nucleotides in the third position

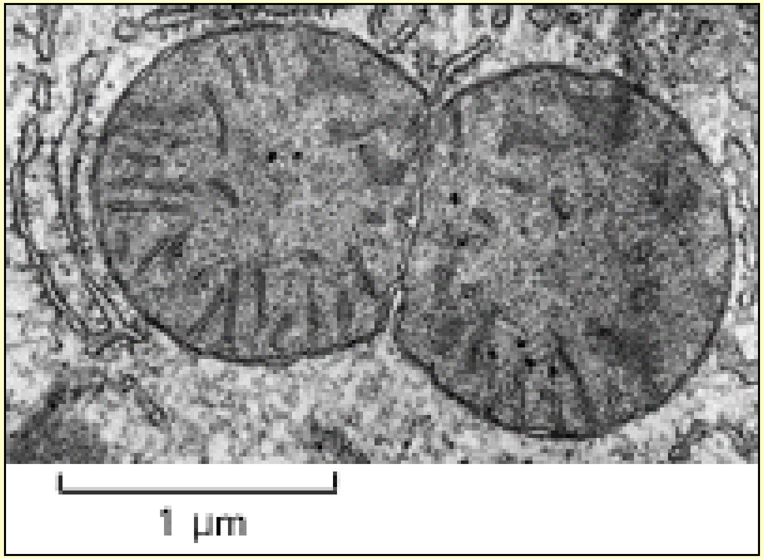
→ 1 tRNA can recognize 4 different codons

Table 14-3. 2002. Alberts et al.



❖ Mitochondrial division

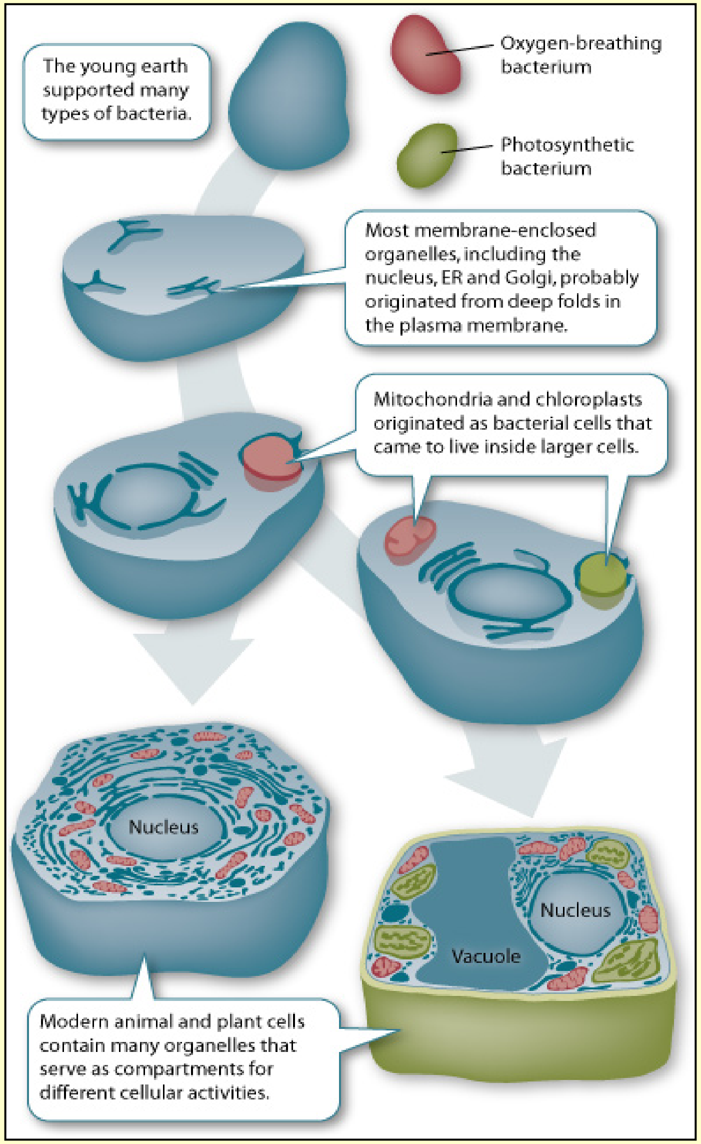
✓ resembles that of bacterial cell division



An electron micrograph of a dividing mitochondrion in a liver cell

Figure 14-54. 2002. Alberts et al.

❖ A suggested evolutionary pathway for the origin of mitochondria and chloroplasts



✓ there is compelling evidence that mitochondria and chloroplasts were once primitive bacterial cells
→ **endosymbiotic theory**

✓ large host cell and ingested bacteria became dependent on one another for survival
→ permanent relationship

✓ over millions of years of evolution, mitochondria and chloroplasts have become more specialized and today they cannot live outside the cell

http://learn.genetics.utah.edu/content/begin/cells/organelles/

The production of mitochondrial and chloroplast proteins by two separate genetic systems

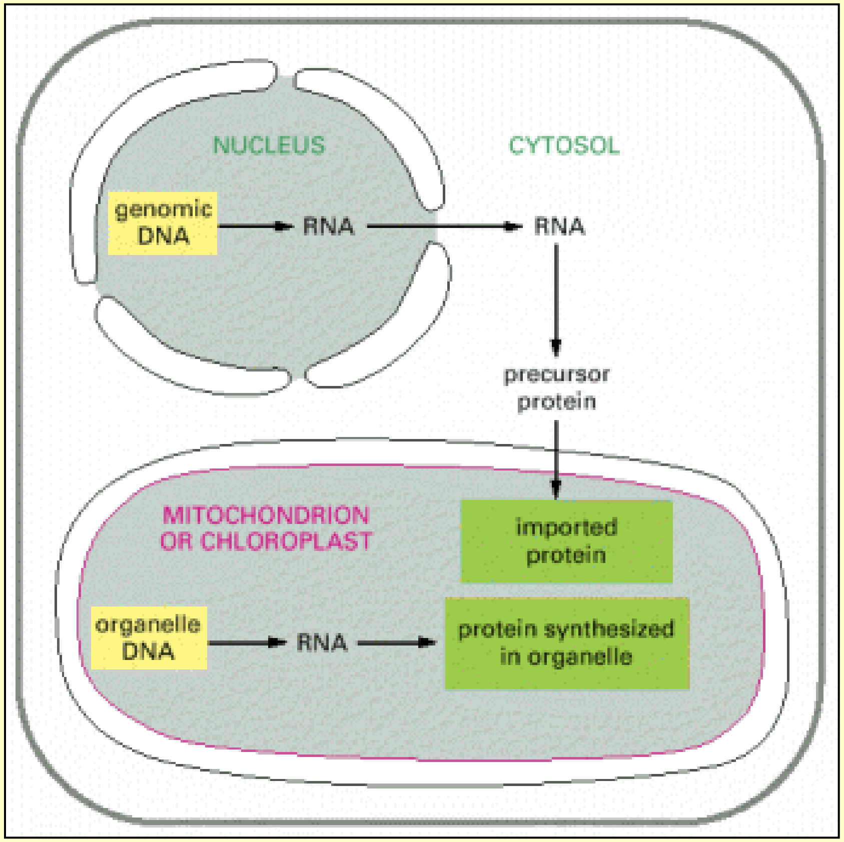
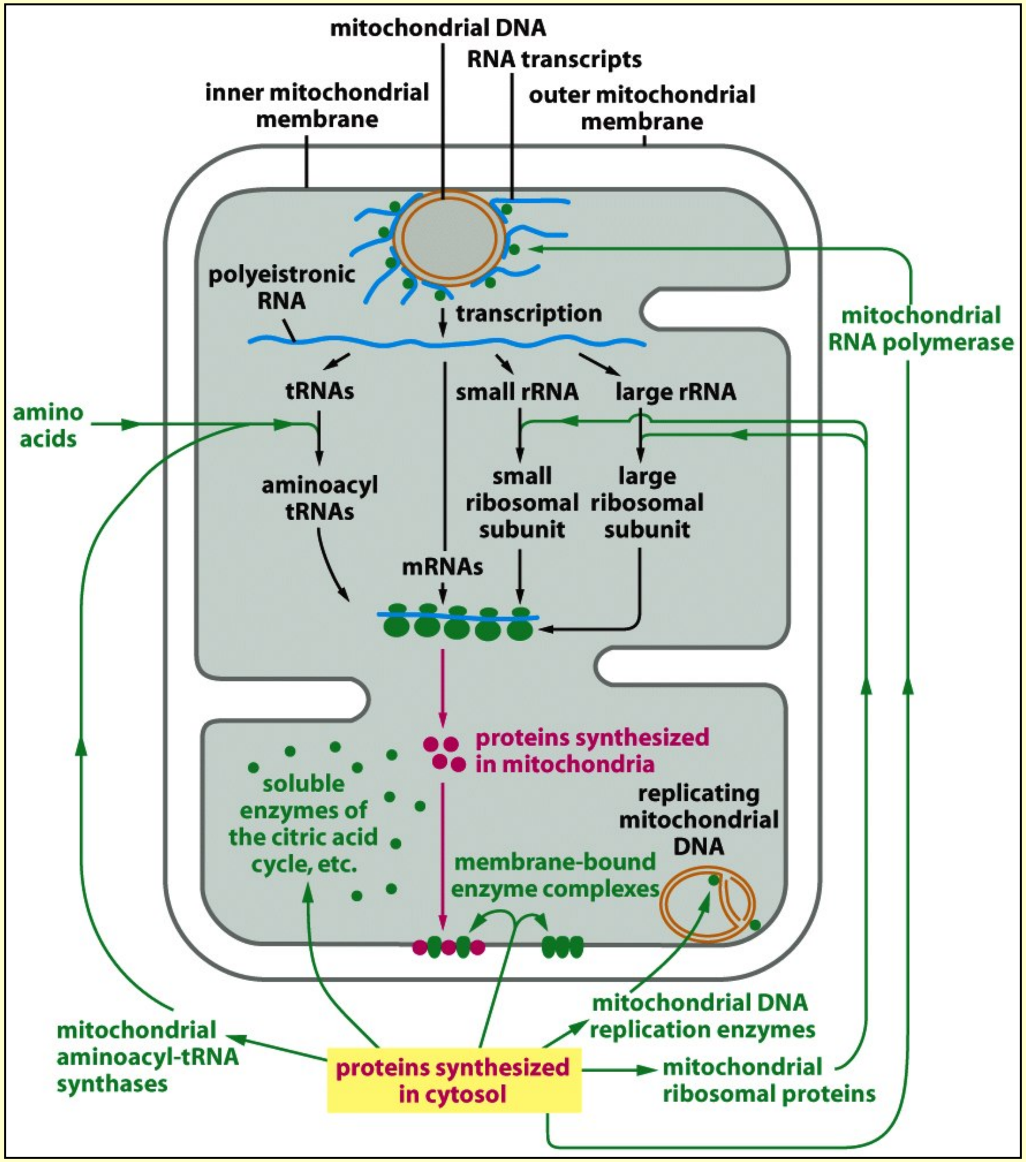


Figure 14-51. 2002. Alberts et al.

✓ **majority of the proteins encoded by nuclear genome**

The origins of mitochondrial RNAs and proteins



✓**proteins encoded in the nucleus and imported from the cytosol** → major role in creating the genetic system of the mitochondrion in addition to contributing most of the organelle's other proteins

✓**organelle contribution** → mitochondrion itself contributes only mRNAs, rRNAs, and tRNAs to its genetic system

Figure 14-66 *Molecular Biology of the Cell* (© Garland Science 2008)

Protein import pathways of mitochondria

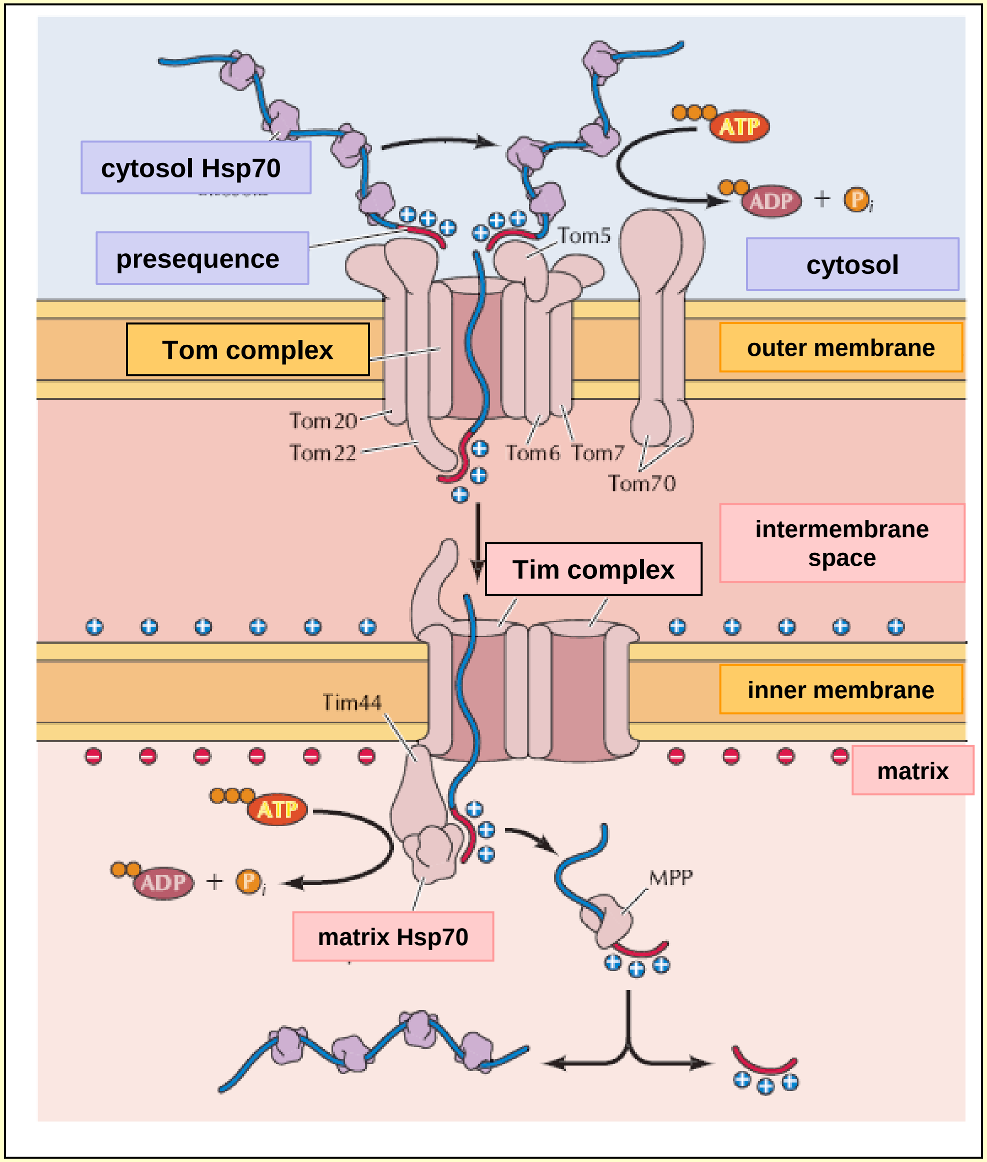
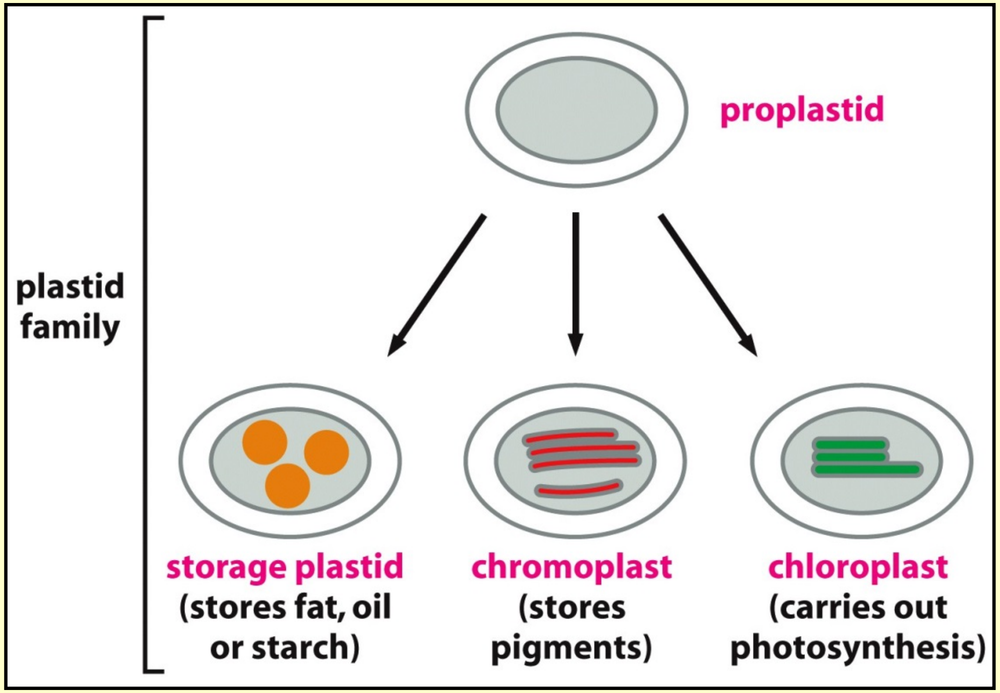


Figure 10-4. 2004. Cooper and Hausman

- ✓ ATP-driven
- ✓presequence at N-end (25-35 ak)
- ✓ cytosol chaperon Hsp70
- ✓ outer membrane receptors – protein complex **Tom** (***T****ranslocase of the* ***O****uter* ***M****embrane*)
- ✓ transfer through the outer membrane
- ✓ inner membrane receptors – protein complex **Tim** (***T****ranslocase of the* ***I****nner* ***M****embrane*)
- ✓ transfer to matrix – presequence is cleaved by MPP (*matrix processing peptidase*)
- ✓ matrix chaperon Hsp70 facilitates protein folding

Chloroplasts

Plastid types



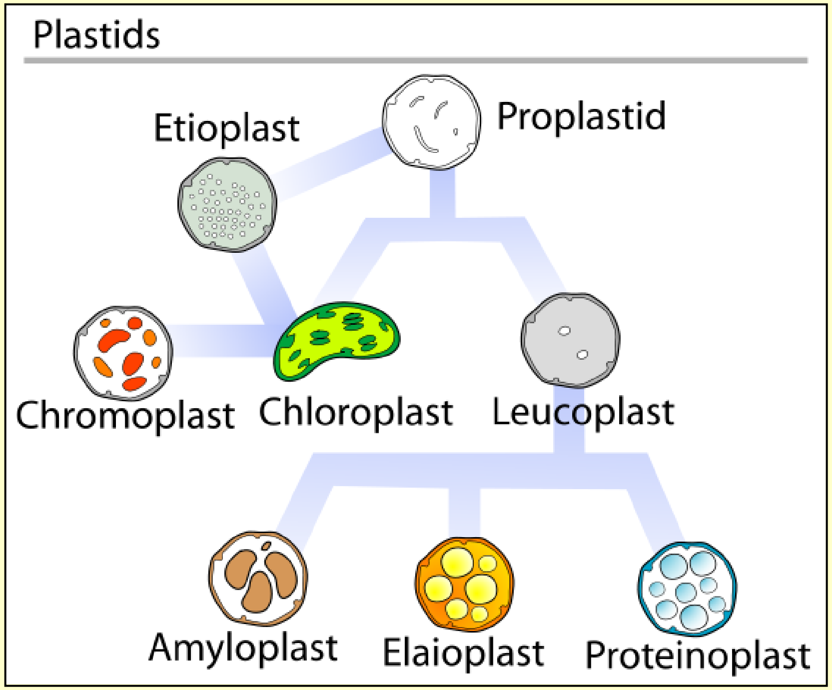


Figure 12-3a *Molecular Biology of the Cell* (© Garland Science 2008)

From Wikipedia, the free encyclopedia

Chloroplasts

- ✓ Plants
- ✓ similarities with mitochondria:
  - produce metabolic energy
  - bounded by a double membrane → chloroplast envelope
  - evolutionary origins from photosynthetic bacteria
  - contain their own genetic system
  - binar division
- ✓ But! Bigger in size and more complex than mitochondria
- ✓ Processes:
  - ATP synthesis
  - conversion of CO_2 in carbohydrates (photosynthesis)
  - synthesis of amino acids, fatty acids and lipids of their membranes

Chloroplast formation

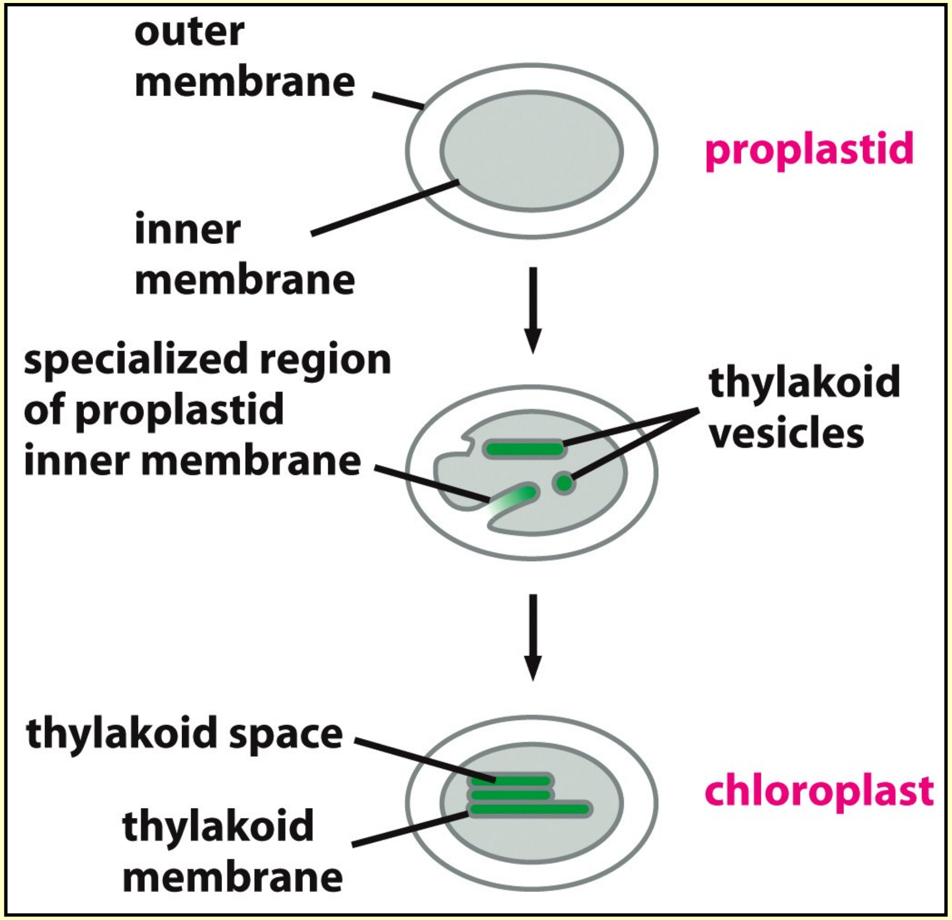


Figure 12-3b *Molecular Biology of the Cell* (© Garland Science 2008)

Chloroplasts in plant cell

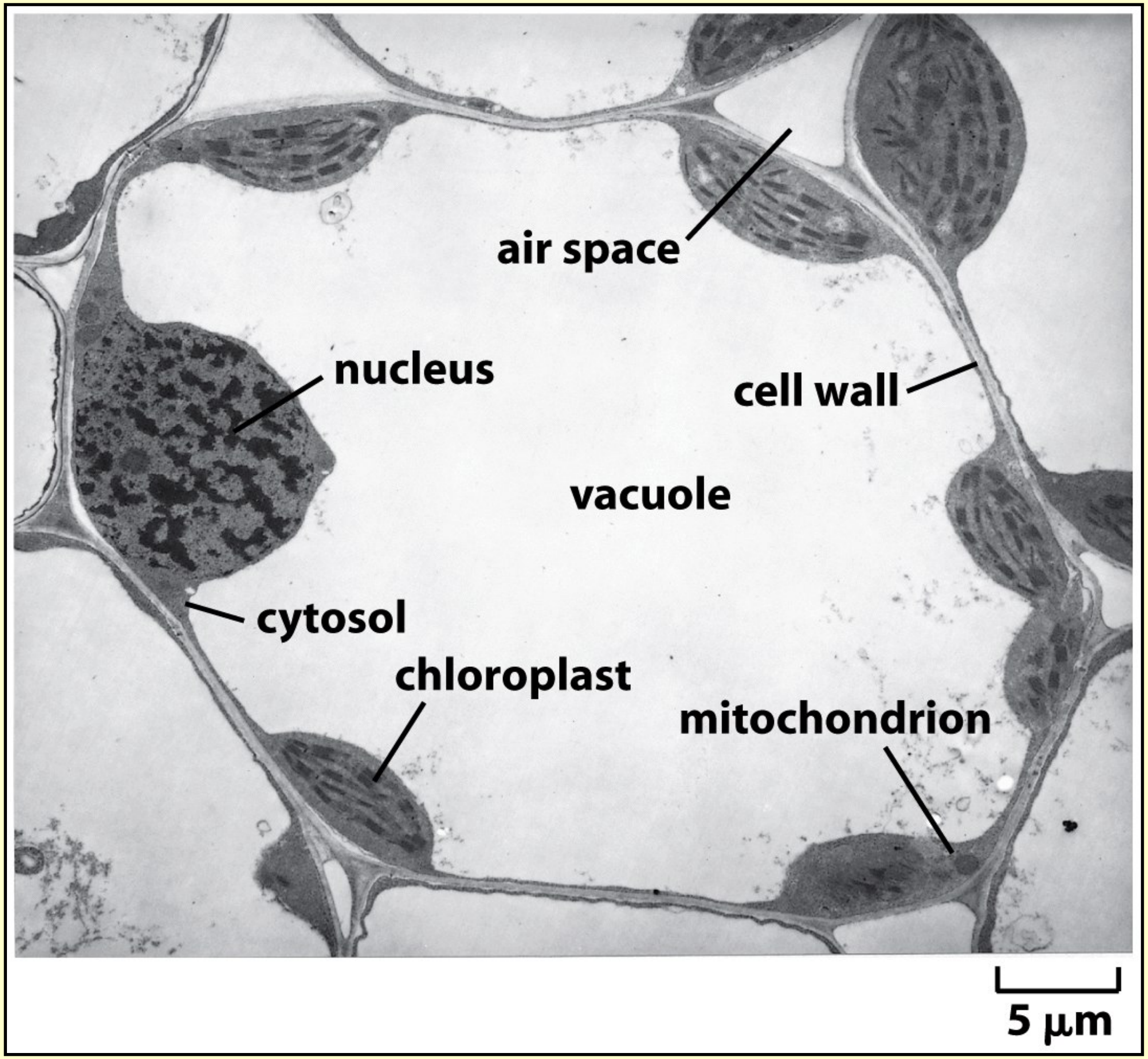
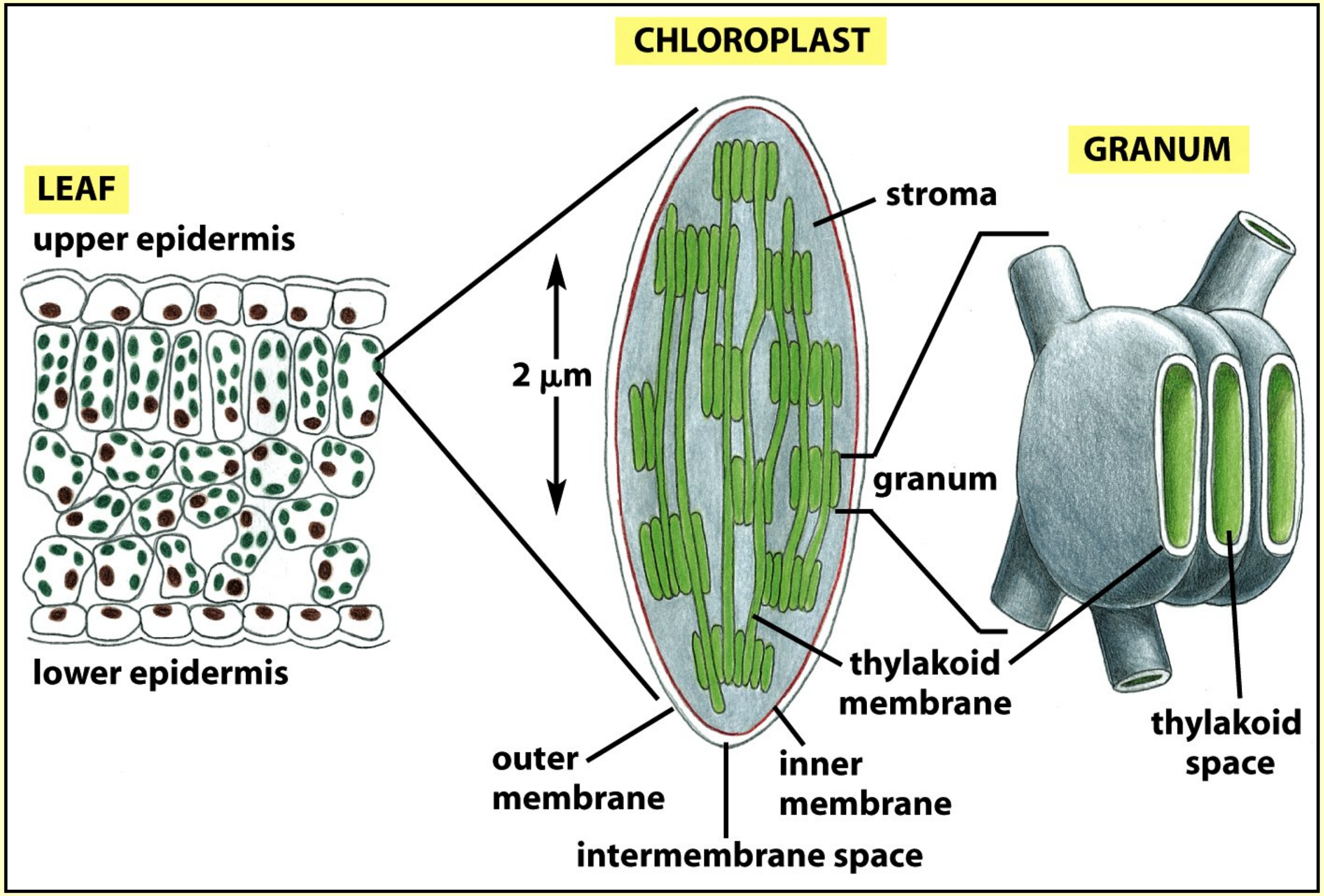


Figure 14-35a *Molecular Biology of the Cell* (© Garland Science 2008)

Structure and ultrastructure



- ✓ size 5 – 10 µm
- ✓ outer membrane
- ✓ inner membrane
- ✓ **thylakoid membrane**

Figure 14-36 *Molecular Biology of the Cell* (© Garland Science 2008)

Structure and ultrastructure

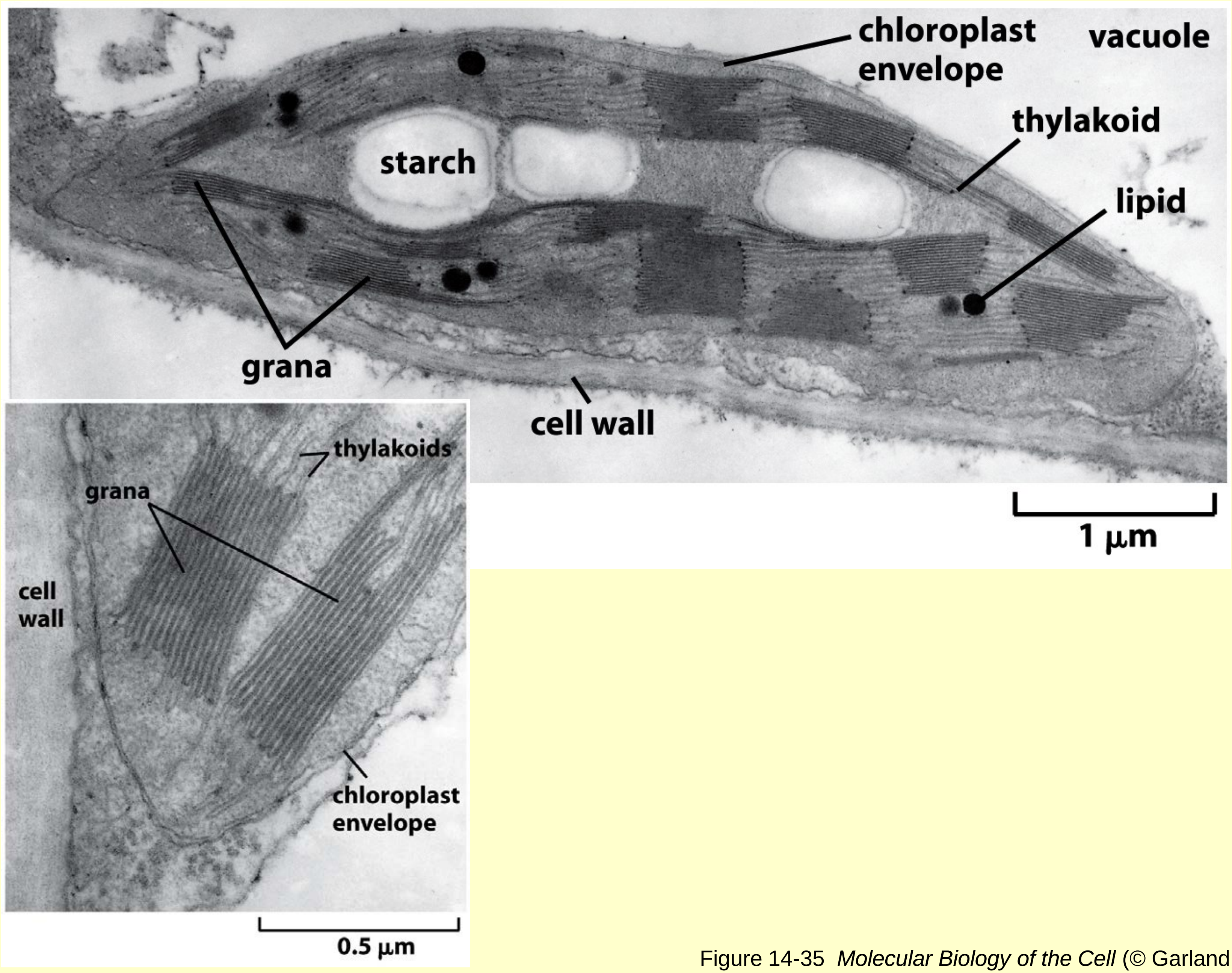


Figure 14-35 *Molecular Biology of the Cell* (© Garland Science 2008)

3 membrane systems make chloroplasts more complex than mitochondria

✓ 3 membranes → 3 different inner compartments:

1. intermembrane space
2. stroma
3. thylakoid lumen

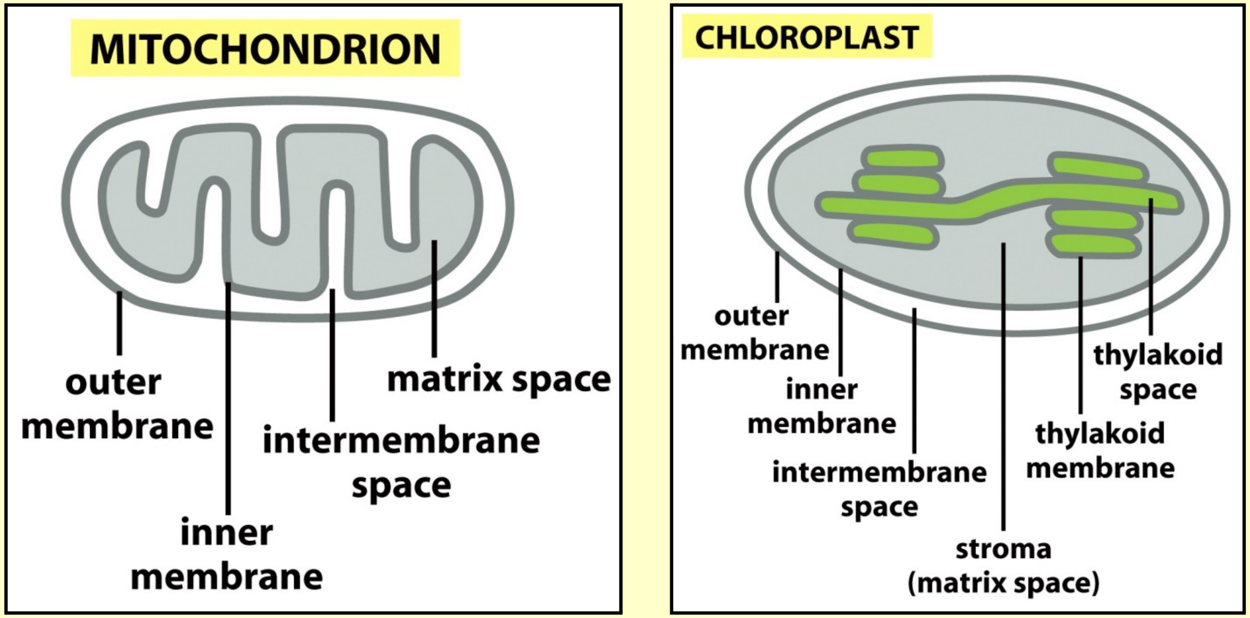
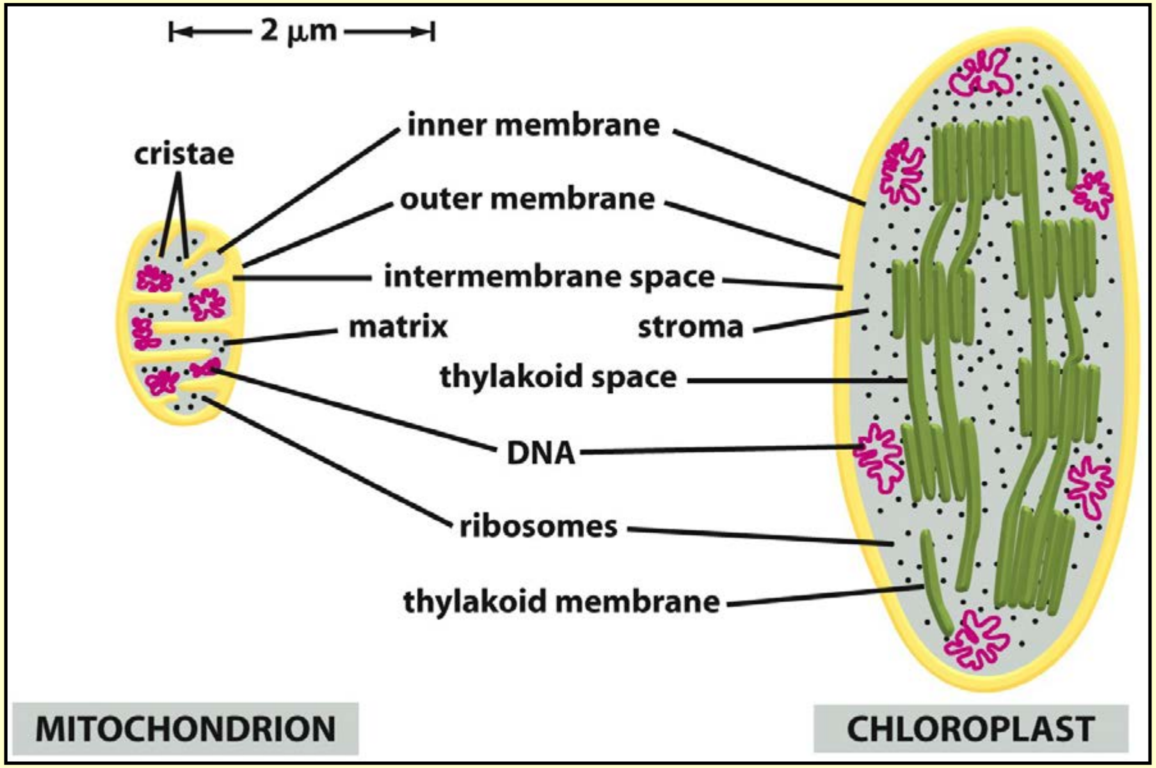


Figure 12-21 *Molecular Biology of the Cell* (© Garland Science 2008)

Mitochondrion vs. chloroplast



✓ **outer membrane** → similar to mitochondrial outer membrane (permeability, porins)

✓ **stroma** equivalent of matrix (genome, metabolic enzymes)

✓ **thylakoid membrane** → e^- transport and ATP synthesis (inner mitochondrial membrane)

✓ **inner membrane** → not involved in photosynthesis

Figure 14-37 *Molecular Biology of the Cell* (© Garland Science 2008)

Chemiosmotic generation of ATP in chloroplasts and mitochondria

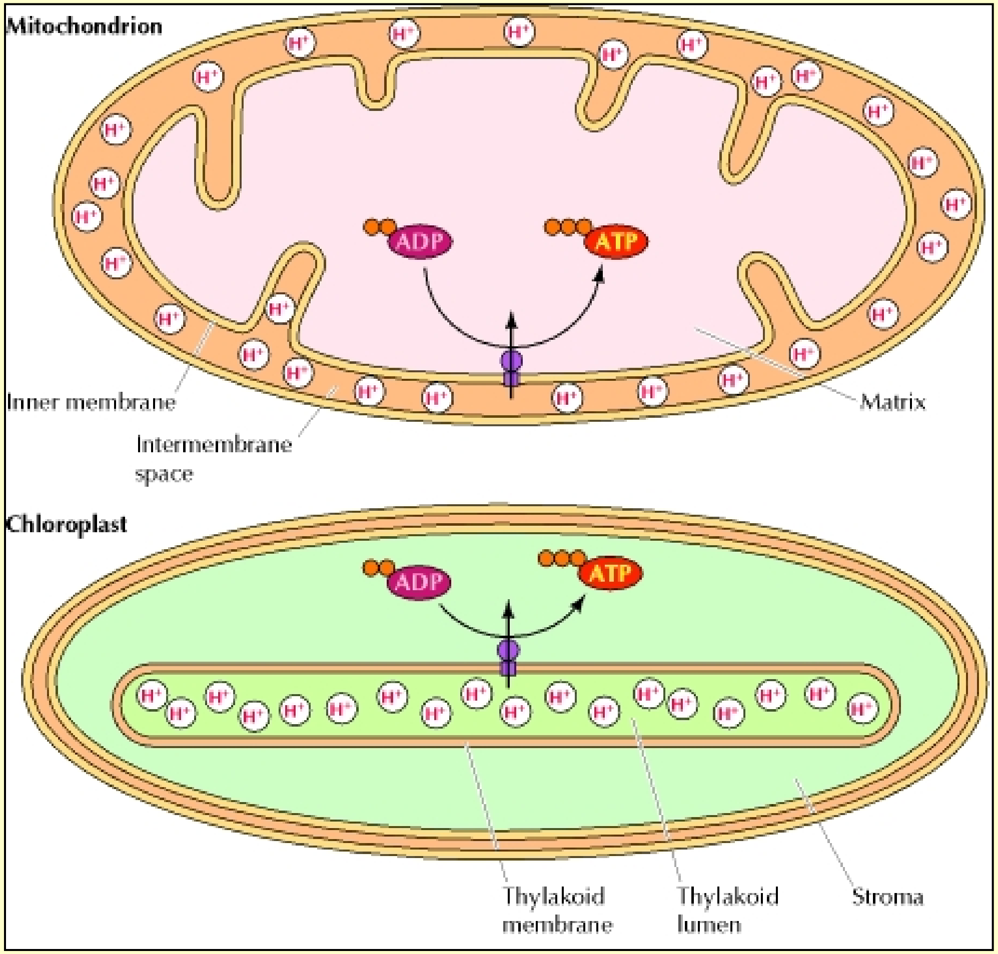


Figure 10.14. 2000. Cooper

→ **mitochondria** - e$^-$ transport generates a H$^+$ gradient across the inner membrane, which is then used to drive ATP synthesis in the matrix

→ **chloroplasts** – H$^+$ gradient is generated across the thylakoid membrane and used to drive ATP synthesis in the stroma

Photosynthesis

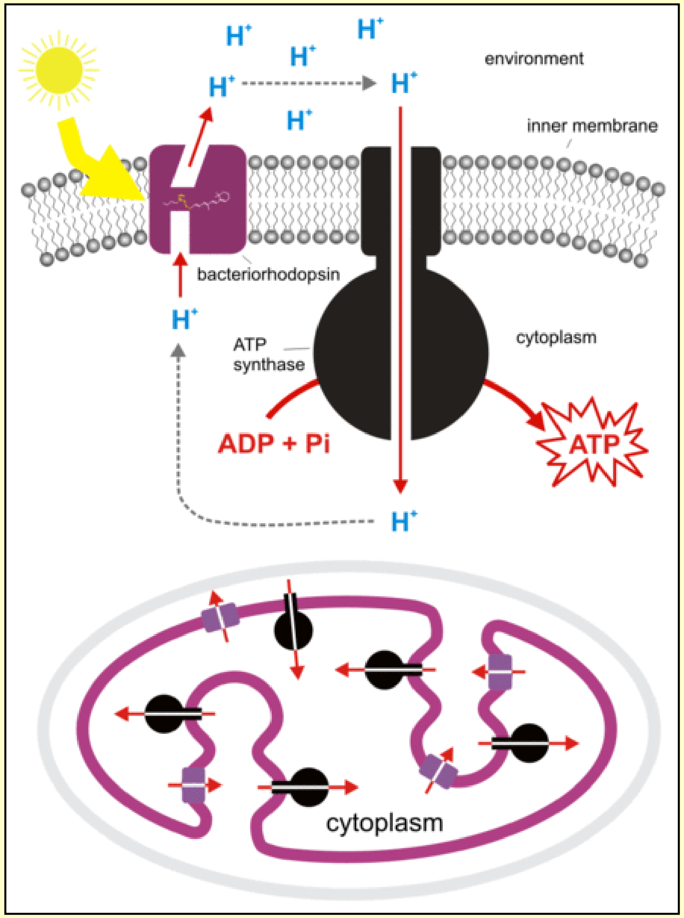
Algae, **bacteria** and **archebacteria**

– photosynthesis on the plasma membrane

– eg. **bacteriorodopsin**

→ photosynthetic proton pump

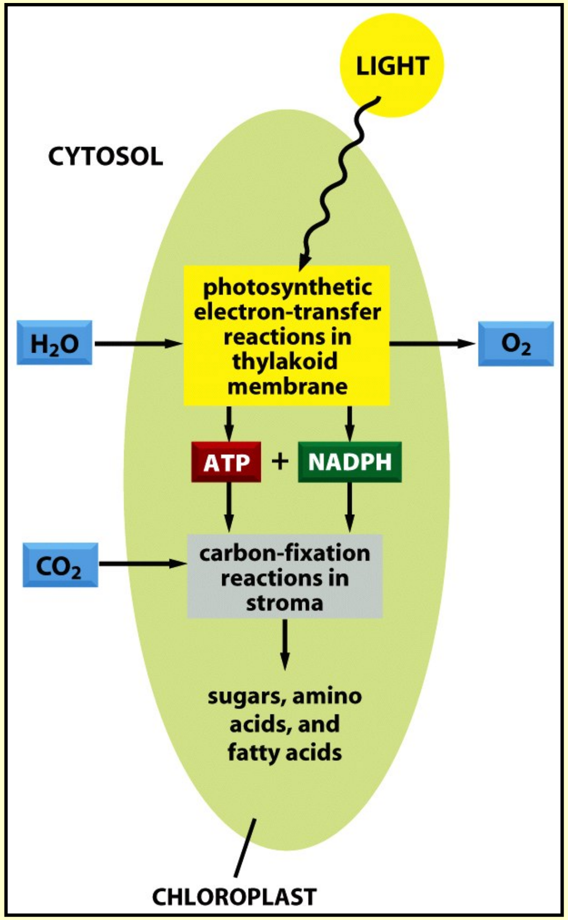
→ *Halobacterium* cell membrane



Photosynthesis in chloroplasts

→ energy from sunlight is harvested and used to drive the synthesis of glucose from CO_2 and H_2O

→ the ultimate source of metabolic energy for all biological systems



→ **takes place in two distinct stages**

1. Light reactions

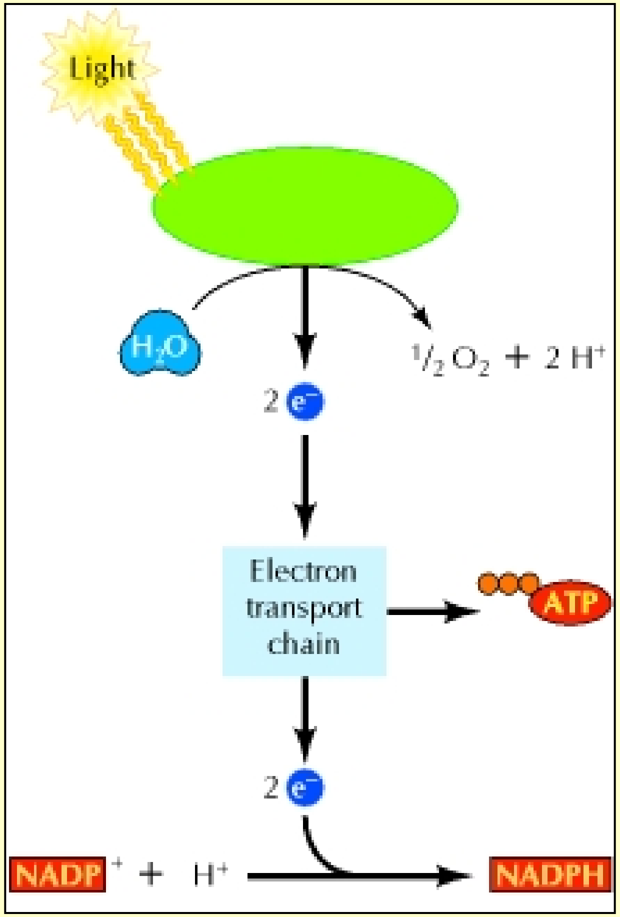
- ✓ energy from sunlight drives the synthesis of ATP and NADPH
- ✓ coupled to the formation of O_2 from H_2O
- ✓ thylakoid membranes

2. Calvin cycle

- ✓ ATP and NADPH produced by the light reactions drive glucose synthesis
- ✓ reduction of CO_2
- ✓ stroma

Figure 14-38 *Molecular Biology of the Cell* (© Garland Science 2008)

Light reactions



✓ energy from sunlight is used to split H_2O to O_2

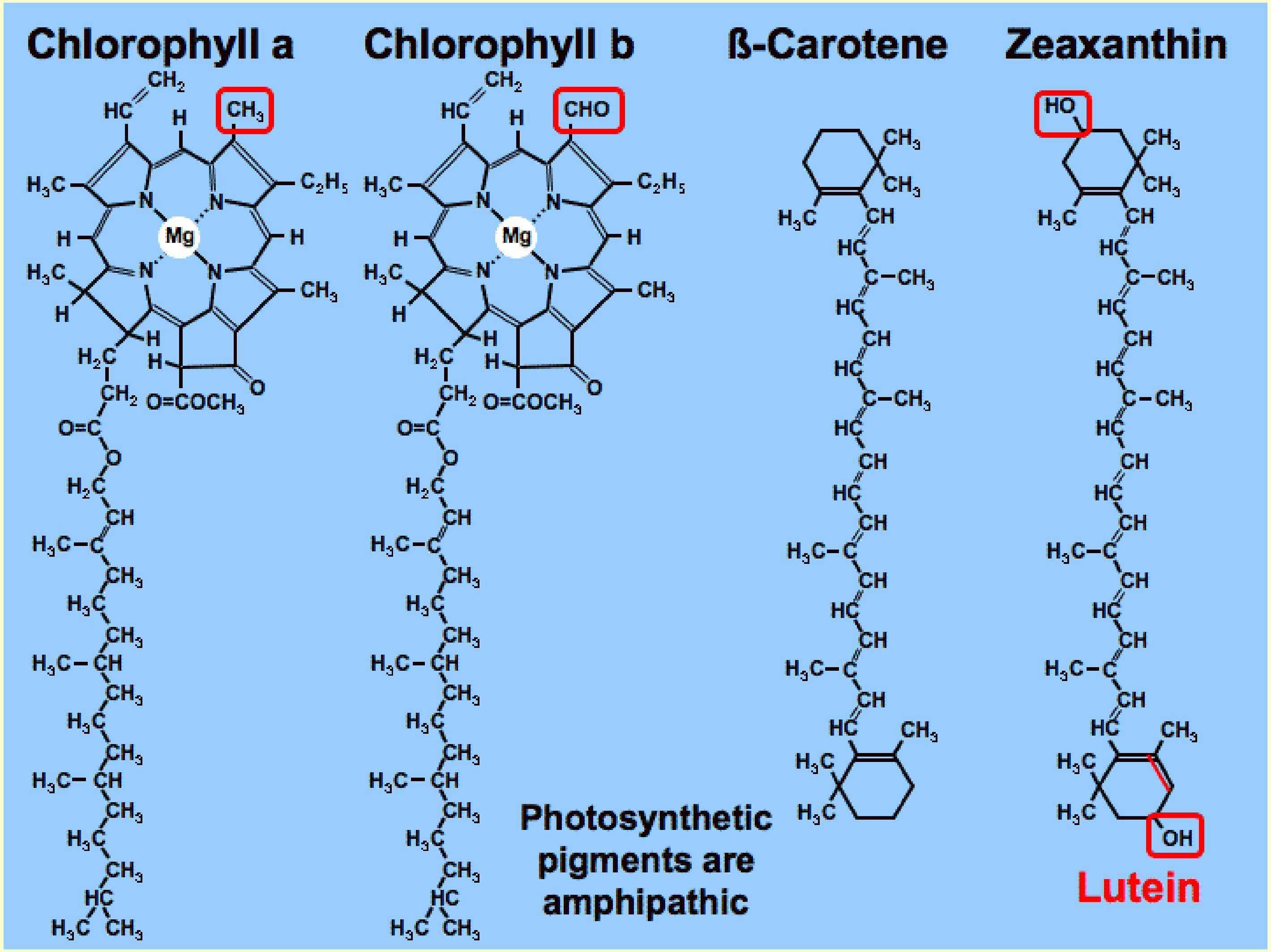
✓ high-energy e^- derived from this process are then transported through a series of carriers and used to convert $NADP^+$ to NADPH

✓ energy derived from the e^- transport reactions also drives the synthesis of ATP

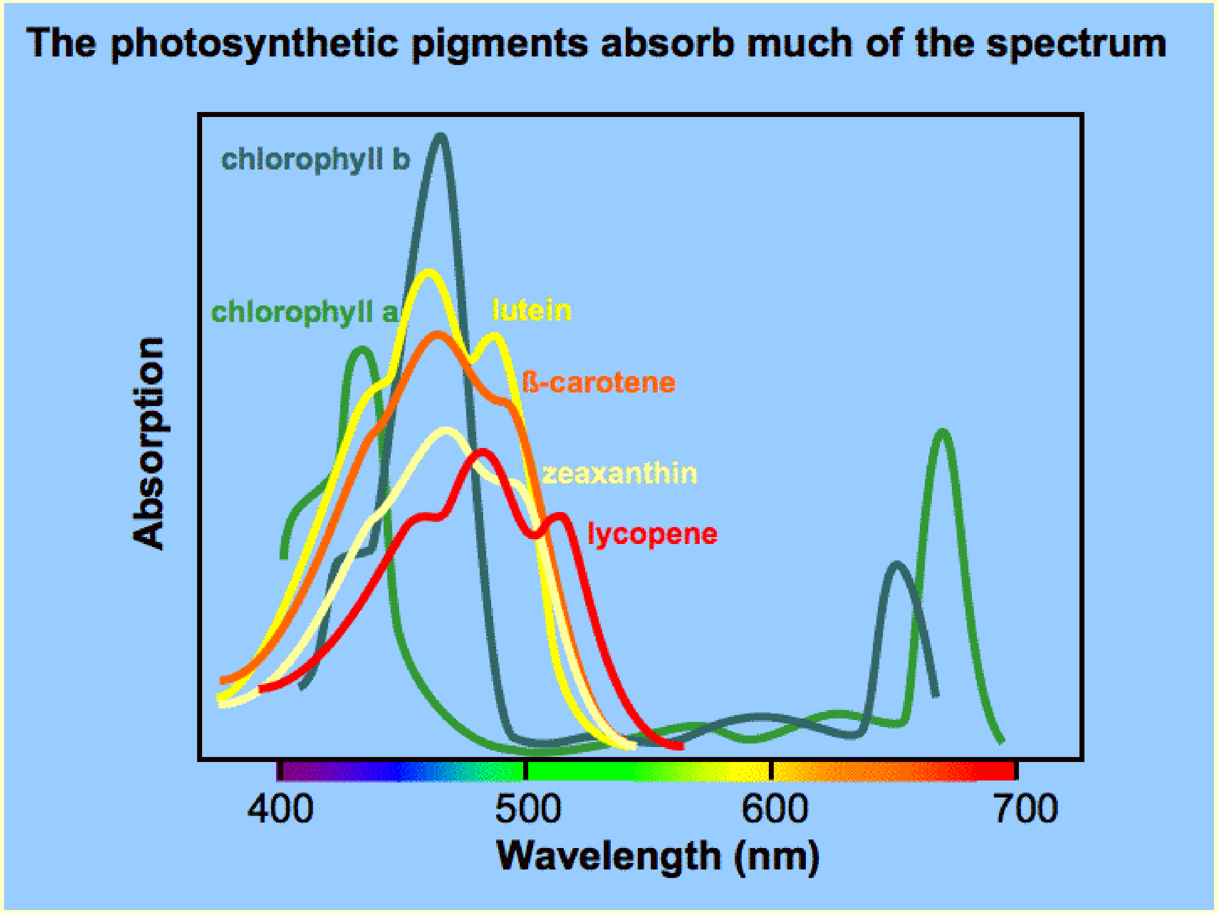
Figure 2.38. 2000. Cooper

Absorption of sunlight

→ photosynthetic pigments – **chlorophyll** *a* and *b* and **carotenoids**



http://plantphys.info/plant_physiology/light.shtml



- ✓**chlorophyll a** – 430 and 662 nm
- ✓**chlorophyll b** – 453 and 642 nm
- ✓**carotenoids** – 400 and 500 nm

Organization of a photocenter

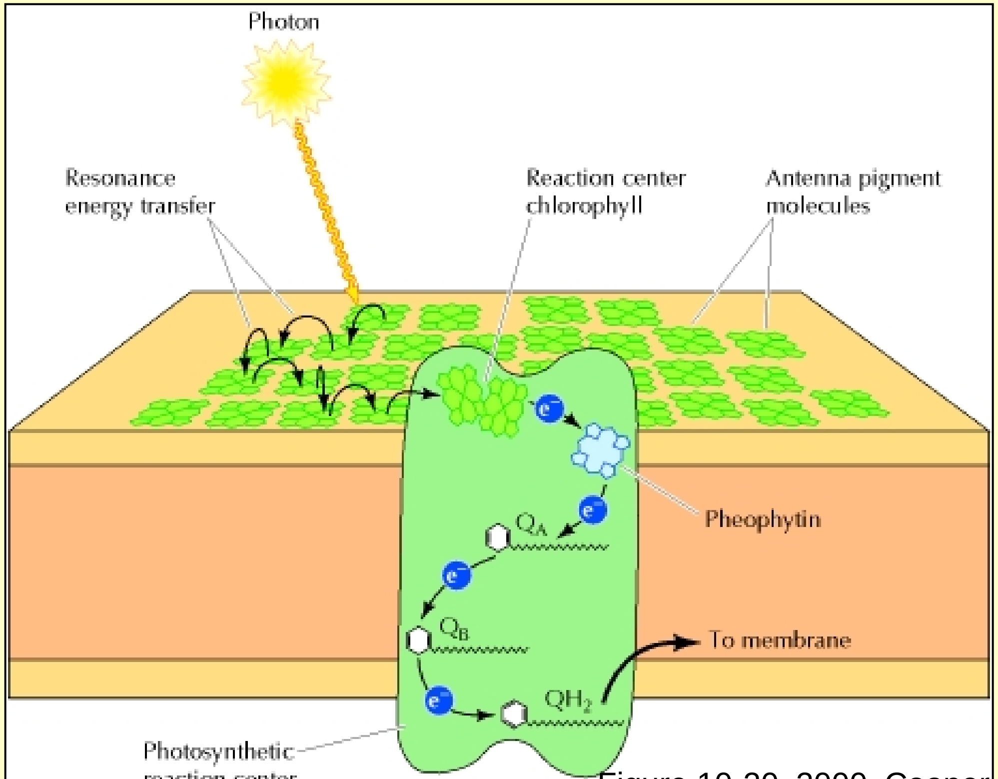
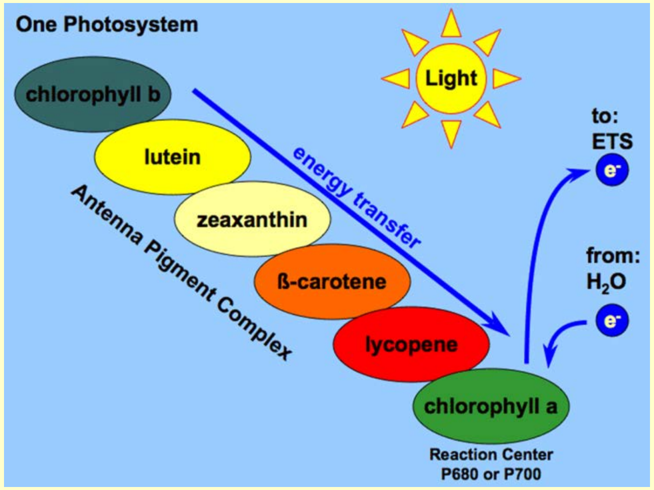


Figure 10-20. 2000. Cooper



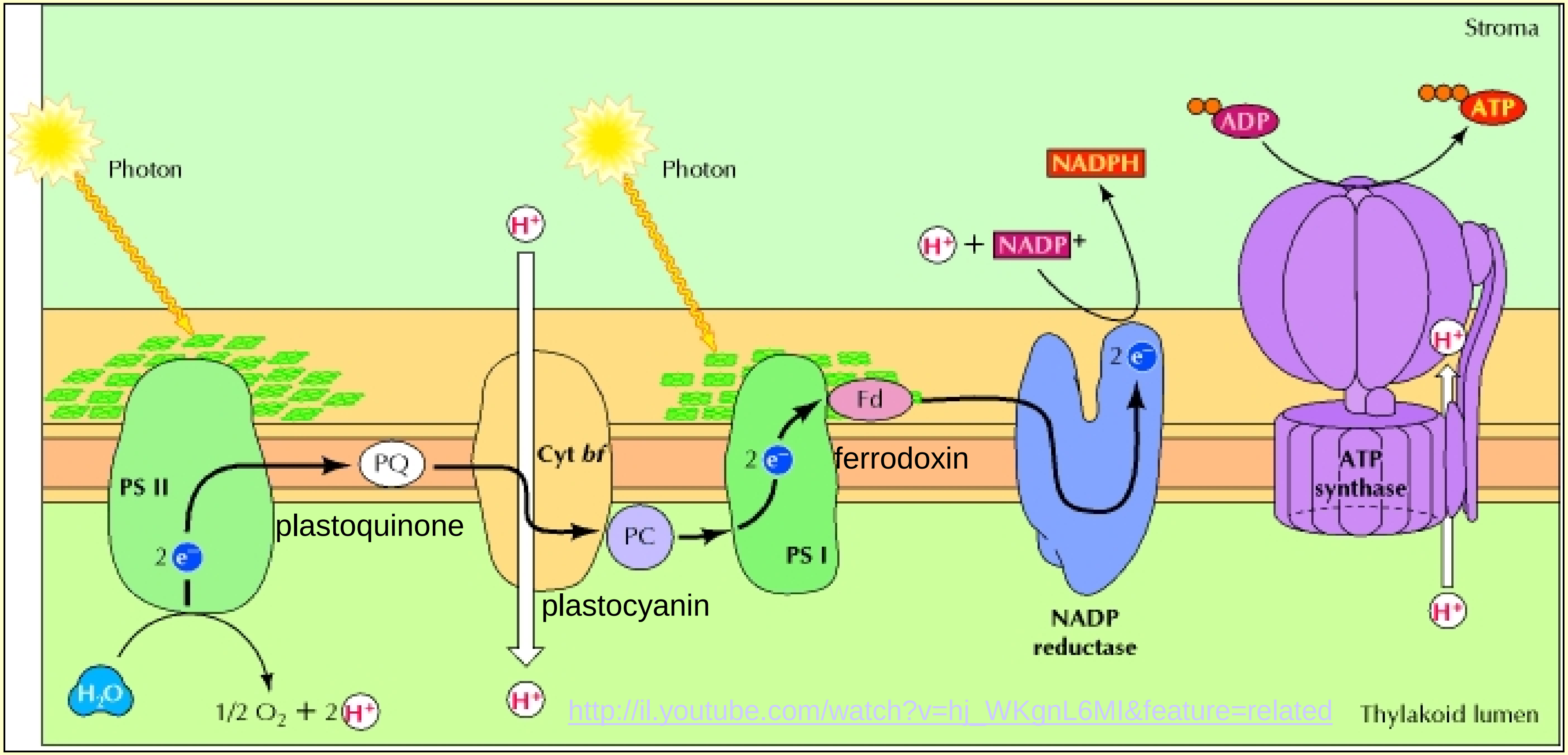
http://plantphys.info/plant_physiology/light.shtml

✓each photocenter → hundreds of antenna pigment molecules → absorb photons and transfer energy to a reaction center chlorophyll

✓reaction center transfers its excited e^- to an acceptor in the e^- transport chain

✓reaction center illustrated is that of **photosystem II**, in which electrons are transferred from the reaction center chlorophyll to pheophytin and then to quinones (Q_A, Q_B, and QH_2)

Electron transport and ATP synthesis during photosynthesis



photosystem II

energy derived from photon absorption is used to split H_2O within the thylakoid lumen

cytochrome *bf* complex

e^- transferred to a lower energy state - H^+ are pumped into the thylakoid lumen

photosystem I

energy derived from light absorption generates high energy e^-

NADP reductase

reduce $NADP^+$ to NADPH in the stroma

ATP synthase

uses the energy stored in the proton gradient to convert ADP to ATP

Calvin cycle

✓ ATP and NADPH produced from the light reactions drive the synthesis of carbohydrates from CO_2 and H_2O

✓ 1 CO_2 at a time is added to a cycle of **reactions** – Calvin cycle

✓ enzyme Rubisco (Ribulose Bisphosphate Carboxylase)
→ adds CO_2 to ribulose-1,5-bisphosphate

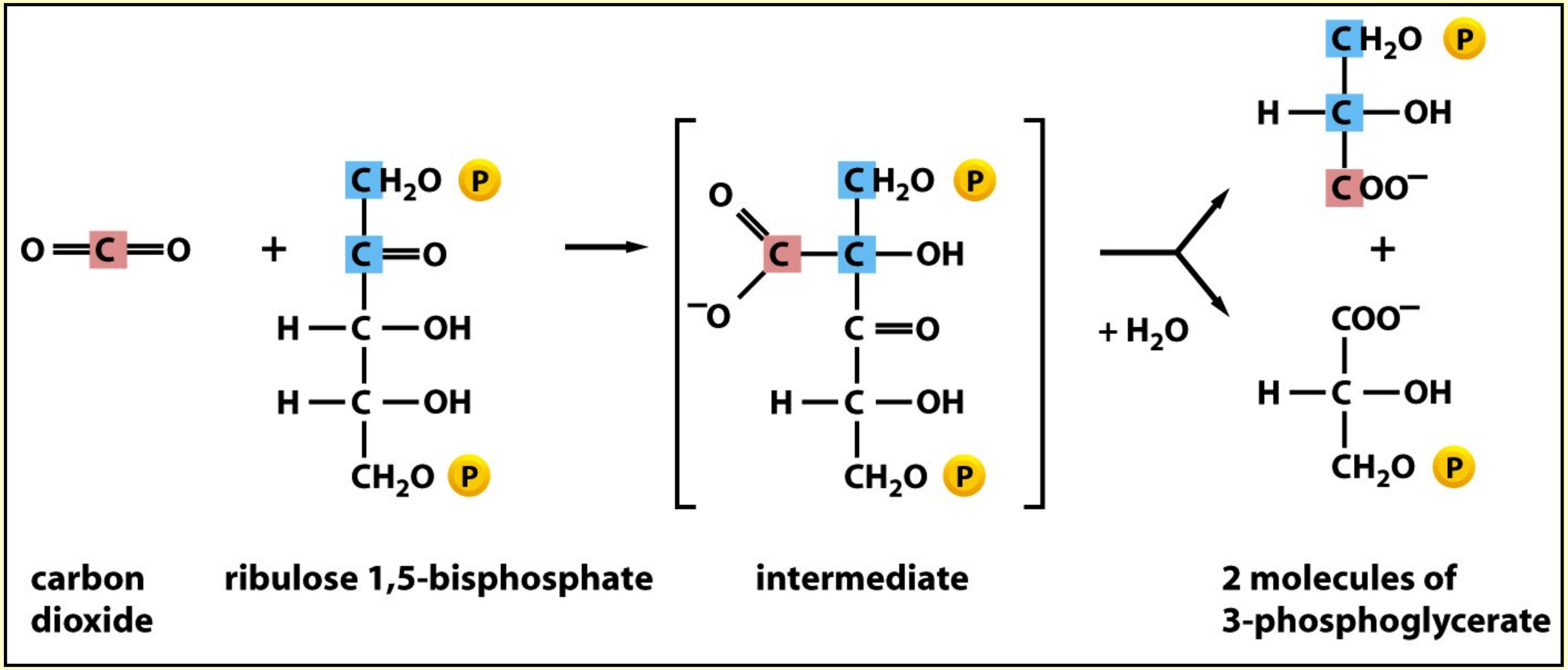
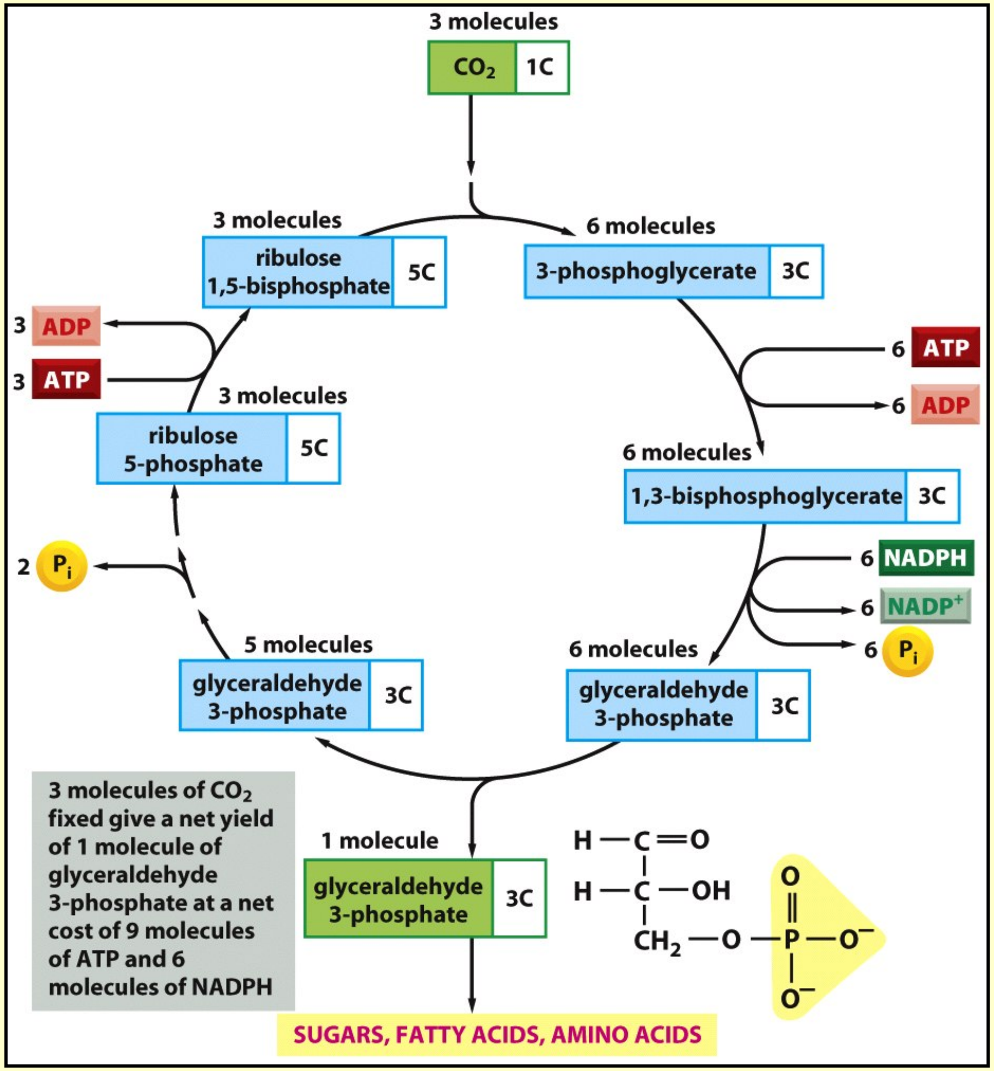


Figure 14-39 *Molecular Biology of the Cell* (© Garland Science 2008)

Calvin cycle



→ synthesis of 1 glyceraldehyde-3-phosphate from 3 CO_2

→ at the cost of 9 molecules of ATP and 6 molecules of NADPH

→ 2 glyceraldehyde-3-phosphate then used for synthesis of glucose

Figure 14-40 *Molecular Biology of the Cell* (© Garland Science 2008)

Chloroplast genome

✓ circular DNA (like mitochondria)

✓ more copies per organelle (like mitochondria)

✓ more complex than mitochondrial (120 - 150 kb; cca 150 genes)

✓ encodes:

- **RNA**: 4 rRNAs and 30 tRNAs (universal genetic code)
- **20 ribosomal proteins**
- **RNA polymerase subunits**
- **30 photosynthesis proteins**
- **30 proteins which need to be identified**

Table 10.2 Genes Encoded by Chloroplast DNA

Function	Number of genes
Genes for the genetic apparatus	
rRNAs (23S, 16S, 5S, 4.5S)	4
tRNAs	30
Ribosomal proteins	21
RNA polymerase subunits	4
Genes for photosynthesis	
Photosystem I	5
Photosystem II	12
Cytochrome *bf* complex	4
ATP synthase	6
Ribulose bisphosphate carboxylase	1

2000. Cooper

Import of chloroplast proteins

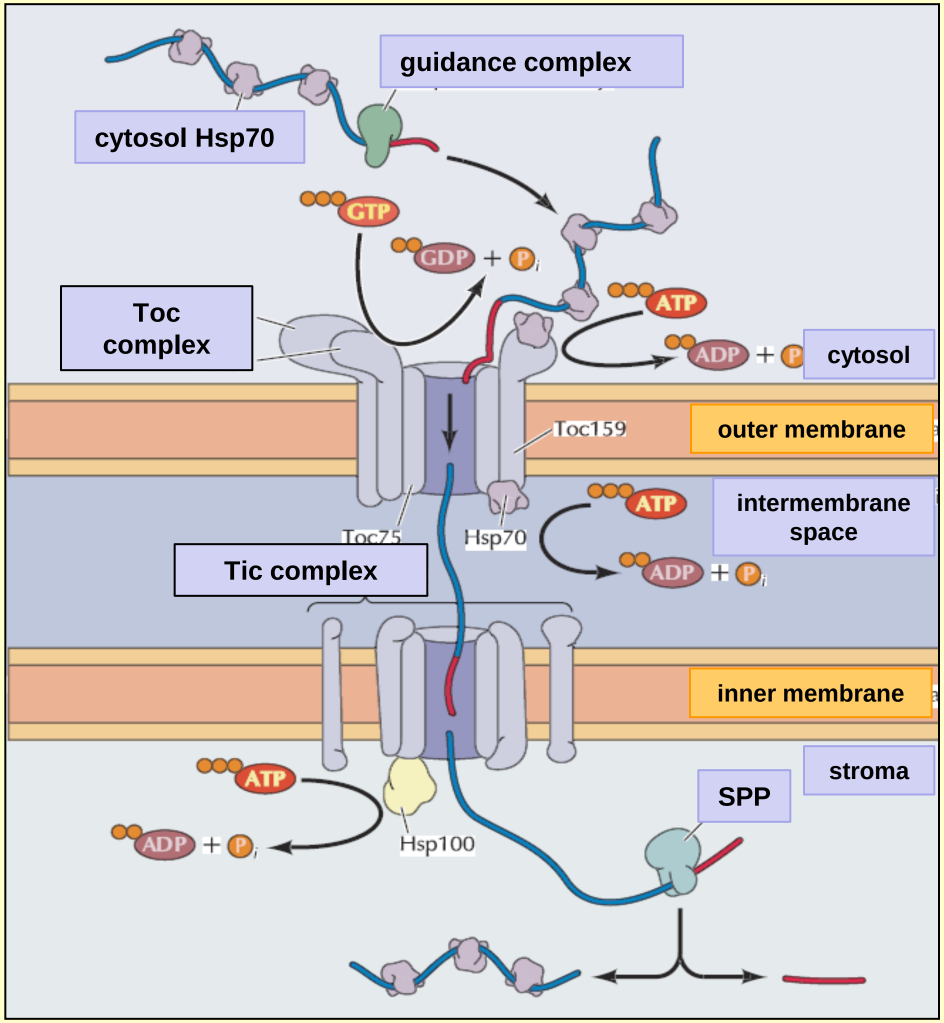


Figure 10-15. 2004. Cooper and Hausman

- ✓ ATP- and GTP-driven
- ✓ N-terminal presequence (30 – 100 ak)
- ✓ **cytosol Hsp70 -** keeps the protein in unfolded state
- ✓**guidance complex**→ recognizes presequence
- ✓ **Toc complex** → receptor in the outer membrane
- ✓ **Tic complex** → receptor in the inner membrane
- ✓ presequence cleavage by **SPP** (*stromal processing peptidase*)
- ✓ **stromal Hsp70** facilitates protein folding

Proteins with thylakoid signal sequence are imported into the thylakoid lumen or membrane

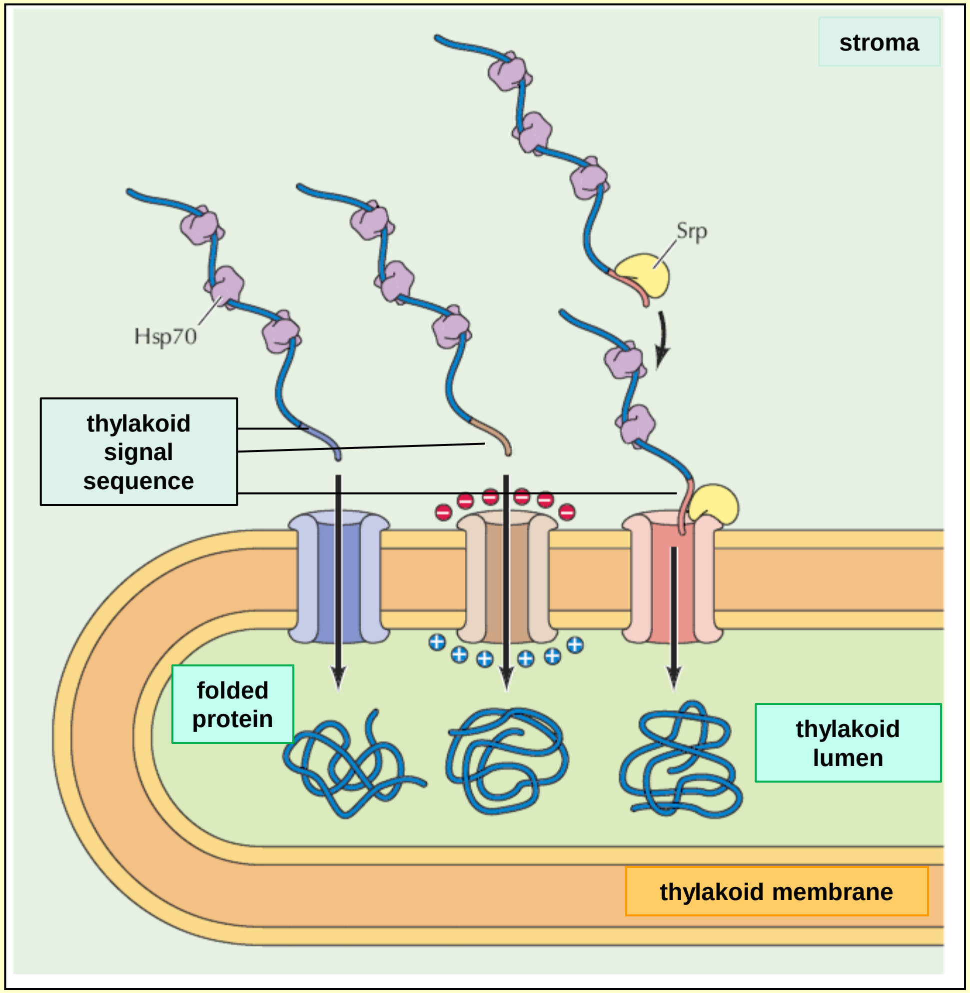
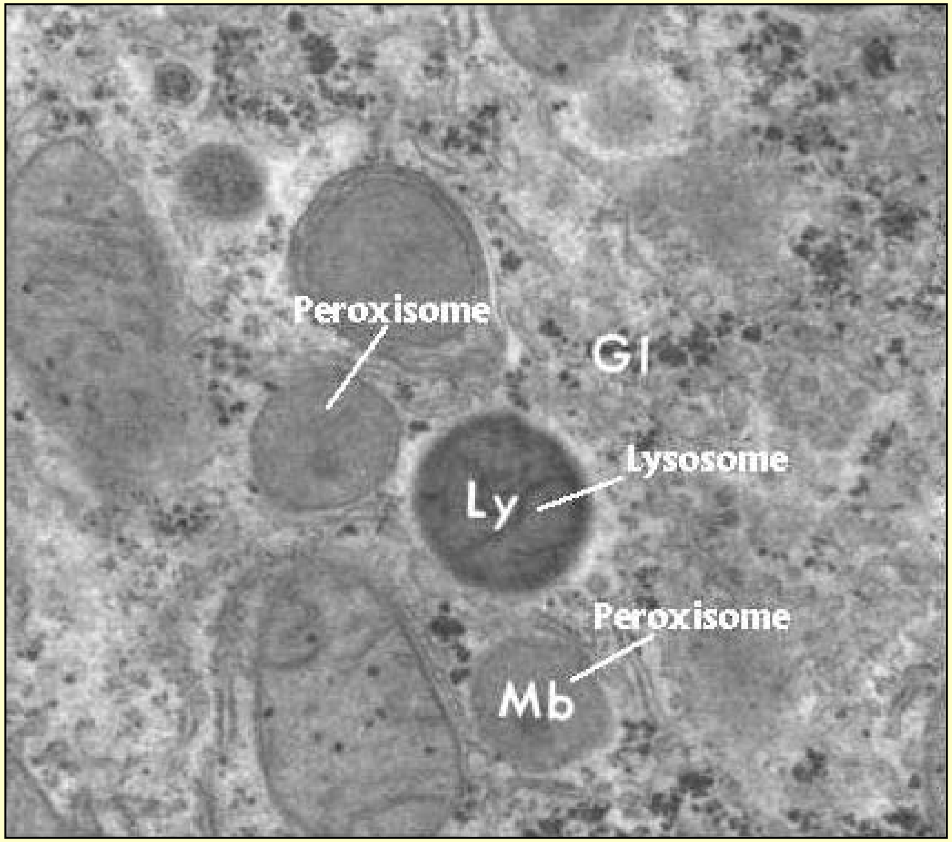


Figure 10-16. 2004. Cooper and Hausman

Peroxisomes

- ✓ small, membrane-enclosed organelles
- ✓ contain enzymes involved in a variety of metabolic reactions
- ✓ morphologically similar to lysosomes
- ✓ do not contain their own genomes
- ✓ mostly assembled from proteins that are synthesized on free ribosomes and then imported into peroxisomes as completed polypeptide chains
- ✓ similar to mitochondria and chloroplasts in that they replicate by binary division



Functions of peroxisomes

✓ contain at least 50 different enzymes → involved in a variety of biochemical pathways in different cell types

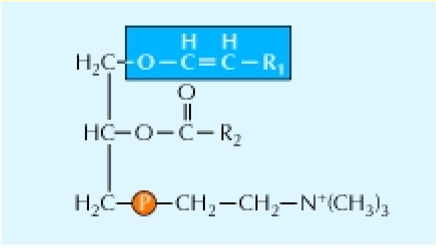
✓ contain the enzyme catalase → decomposes H_2O_2 either by converting it to water or by using it to oxidize another organic compound

✓oxidation of fatty acids is a particularly important since it provides a major source of metabolic energy

- animal cells → in peroxisomes and mitochondria
- yeast and plants → only in peroxisomes

✓ biosynthesis of lipids → in animal cells, cholesterol and dolichol are synthesized in peroxisomes as well as in the ER

✓ contain enzymes required for the synthesis of plasmalogens - a family of phospholipids in heart and brain



Two particularly important roles in plants

1. In seeds (glyoxysomes)

- ✓ responsible for the conversion of stored fatty acids to carbohydrates
- ✓ critical for providing energy and raw materials for growth of the germinating plant
- ✓ this occurs via a series of reactions termed the glyoxylate cycle, which is a variant of the citric acid cycle

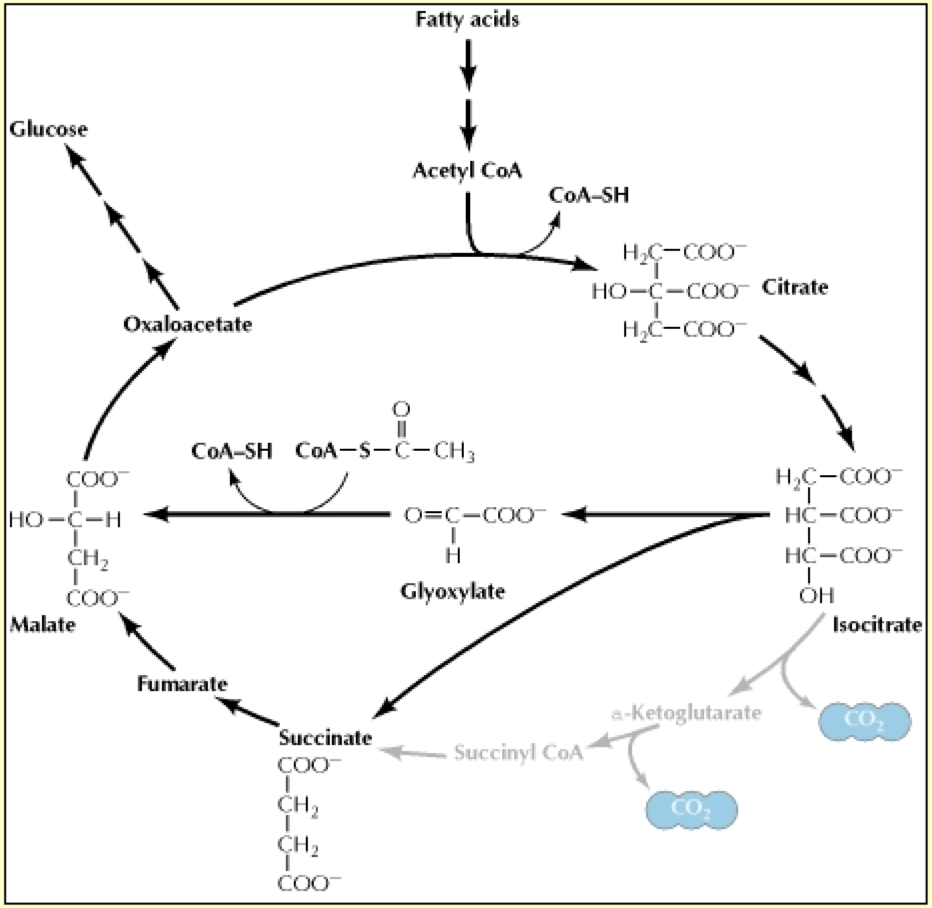


Figure 10-27. 2000. Cooper

2. In leaves

- ✓ **photorespiration → adaptation on hot and dry environment**
- ✓ serves to metabolize a side product formed during photosynthesis
- ✓ Rubisco adds CO_2 = **photosynthesis**
- ✓ Rubisco sometimes catalyzes **the addition of O_2** instead of CO_2

→ result - one 3-phosphoglycerate and one **phosphoglycolate**

→ a side reaction, and **phosphoglycolate** is <u>not a useful metabolite!</u>

Role of peroxisomes in photorespiration

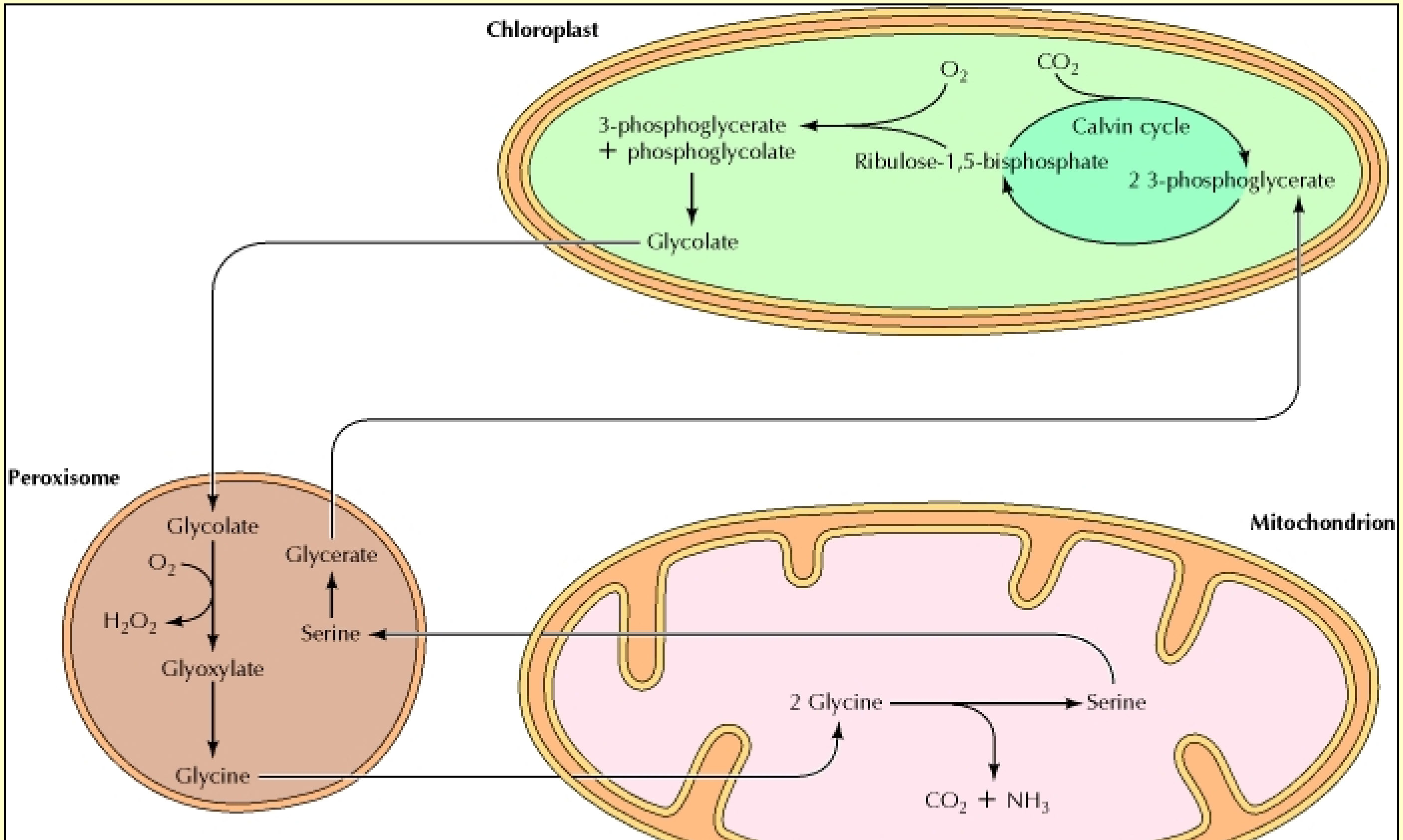


Figure 10-28. 2000. Cooper

- ✓ phosphoglycolate is converted to glycolate
- ✓ transfer to peroxisomes → oxidation and convertion to glycine
- ✓ glycine is then transferred to mitochondria and converted to serine.
- ✓ serine is returned to peroxisomes and converted to glycerate
- ✓ glycerate is transferred back to chloroplasts

❖ Peroxisome assembly

✓ peroxins – peroxisome proteins

✓ matrix peroxisome proteins are translated on free cytosolic ribosomes

✓membrane peroxisome proteins synthesized on rough ER

✓assembly starts on ER membrane:
- Pex3 (ER) i Pex19 (cytosol) interact
- initiates vesicle formation on ER

✓ other Pex proteins (ER) serve as receptors for import of matrix proteins (cytosol)

✓ import:
- signal sequence Ser-Lys-Leu on C-end (PTS1)
- 9 aa on N-end (PTS2)
- no cleavage of signal sequence!

✓ phospholipids are imported to peroxisomes, via phospholipid transfer proteins, from the ER

✓ import of proteins and phospholipids results in peroxisome growth, and new peroxisomes are then formed by division of old ones

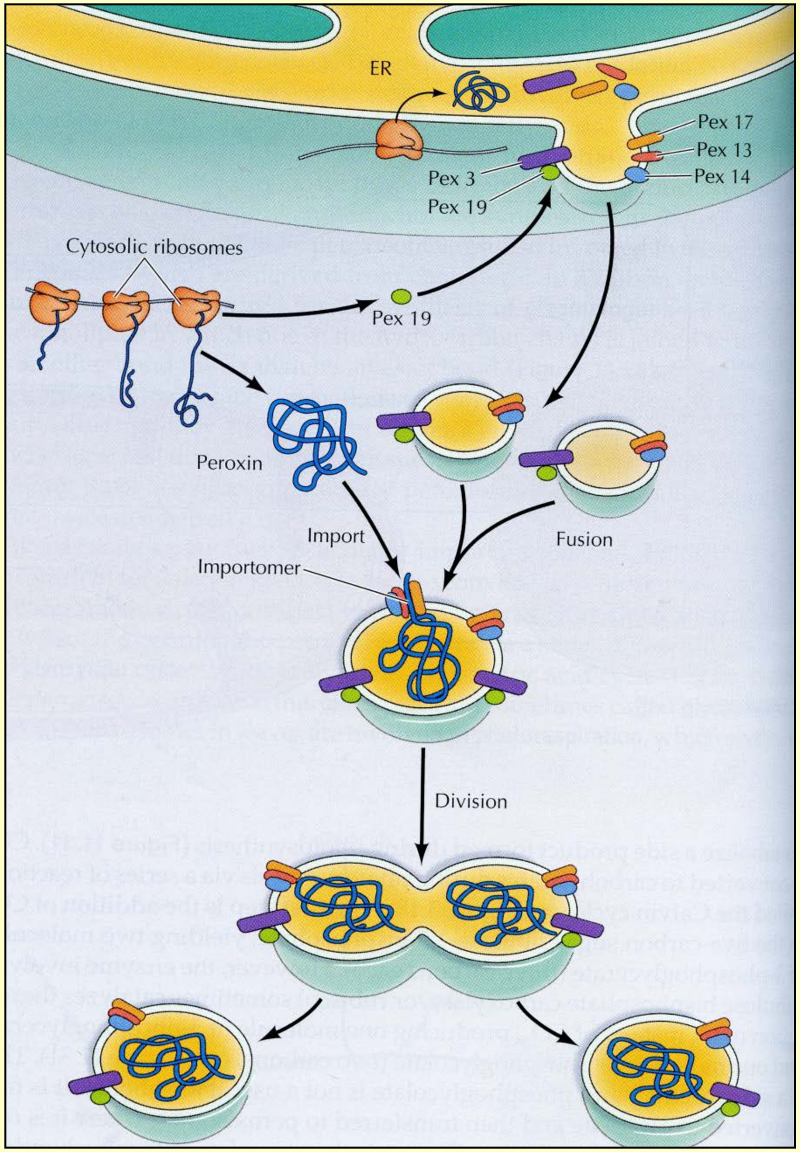


Figure 11-32. 2013. Cooper