

# Bioenergetics and metabolism

➤ Mitochondria

➤ Chloroplasts

➤ Peroxisomes

# 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

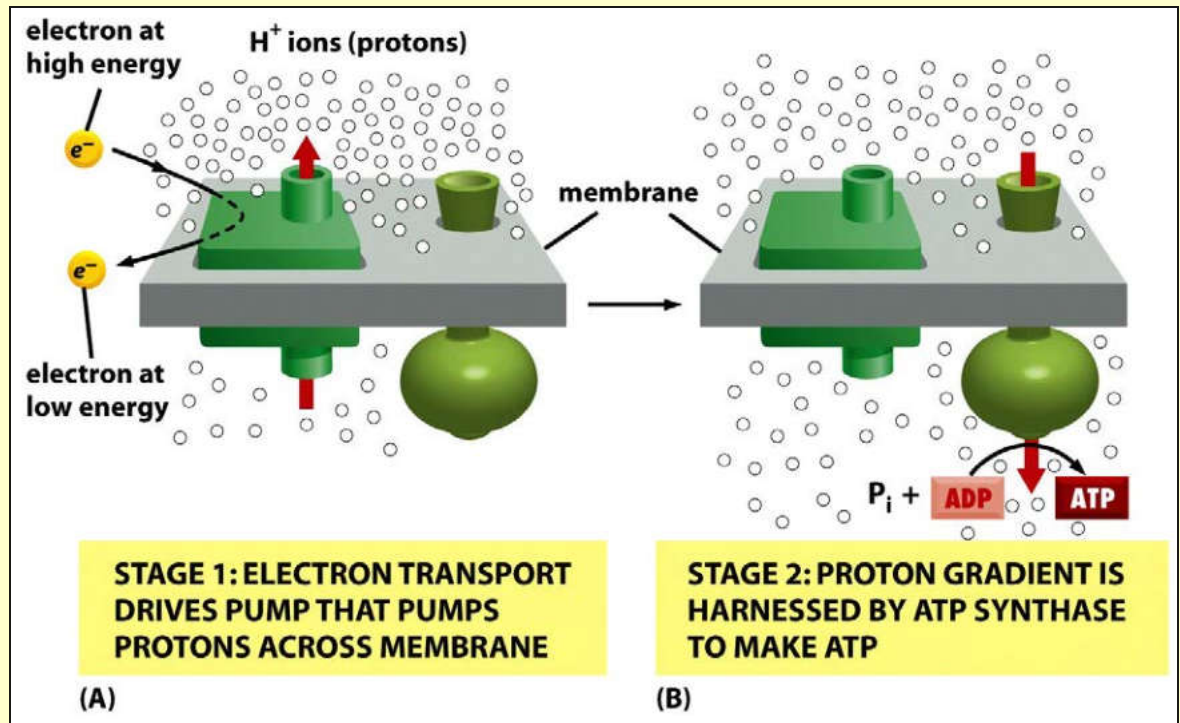
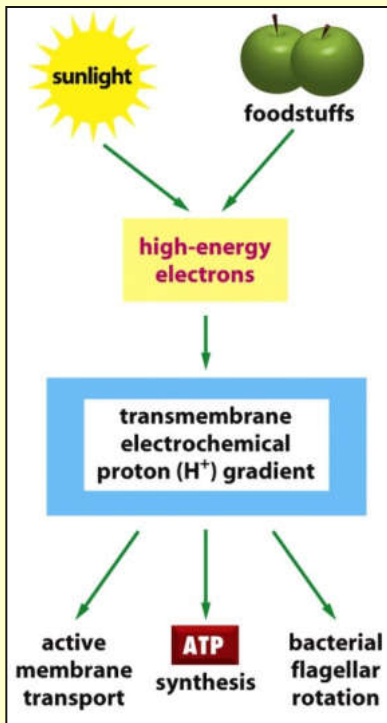
– membranes of cell compartments – *energy-converting organelles*  
→ production of ATP

- Mitochondria – fungi, animals, plants

- Plastids (chloroplasts) – plants

# The essential requirements for chemiosmosis

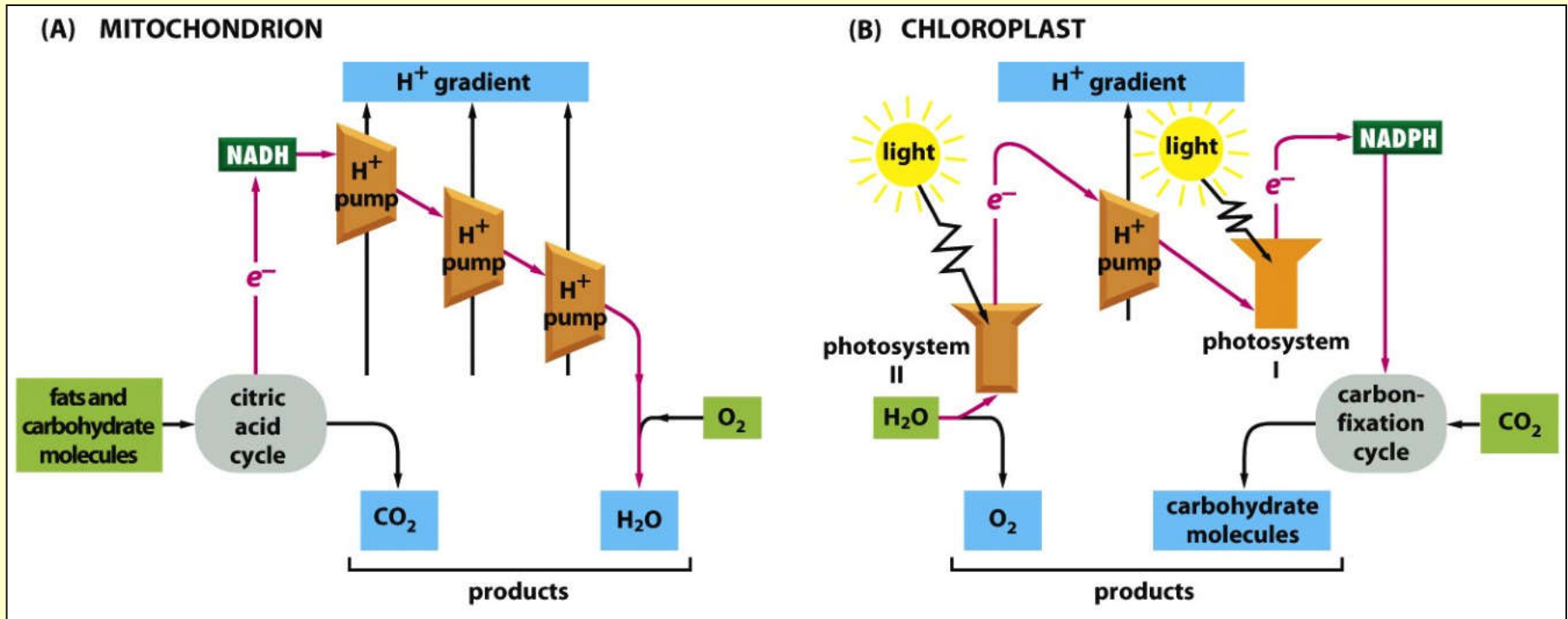
- ✓ source of high-energy  $e^-$
- ✓ membrane with embedded **proton pump** and **ATP synthase**



✓ 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  $H^+$  → proton gradient across the membrane
- ✓  $H^+$  gradient serves as an energy store that can be used to drive ATP synthesis

# Electron transport processes



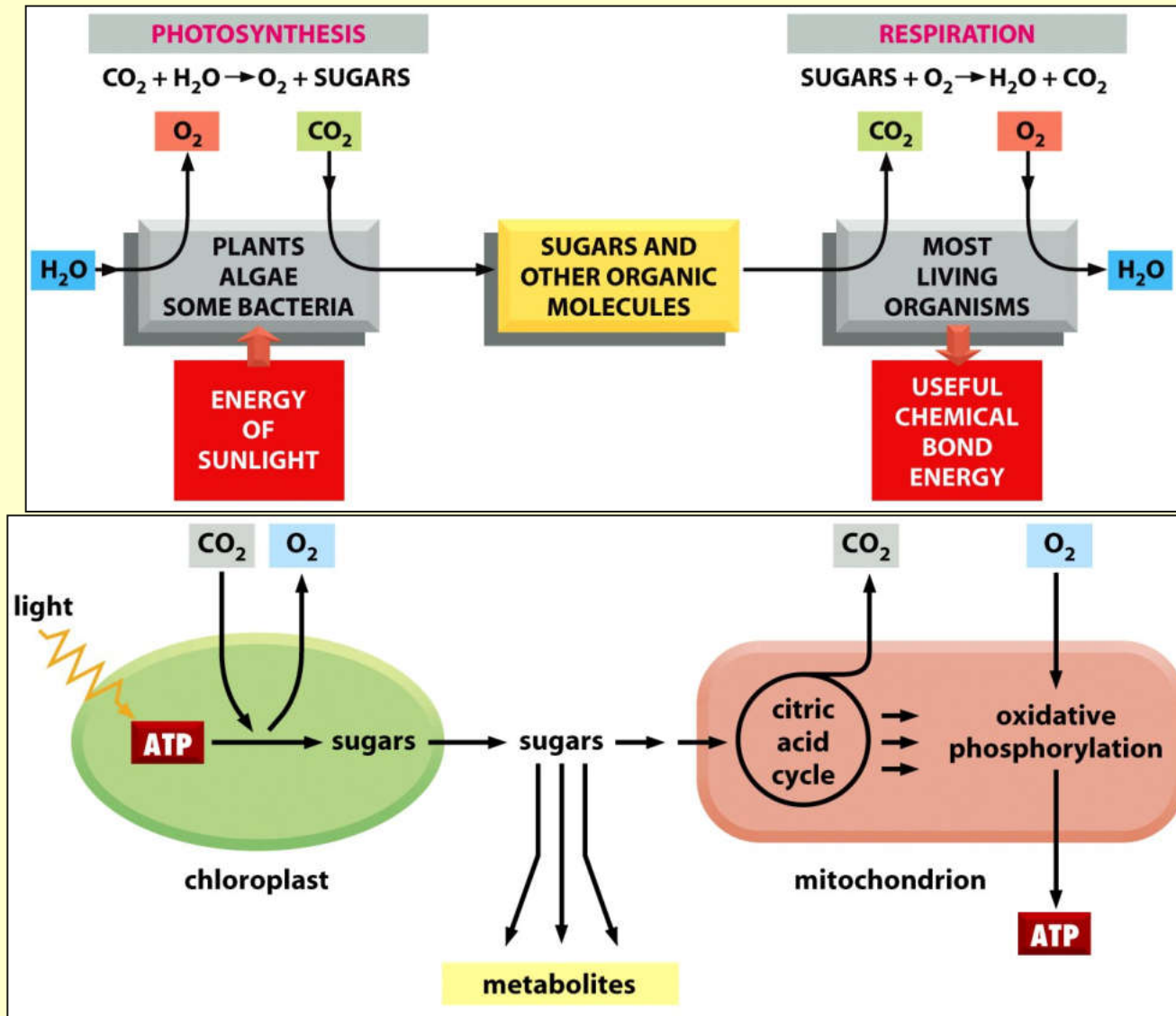
(A) mitochondrion converts energy from chemical fuels

(B) chloroplast converts energy from sunlight

→ electron-motive force generated by the 2 photosystems enables the chloroplast to drive electron transfer from H<sub>2</sub>O to carbohydrate

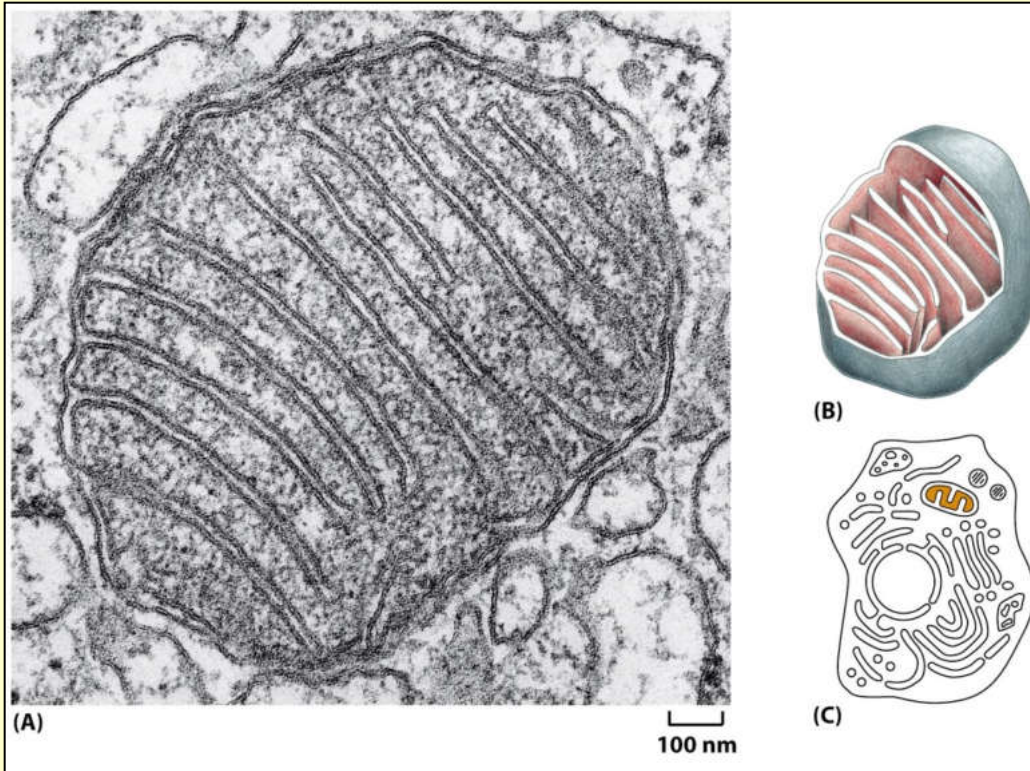
→ chloroplast electron transfer is *opposite* of electron transfer in a mitochondrion

# Carbohydrate molecules and O<sub>2</sub> are products of the chloroplast and inputs for the mitochondrion



# **Mitochondria**

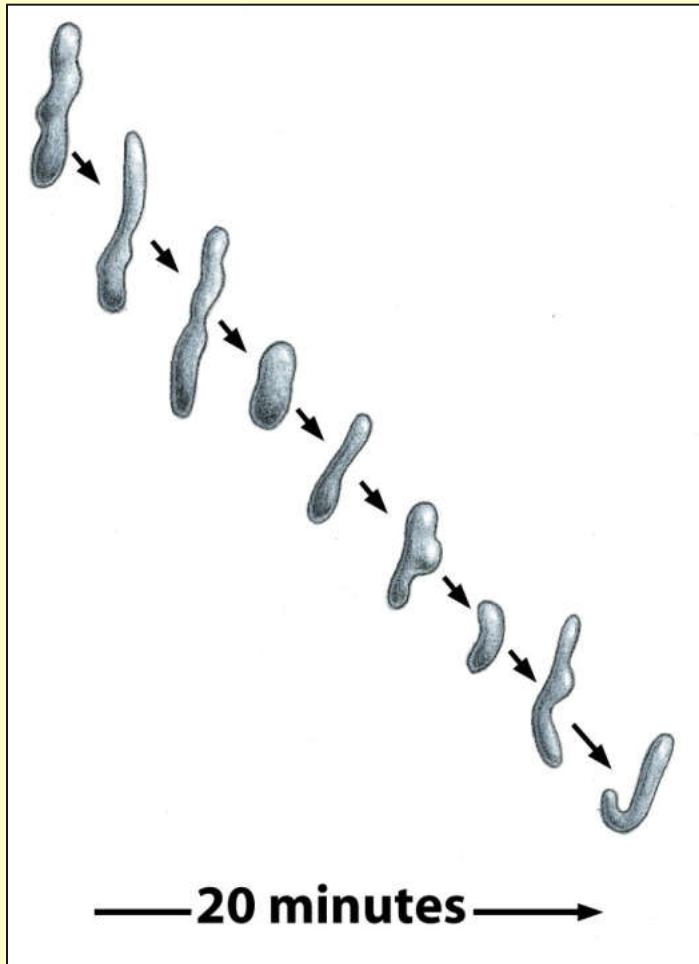
# Structure and ultrastructure



- ✓ all eukaryotic cells  
→ diameter 0,5 - 1  $\mu\text{m}$
- ✓ discovered in 19<sup>th</sup> century  
→ light microscopy
- ✓ 1948. isolated from liver cells
- ✓ key function in metabolic energy production

- ✓ double membrane
  - outer membrane
  - intermembrane space
  - inner membrane

# Mitochondria mobility



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

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

Animation

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



# Ultrastructure

## ✓ Outer membrane

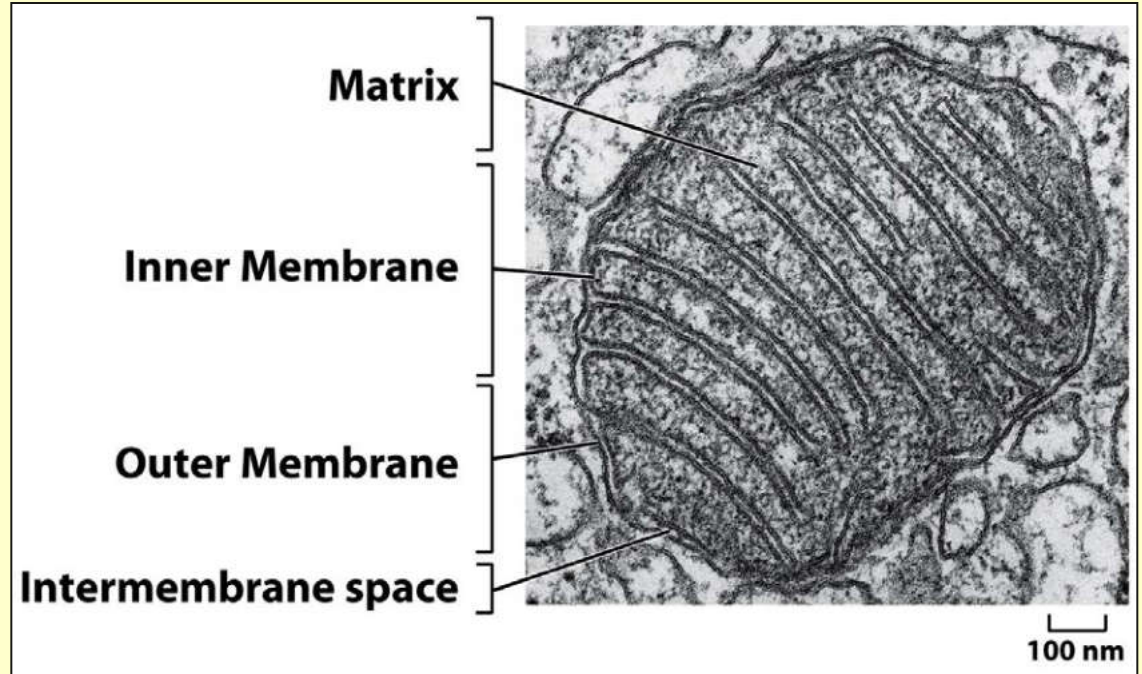
- porins (water channels)
- < 5000 Da free passage

## ✓ Inner membrane

- christae
- cardiolipin
- impermeability for ions

## ✓ Matrix

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



# Cardiolipin

→ impermeability of the inner membrane for ions

→ phospholipid with 4 fatty acid chains

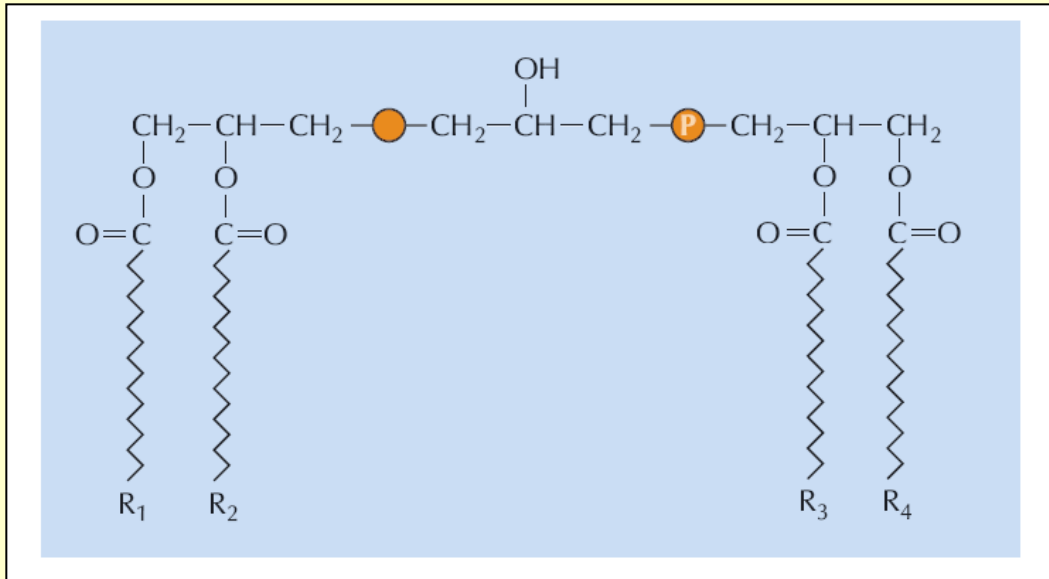


Figure 10-7. 2004. Cooper and Hausman

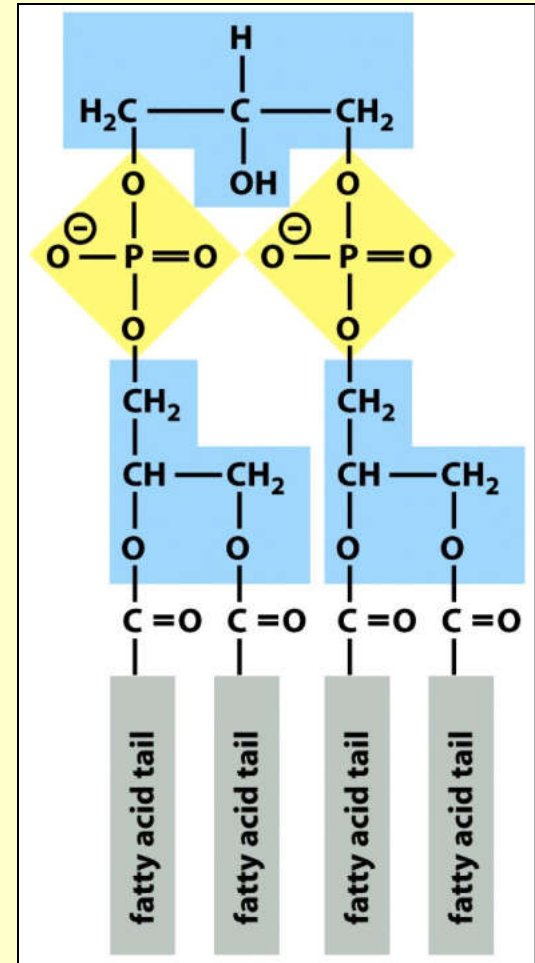


Figure 14-65 *Molecular Biology of the Cell* (© Garland Science 2008)

# Biochemical activity of mitochondria

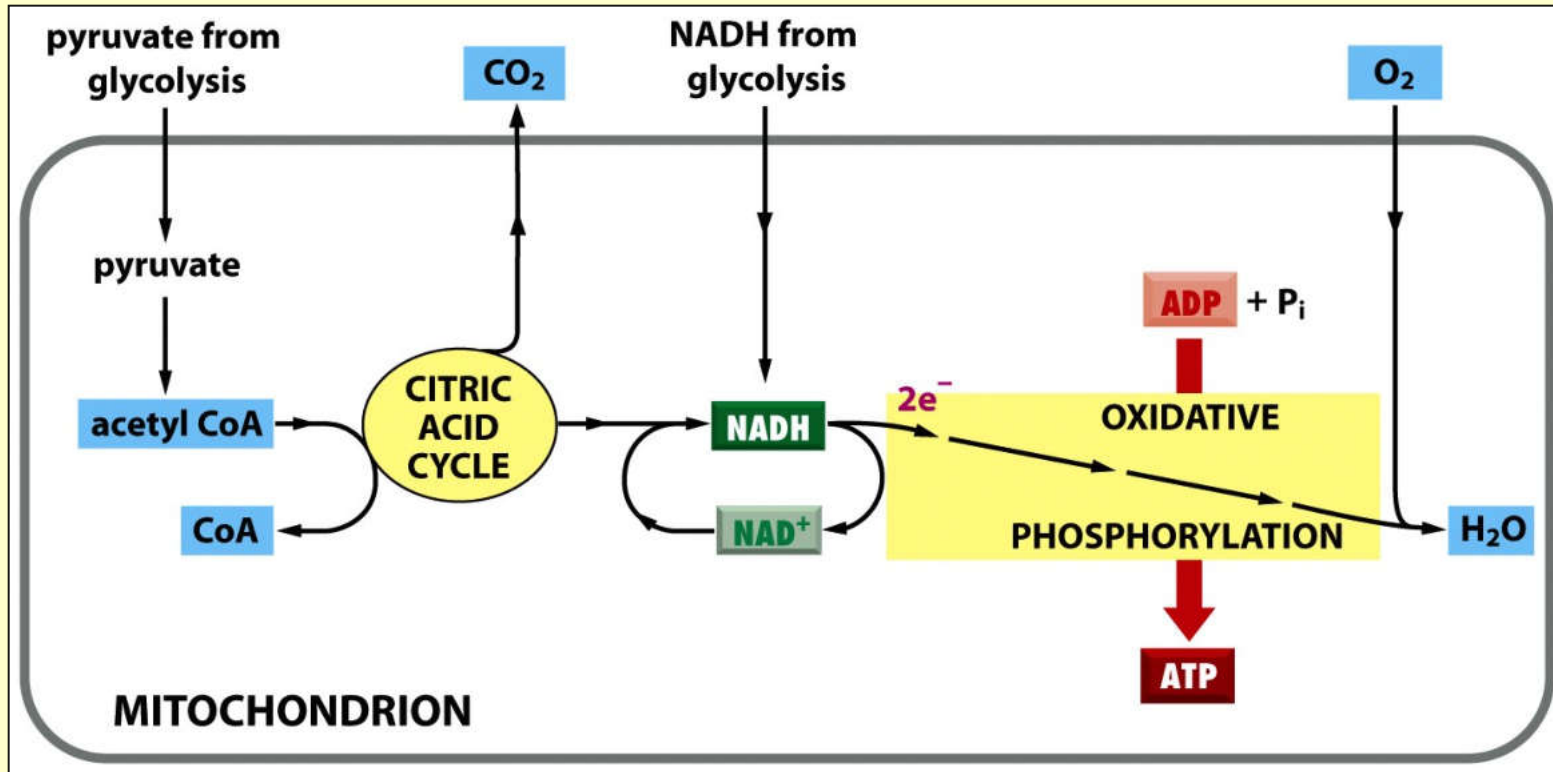


Figure 2-86 *Molecular Biology of the Cell* (© Garland Science 2008)

✓ 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

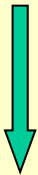
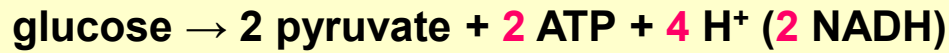
→ 2 ATP molecules

✓ Pyruvate is transported to mitochondria

→ complete oxidation to CO<sub>2</sub>

→ gain in energy is **15 x** higher than in glycolysis

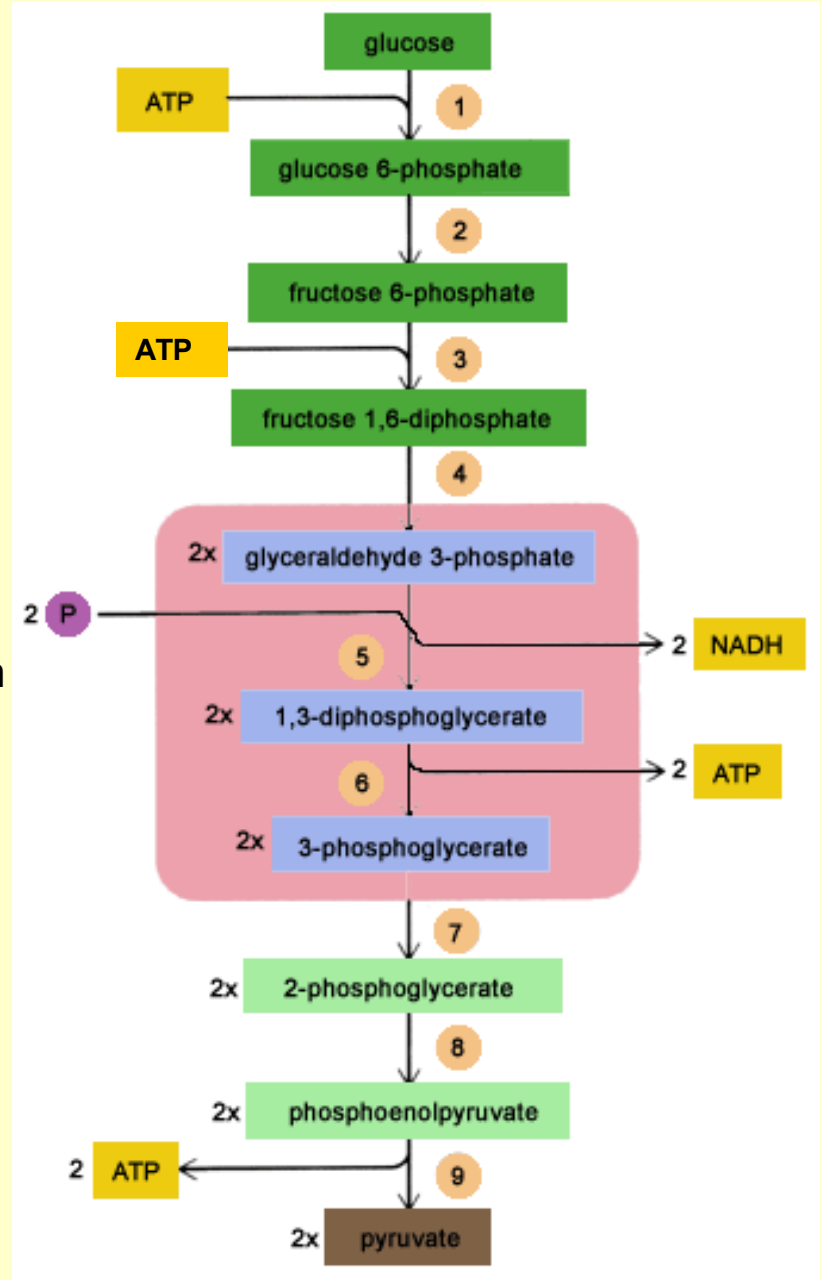
# ❖ Glycolysis - cytosol



**mitochondria  
matrix**  
citric acid cycle



**mitochondria  
inner membrane**  
oxidative phosphorylation



# Reactions that take place in mitochondrion

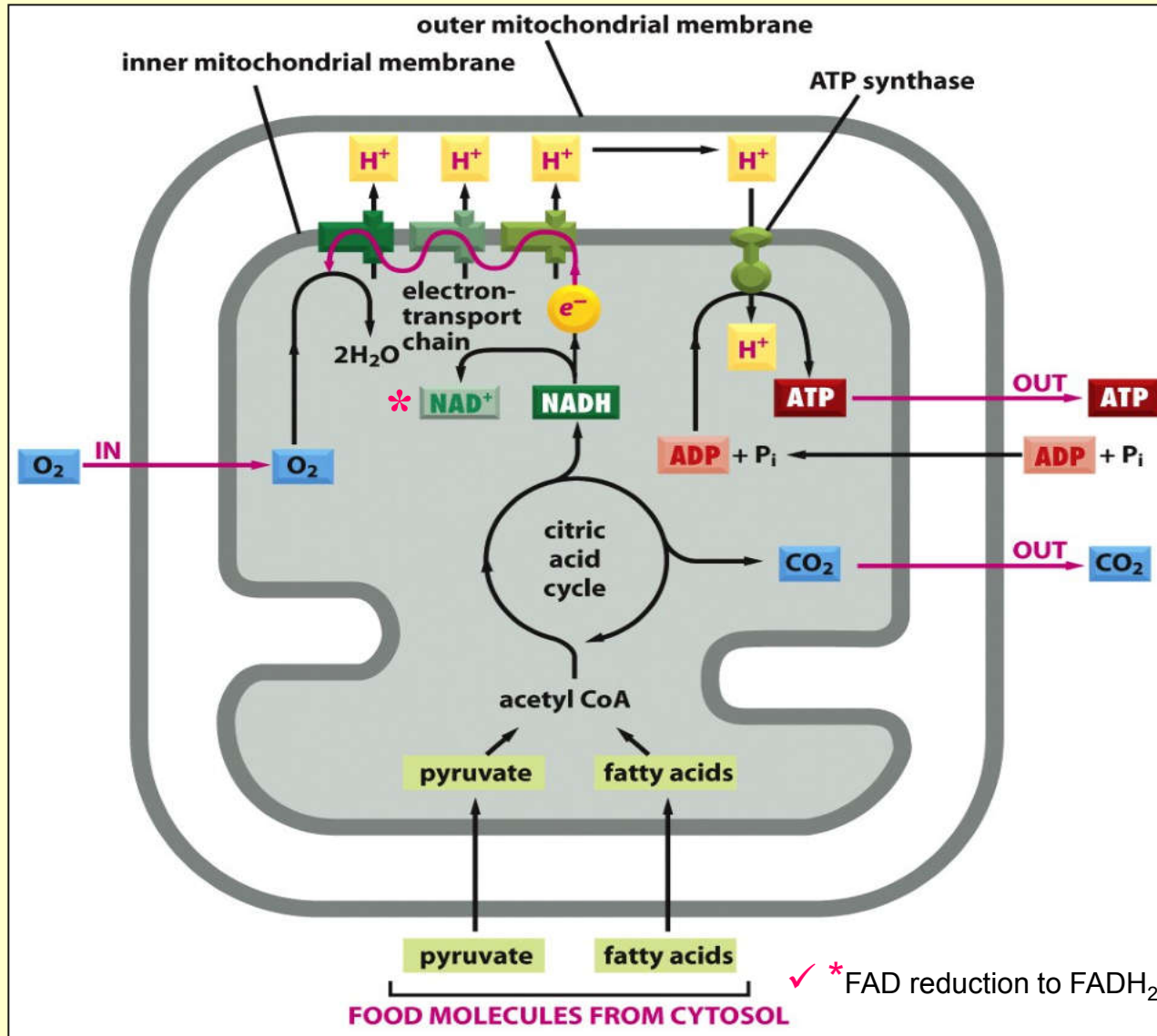


Figure 14-10 *Molecular Biology of the Cell* (© Garland Science 2008)

## ❖ NADH - nicotinamide adenine dinucleotide

✓ coenzyme found in all living cells

✓ dinucleotide

→ consists of two nucleotides joined through their phosphate groups

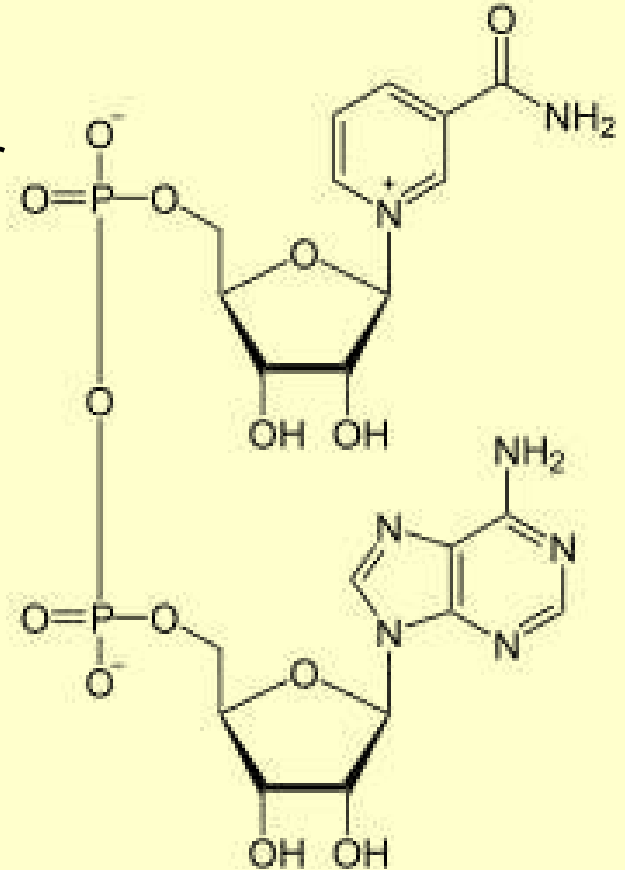
→ one nucleotide contains an adenine base and the other nicotinamide

✓ involved in redox reactions

✓ found in two forms in cells:

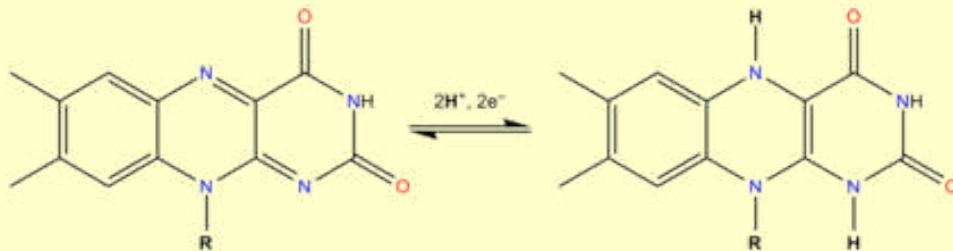
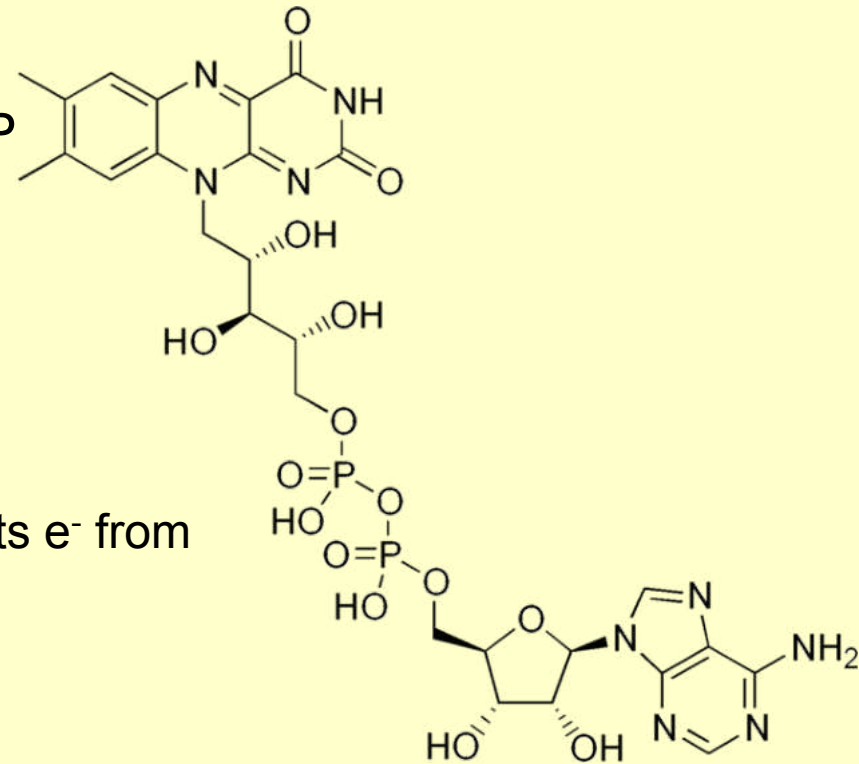
- **NAD<sup>+</sup>** - is an oxidizing agent → it accepts e<sup>-</sup> from other molecules and becomes reduced
- **NADH** - reducing agent → donates e<sup>-</sup>

✓ the main function of NAD<sup>+</sup> - e<sup>-</sup> transfer reactions



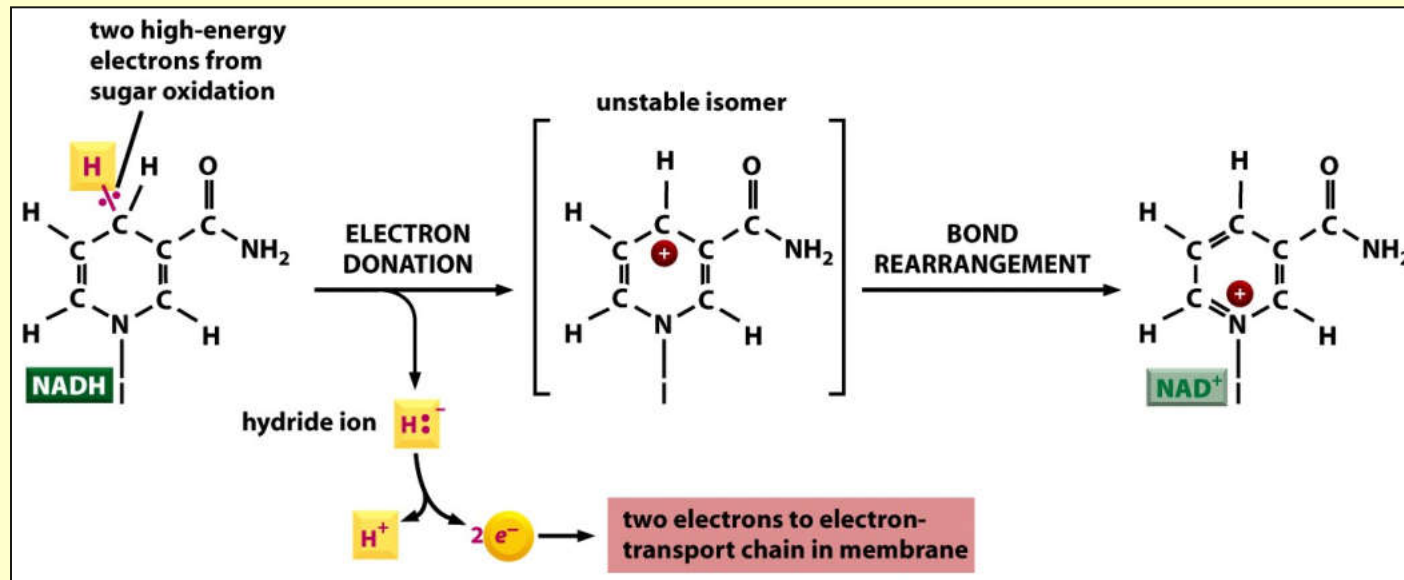
# ❖ FADH<sub>2</sub> - flavin adenine dinucleotide

- ✓ 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<sup>-</sup> from other molecules and becomes reduced
  - **FADH<sub>2</sub>** - reducing agent → donates e<sup>-</sup>





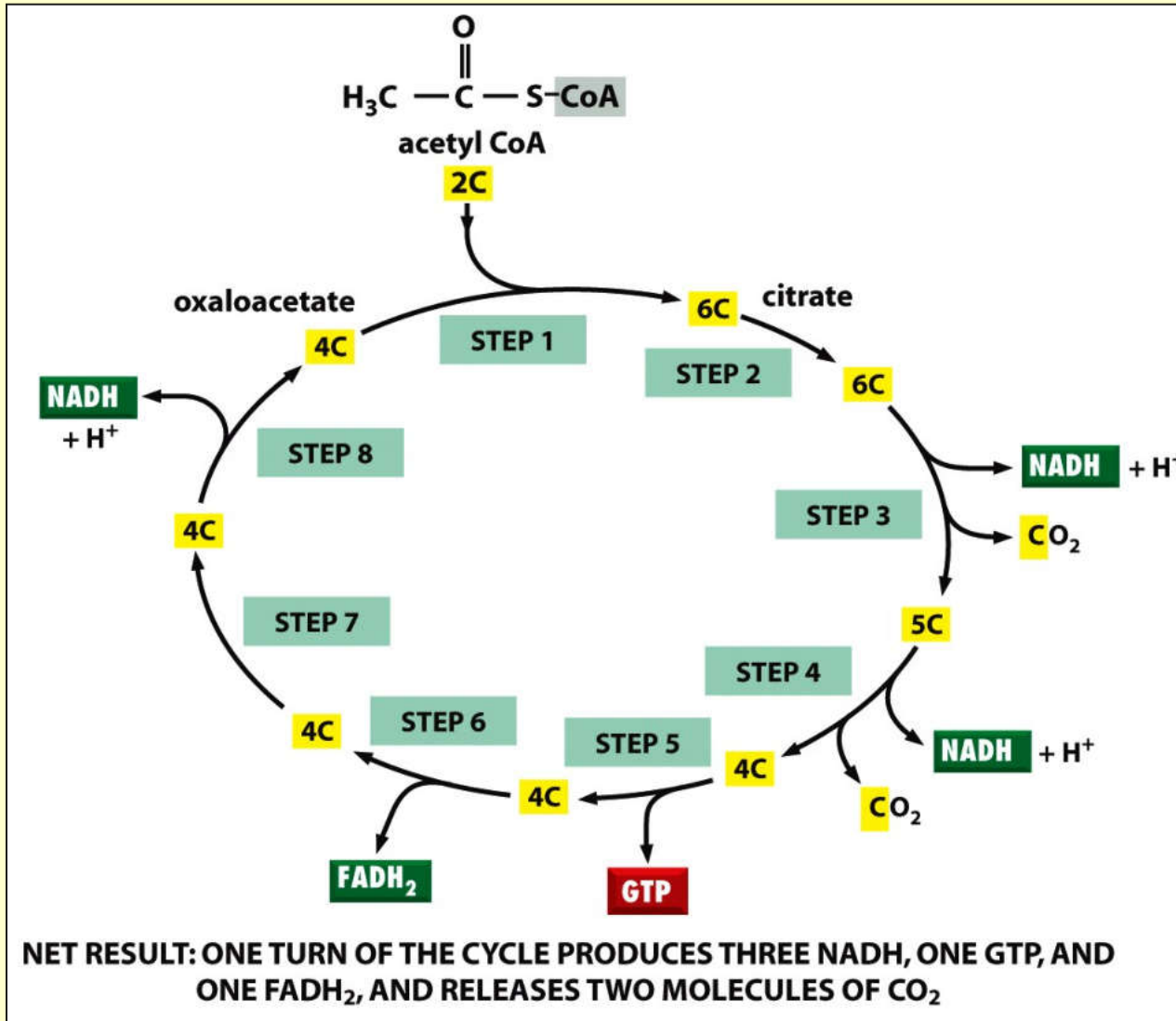
- ✓ mitochondria use both pyruvate and fatty acids → acetyl CoA
- ✓ acetyl groups of acetyl CoA are being oxidized in **citric acid cycle**
  - C-atoms from acetyl CoA converted to CO<sub>2</sub>
  - cycle forms high-energy e<sup>-</sup> transferred by NADH and FADH<sub>2</sub>



- ✓ hydride ion is removed from NADH and is converted into H<sup>+</sup> and 2 high-energy e<sup>-</sup>



# Citric acid cycle (Kreb's cycle)



✓ Matrix

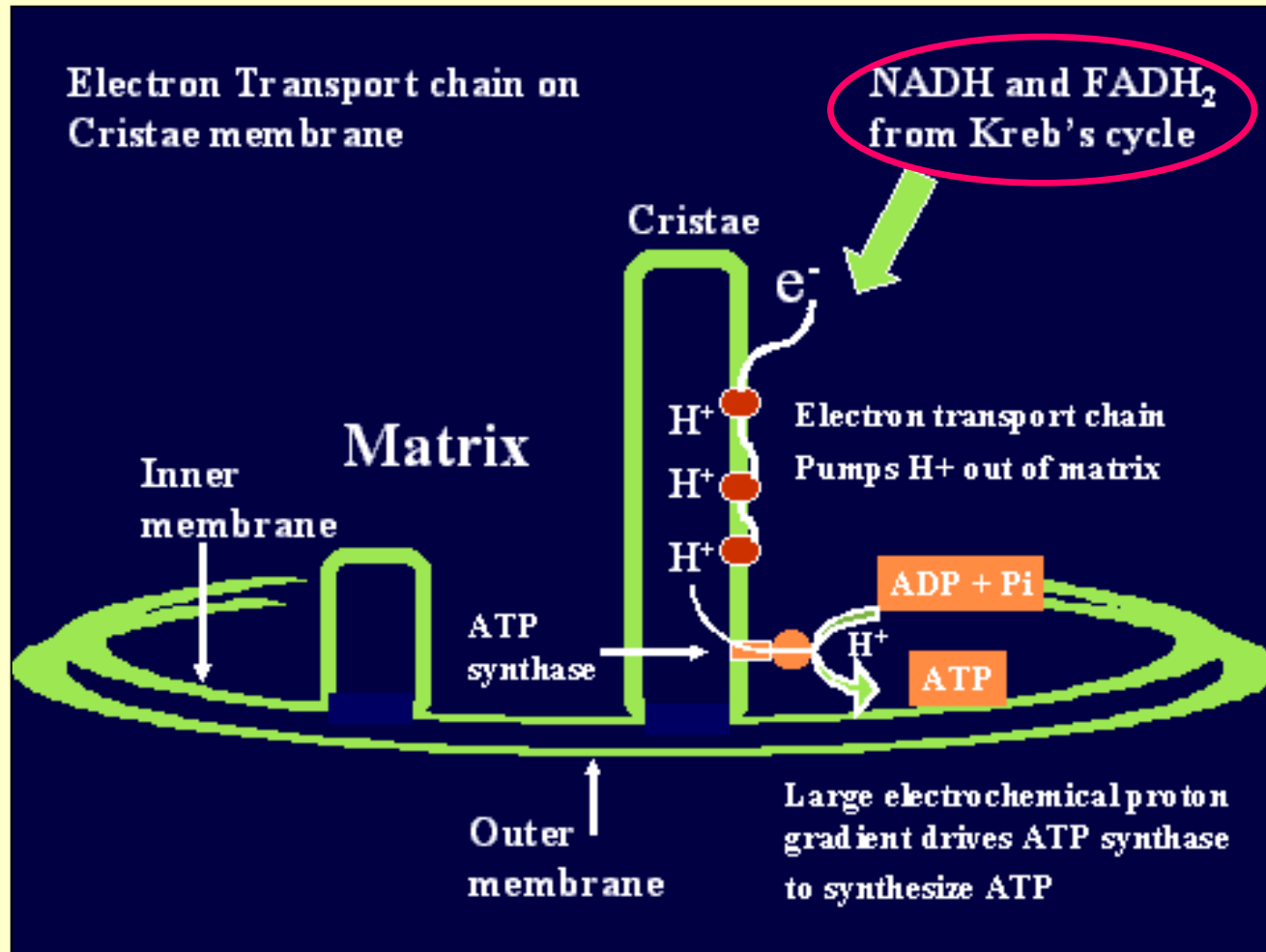
✓ Yield:

• 3 NADH molecules

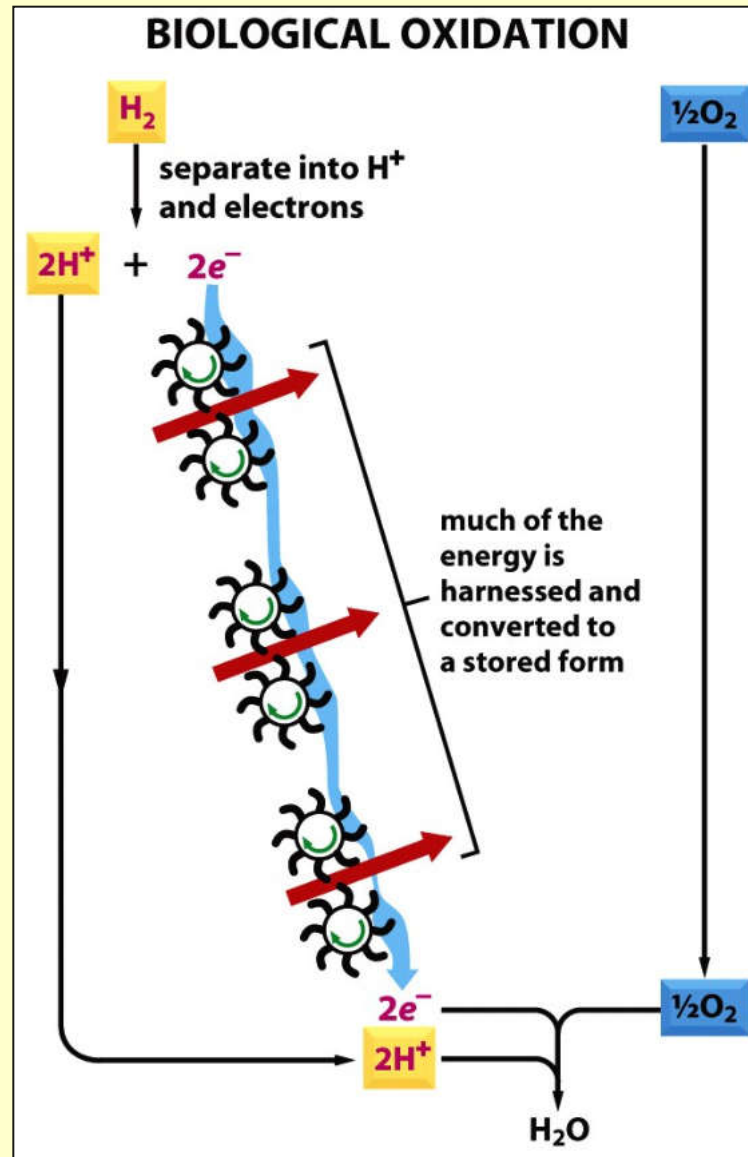
• 1  $\text{FADH}_2$  molecule

• 1 GTP

# Mitochondrion inner membrane



# e<sup>-</sup> transport in respiratory chain



# Oxidative phosphorylation

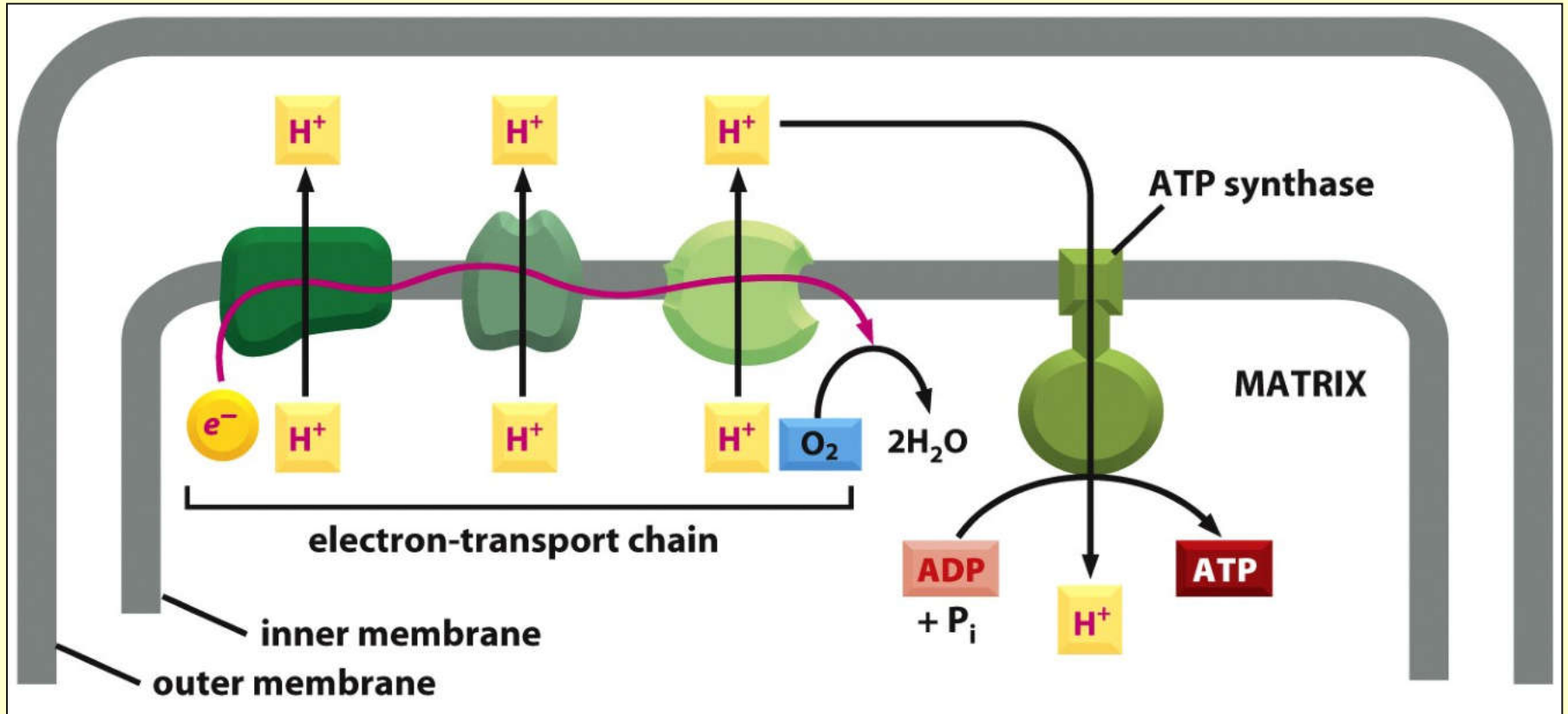
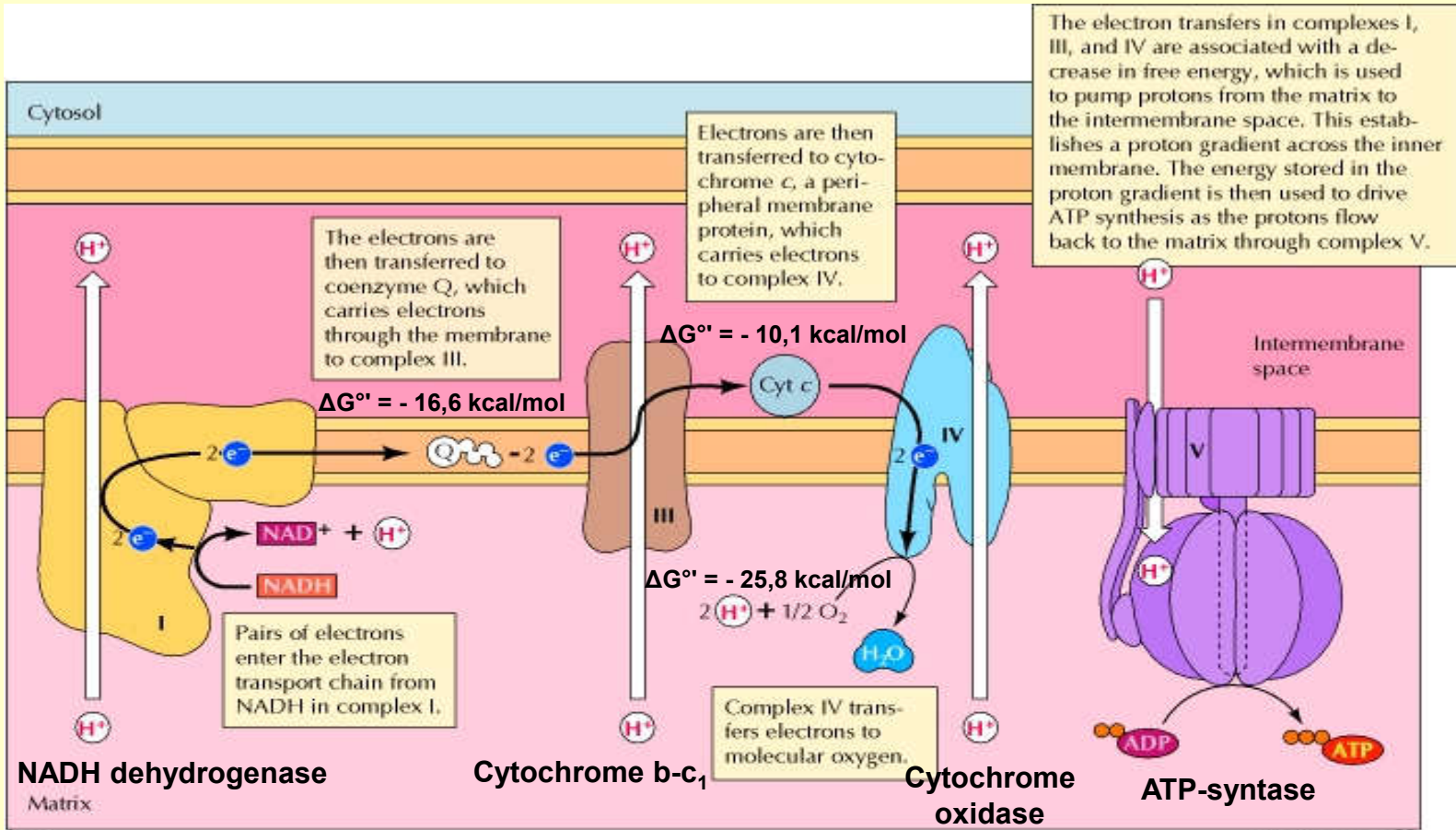


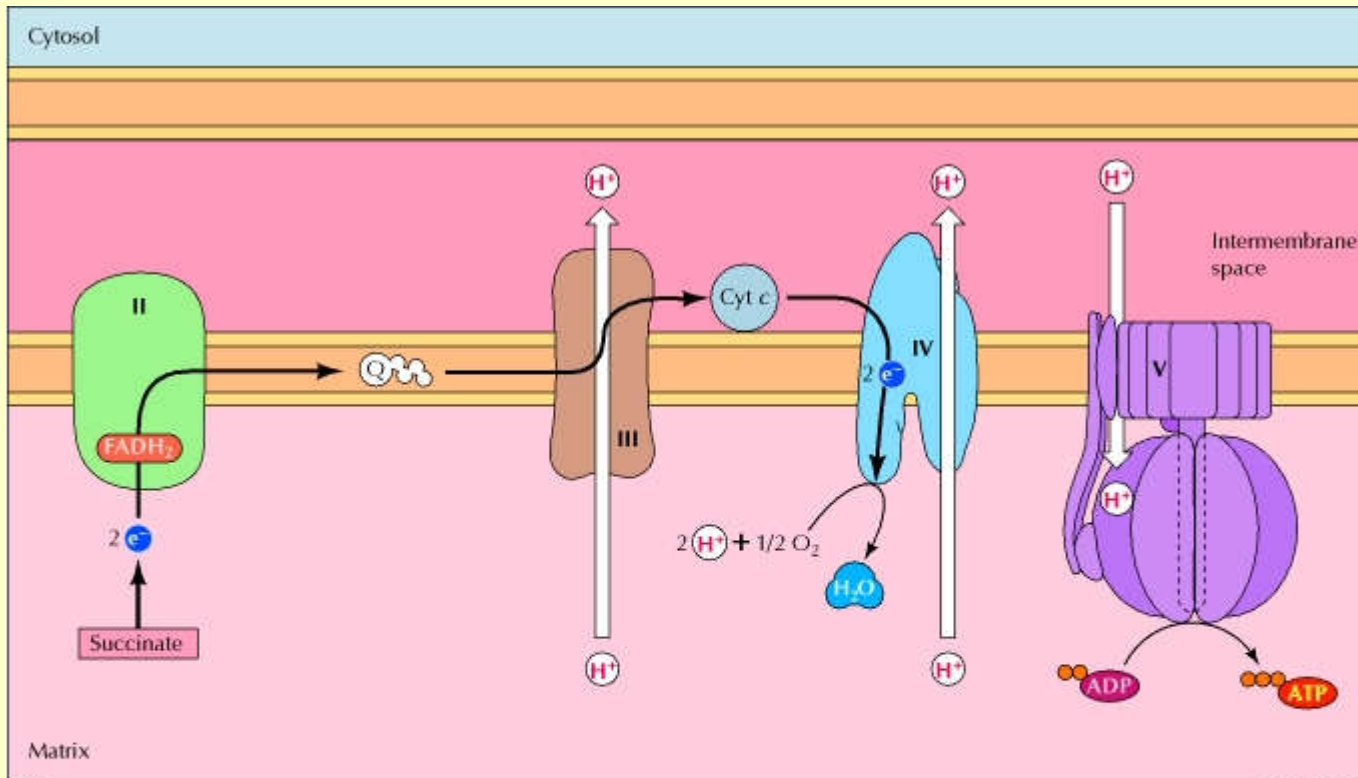
Figure 14-14 *Molecular Biology of the Cell* (© Garland Science 2008)

# Transport of electrons from NADH



complexes I, III and IV – proton pumps

# Transport of electrons from FADH<sub>2</sub>



- e<sup>-</sup> from succinate enter the electron transport chain via FADH<sub>2</sub> in complex II
- they are then transferred to coenzyme Q and carried through the rest of the e<sup>-</sup> transport chain
- e<sup>-</sup> transfer from FADH<sub>2</sub> 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,  $F_0$  and  $F_1$ , which are linked by a slender stalk
- ✓  $F_0$  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  $F_0$  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<sub>2</sub>**, which enters the electron transport chain at complex II, yields only 2 **ATP**

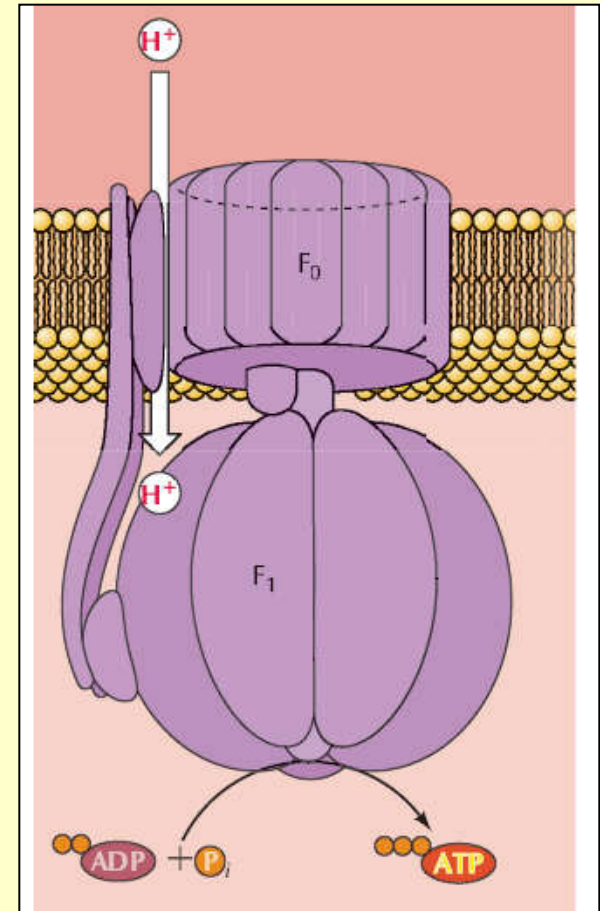
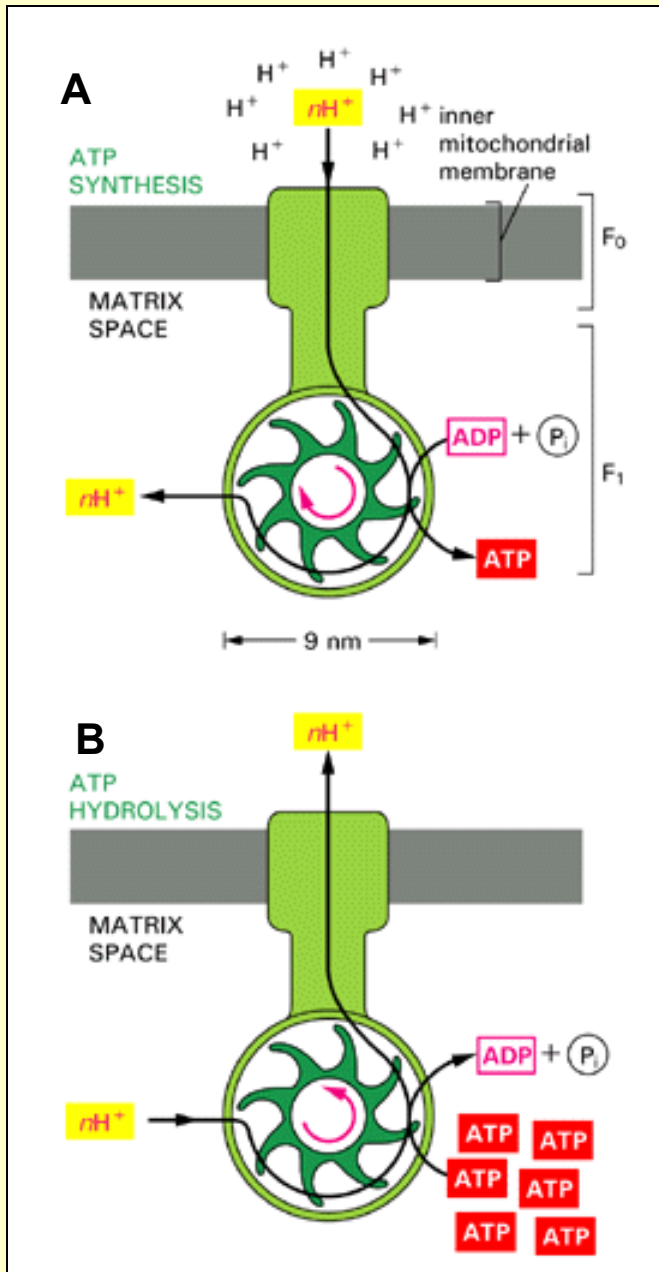


Figure 10-11. 2000. Cooper

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

## ❖ Net yield in energy

During oxidative phosphorylation:

- each e<sup>-</sup> pair from NADH (citric acid cycle) → 2.5 ATP
- each e<sup>-</sup> pair from FADH<sub>2</sub> (citric acid cycle) → 1.5 ATP
- each e<sup>-</sup> pair from NADH (glycolysis) → 1.5 - 2.5 ATP

### **A – 38 ATP**

**2 ATP (glycolysis) + 2 ATP (citric acid cycle) + 34 ATP (e<sup>-</sup> transport)**

### **B – 36 ATP**

- in some cells → 2 NADH (glycolysis) can not enter mitochondria directly
- their enter through “*shuttle*” system → their e<sup>-</sup> might enter the chain at complex II

# Mitochondrial genome

- ✓ contain their own genetic system, which is separate and distinct from the nuclear genome of the cell
- ✓ autoreplicative and semiautonomous organelle
- ✓ circular DNA molecules (like those of bacteria); present in multiple copies per organelle

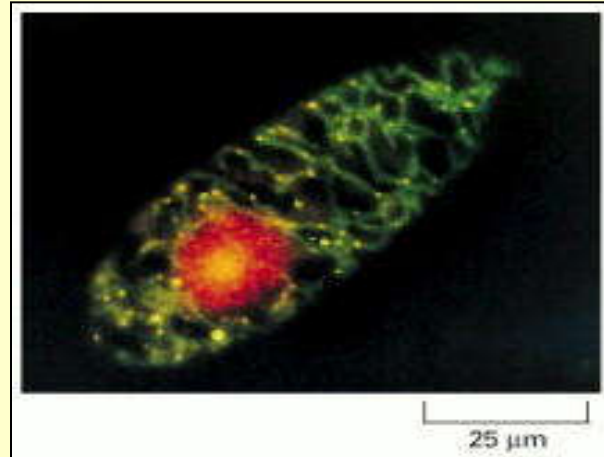


Figure 14-50. 2002. Alberts et al.

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

- ✓ genome – red (ethyidium 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

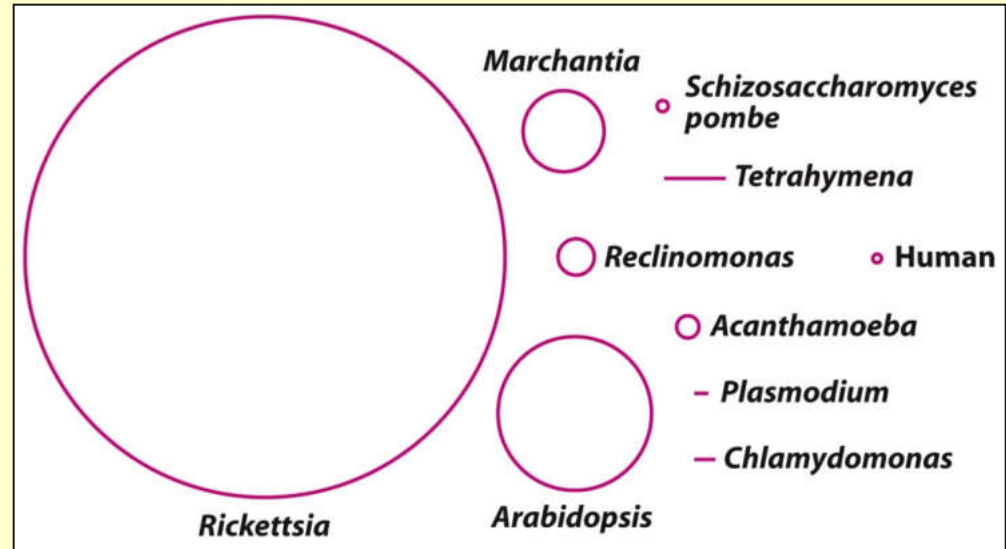


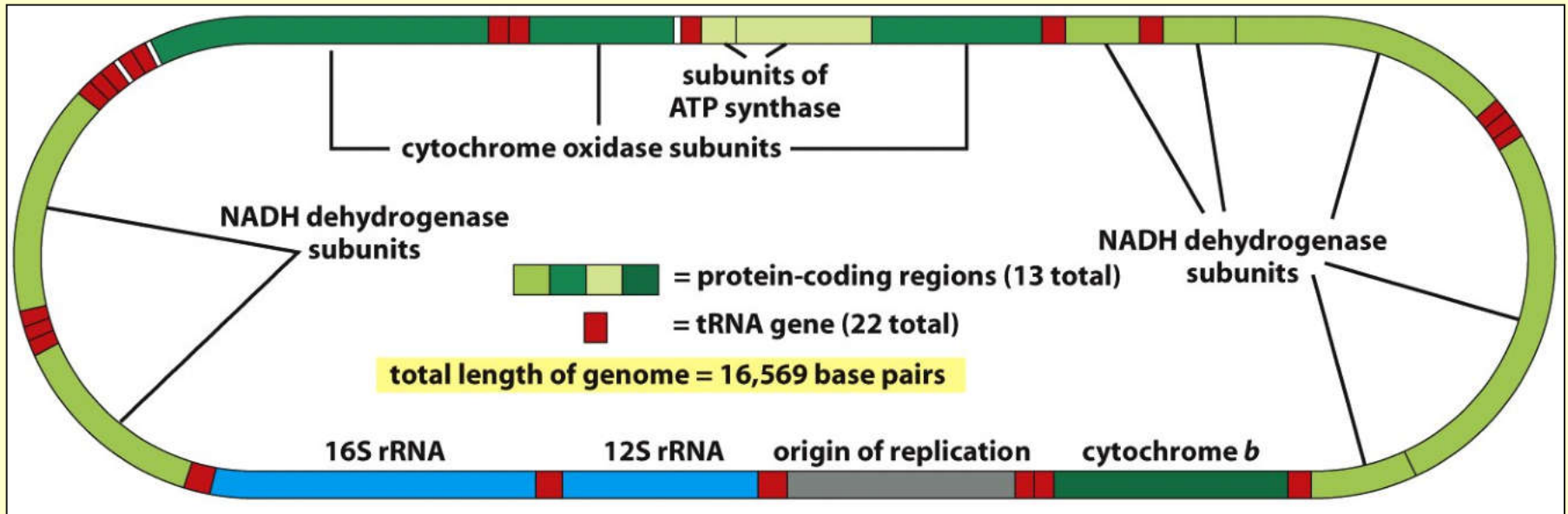
Figure 14-57 *Molecular Biology of the Cell* (© Garland Science 2008)

✓ 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 <sub>m</sub>genome

- ✓ 13 proteins involved in e<sup>-</sup> transport chain and oxidative phosphorylation
- ✓ 16S and 12S rRNA
- ✓ 22 tRNA



## **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 from 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\***

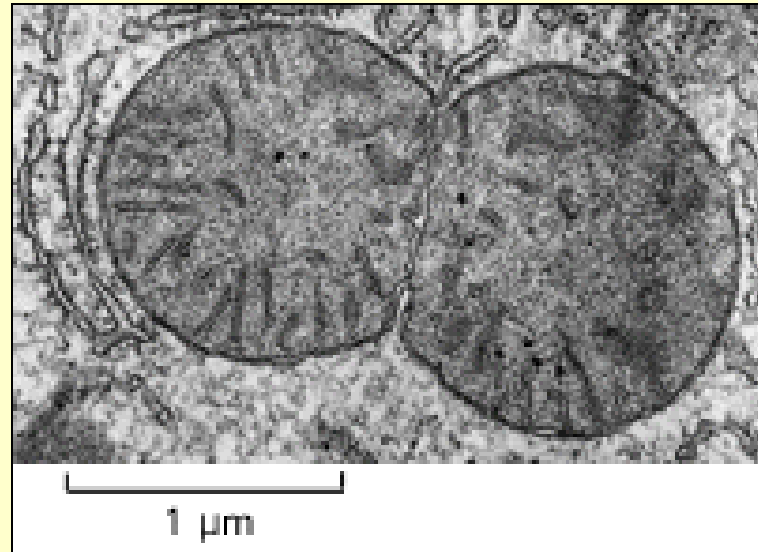
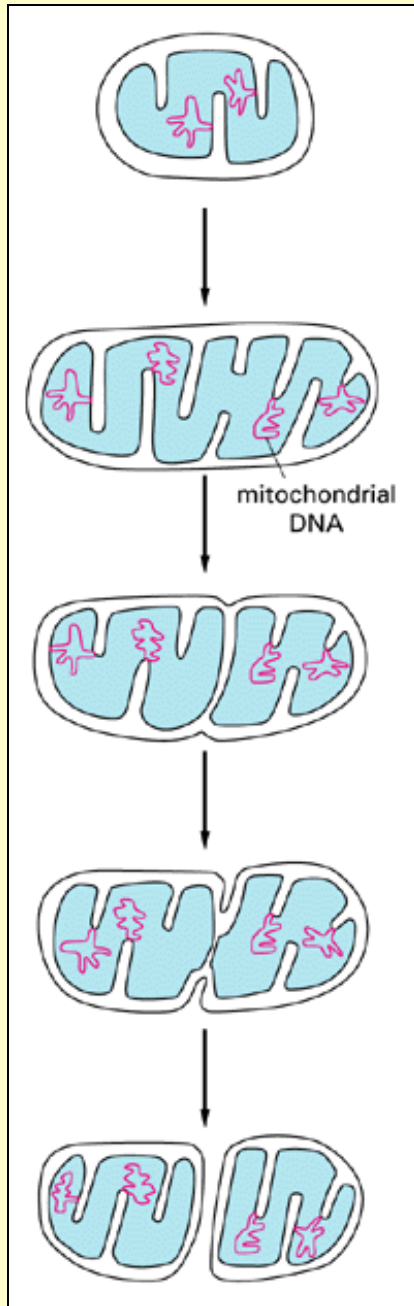
CODON	“UNIVERSAL” CODE	MITOCHONDRIAL CODES			
		MAMMALS	INVERTEBRATES	YEASTS	PLANTS
UGA	STOP	<i>Trp</i>	<i>Trp</i>	<i>Trp</i>	STOP
AUA	Ile	<i>Met</i>	<i>Met</i>	<i>Met</i>	Ile
CUA	Leu	Leu	Leu	<i>Thr</i>	Leu
AGA } AGG }	Arg	<i>STOP</i>	<i>Ser</i>	Arg	Arg

\*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

## ❖ Mitochondrial division

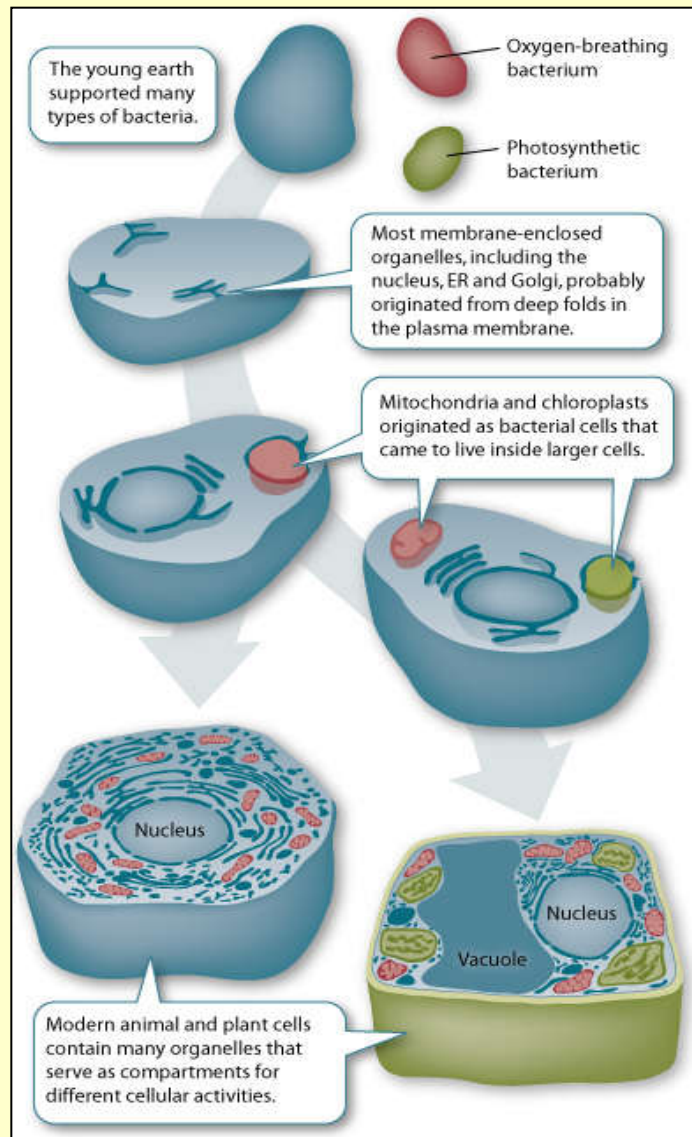
✓ resembles that of bacterial cell division



An electron micrograph of a dividing mitochondrion in a liver cell



# ❖ 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

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

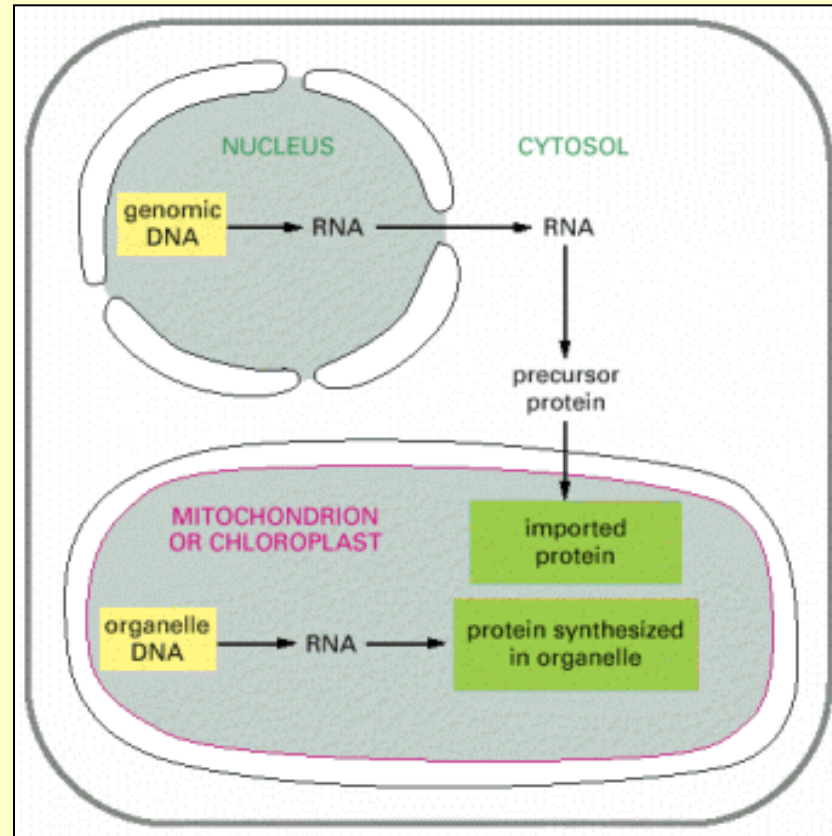
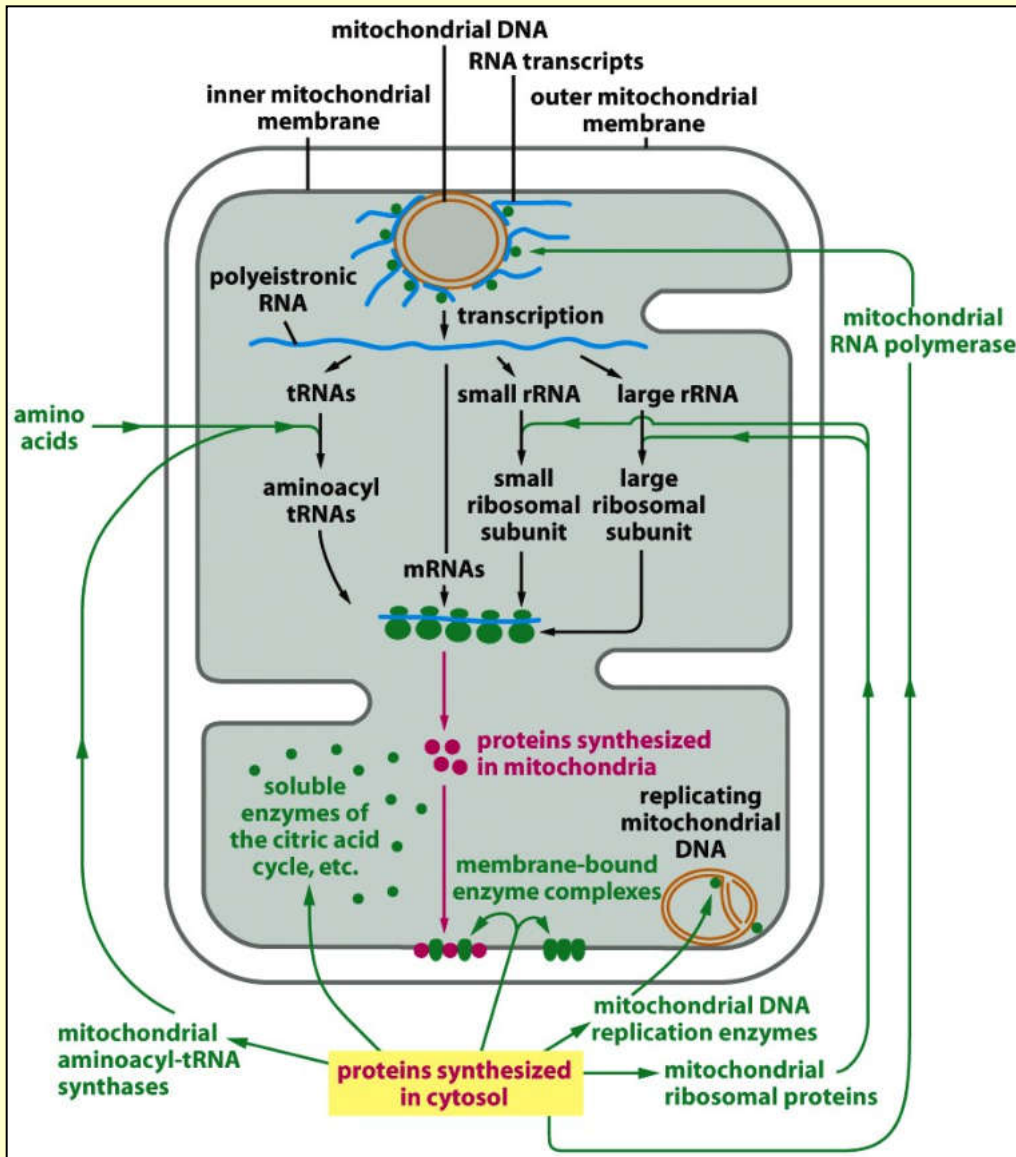


Figure 14-51. 2002. Alberts et al.

- ✓ 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

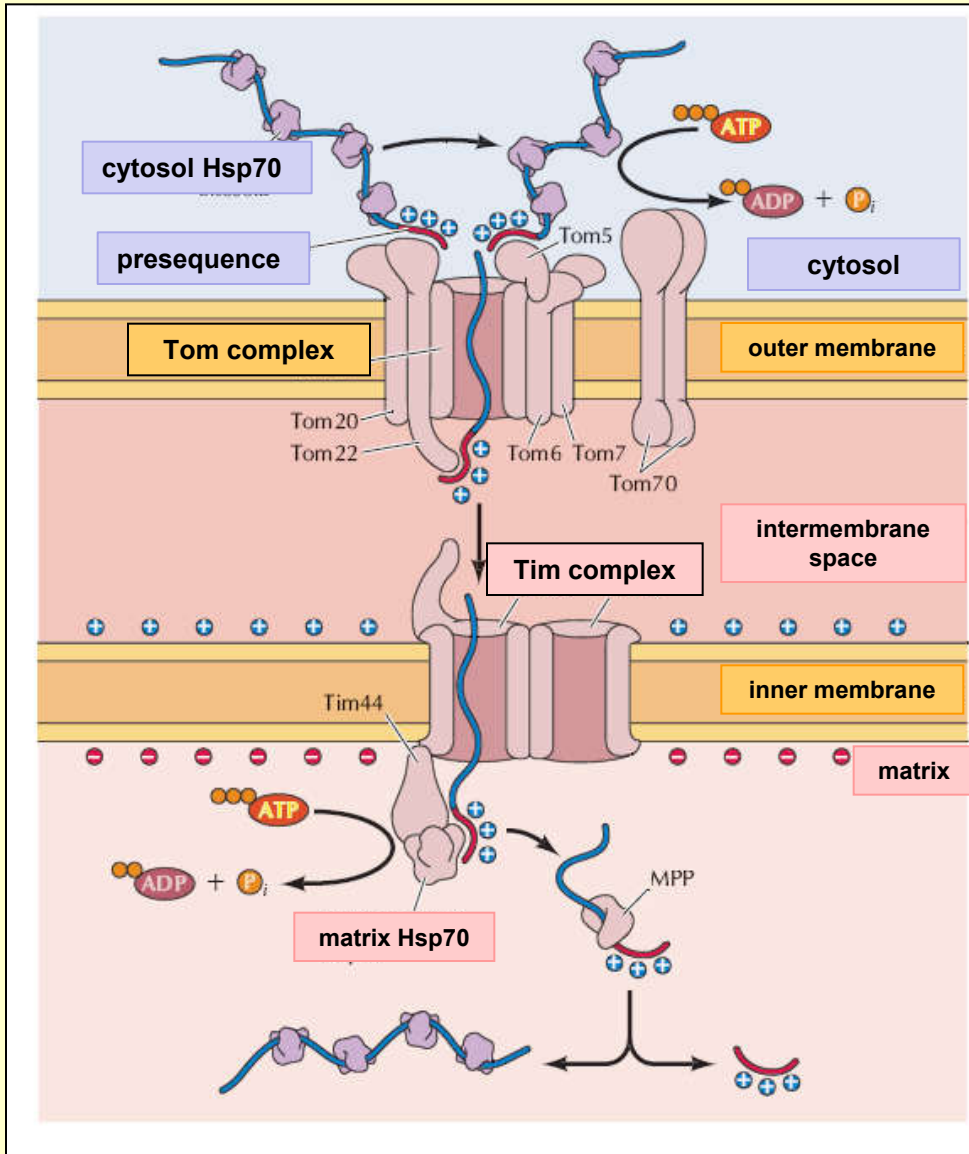


Figure 10-4. 2004. Cooper and Hausman

- ✓ ATP-driven
- ✓ presequence at N-end (25-35 aa)
- ✓ cytosol chaperon Hsp70
- ✓ outer membrane receptors – protein complex **Tom**  
(*Translocase of the Outer Membrane*)
- ✓ transfer through the outer membrane
- ✓ inner membrane receptors – protein complex **Tim**  
(*Translocase of the Inner Membrane*)
- ✓ transfer to matrix – presequence is cleaved by MPP (*matrix processing peptidase*)
- ✓ matrix chaperon Hsp70 facilitates protein folding

# **Chloroplasts**

# Plastid types

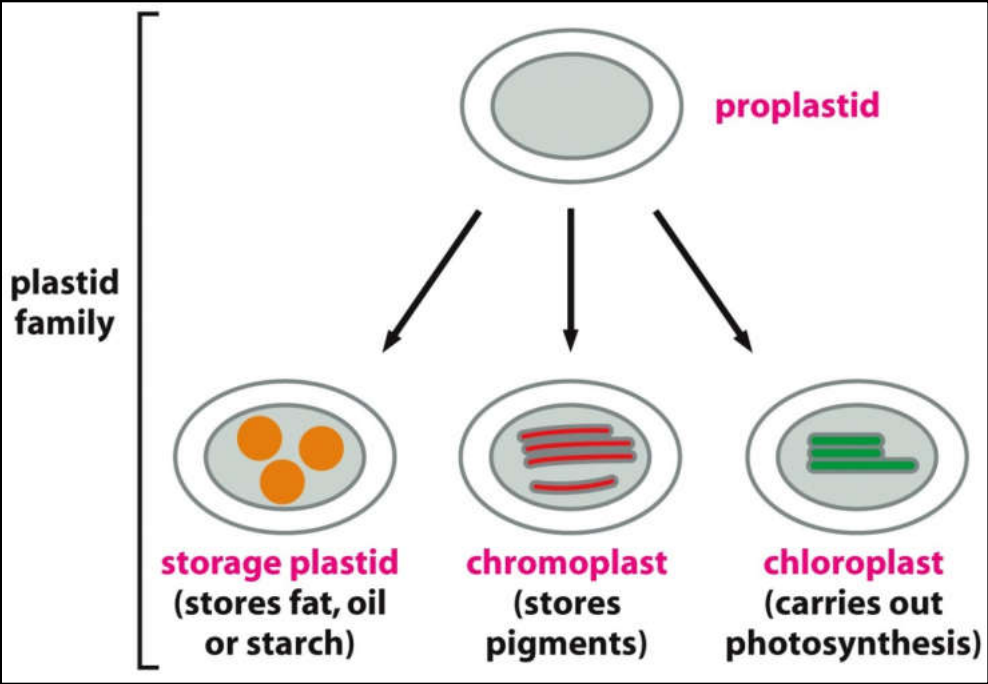
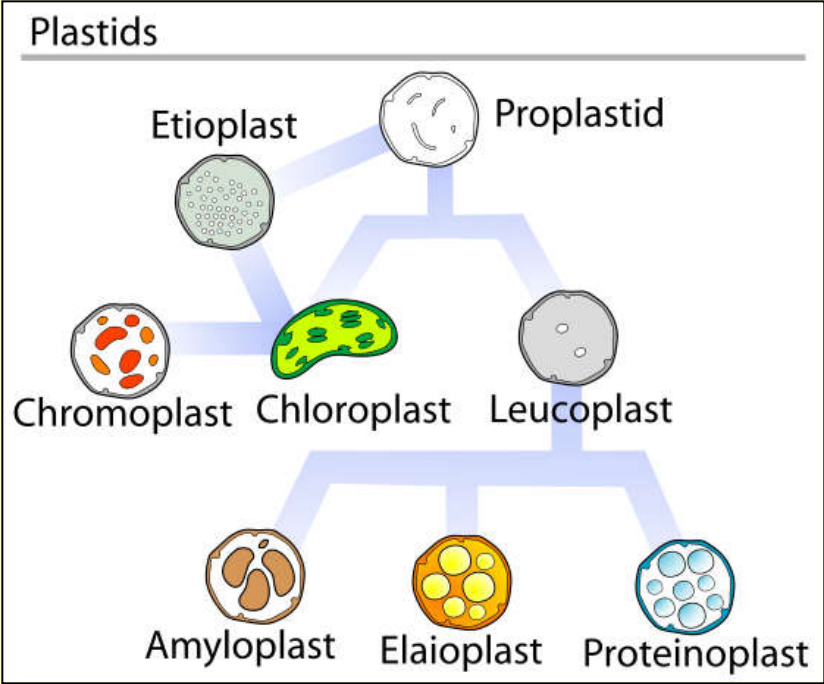


Figure 12-3a *Molecular Biology of the Cell* (© Garland Science 2008)



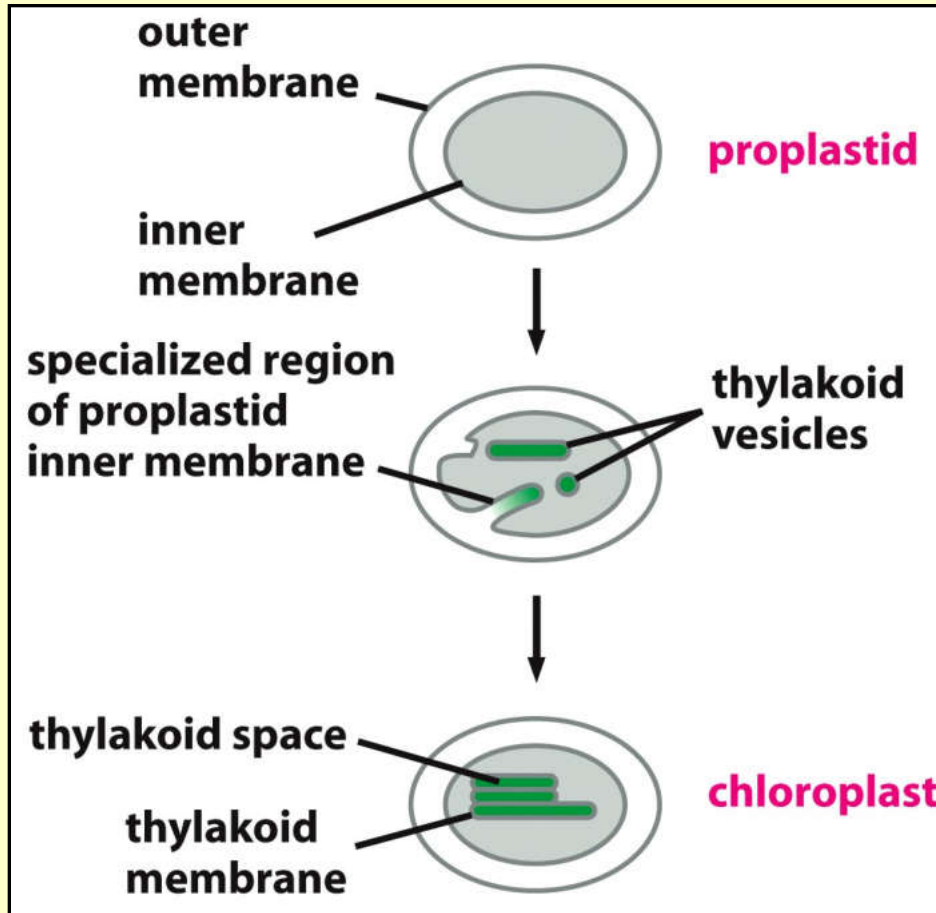
From Wikipedia, the free encyclopedia

# 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<sub>2</sub> in carbohydrates (photosynthesis)
  - synthesis of amino acids, fatty acids and lipids of their membranes

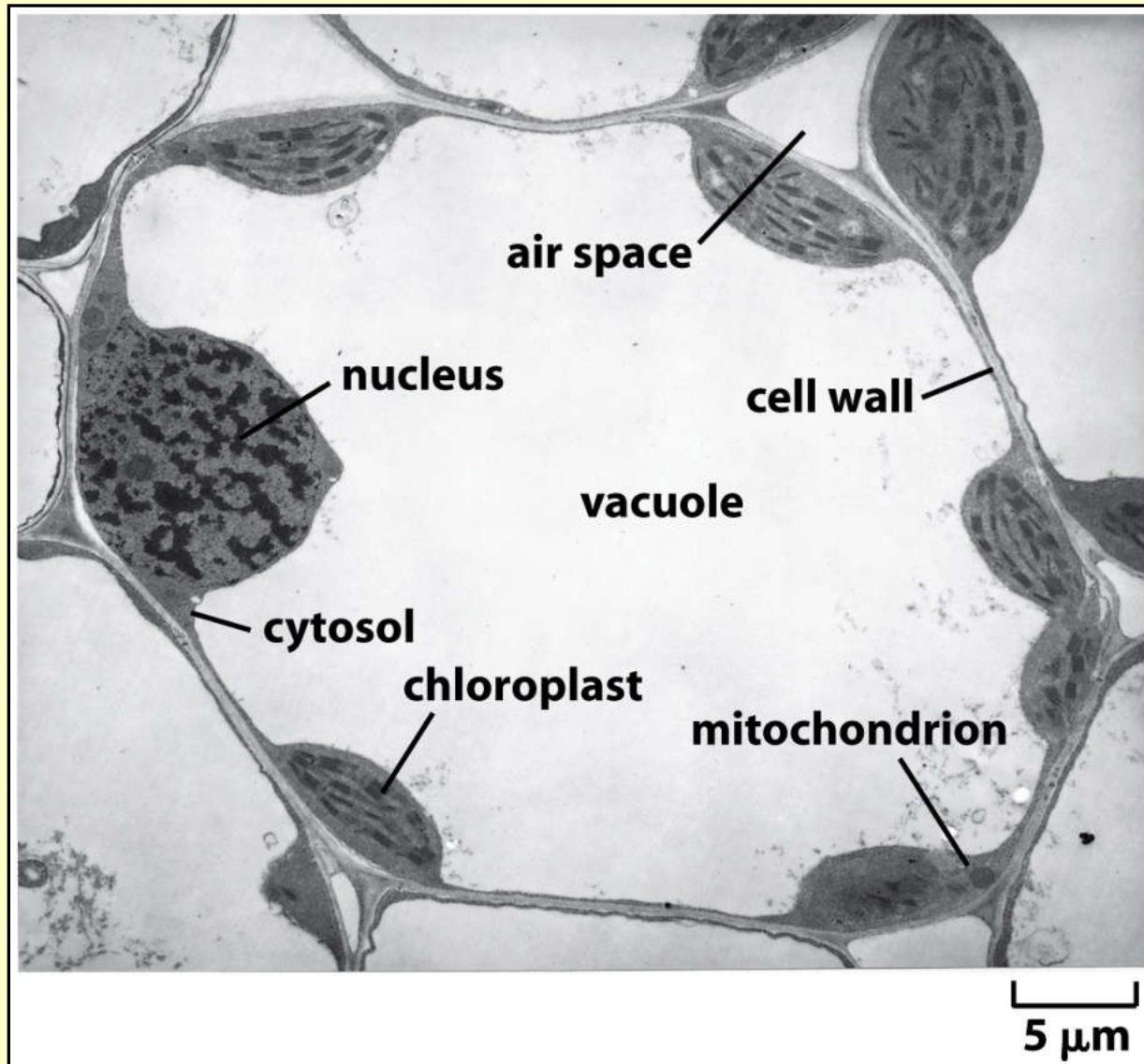


# Chloroplast formation

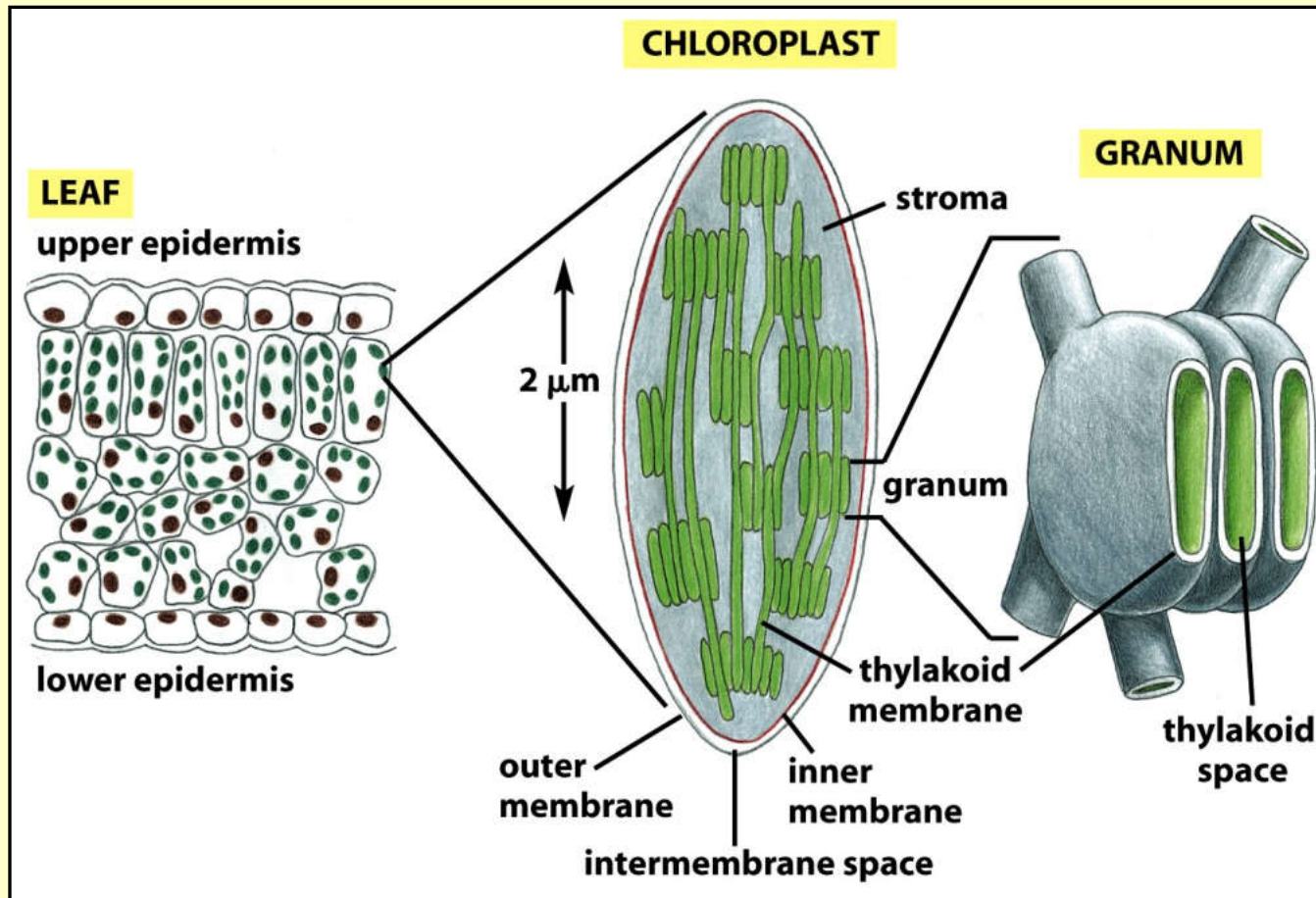




# Chloroplasts in plant cell



# Structure and ultrastructure



✓ size 5 – 10 μm

✓ outer membrane

✓ inner membrane

✓ thylakoid membrane

# Structure and ultrastructure

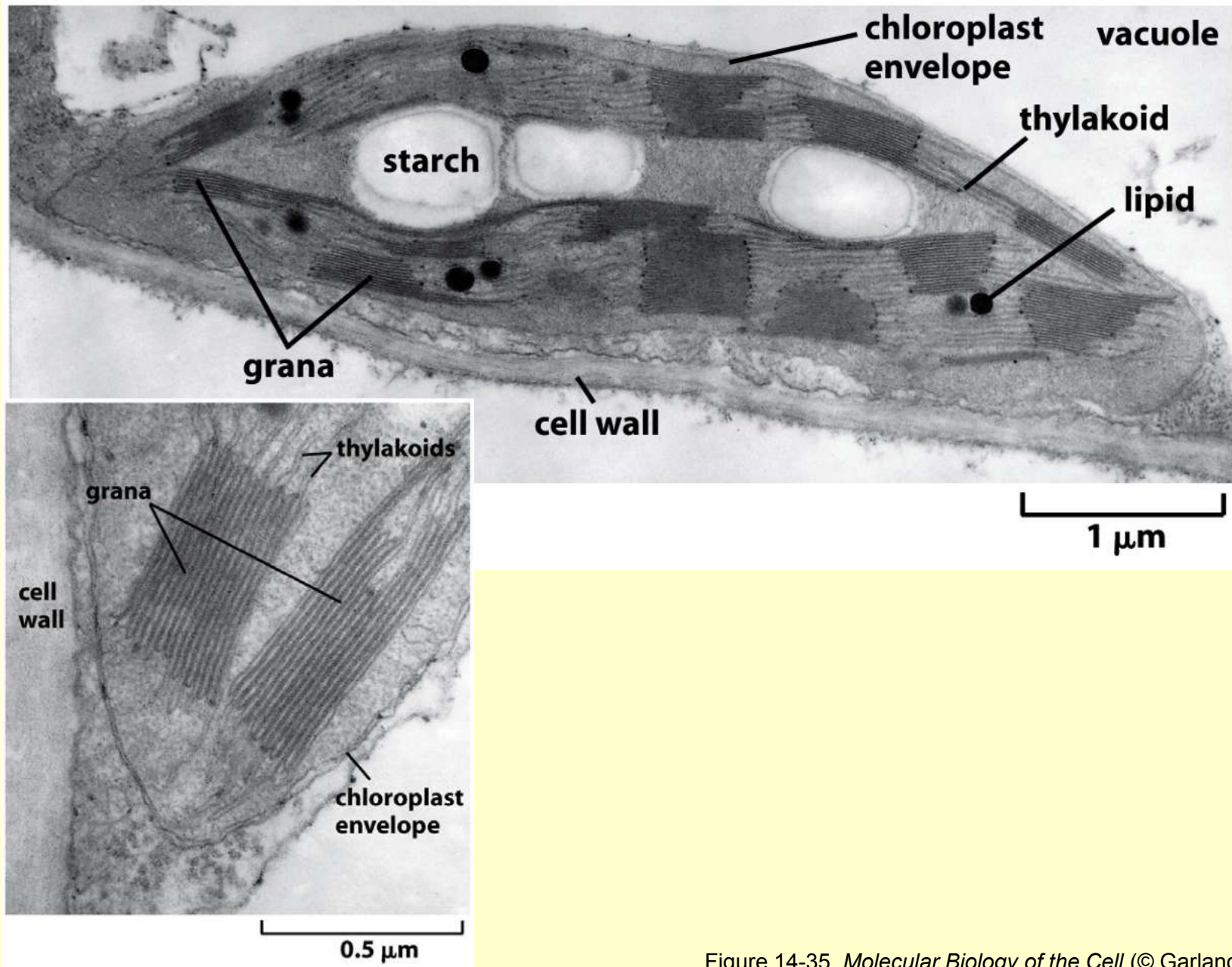
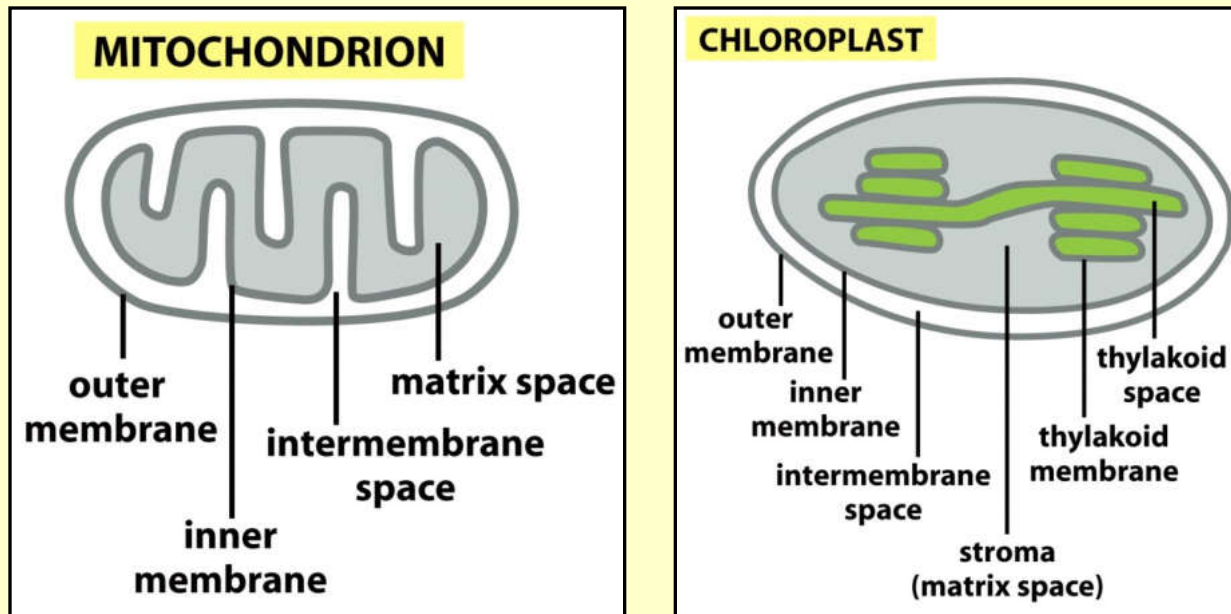


Figure 14-35 *Molecular Biology of the Cell* (© Garland Science 2008)

## 3 membrane systems make chloroplasts more complex than mitochondria

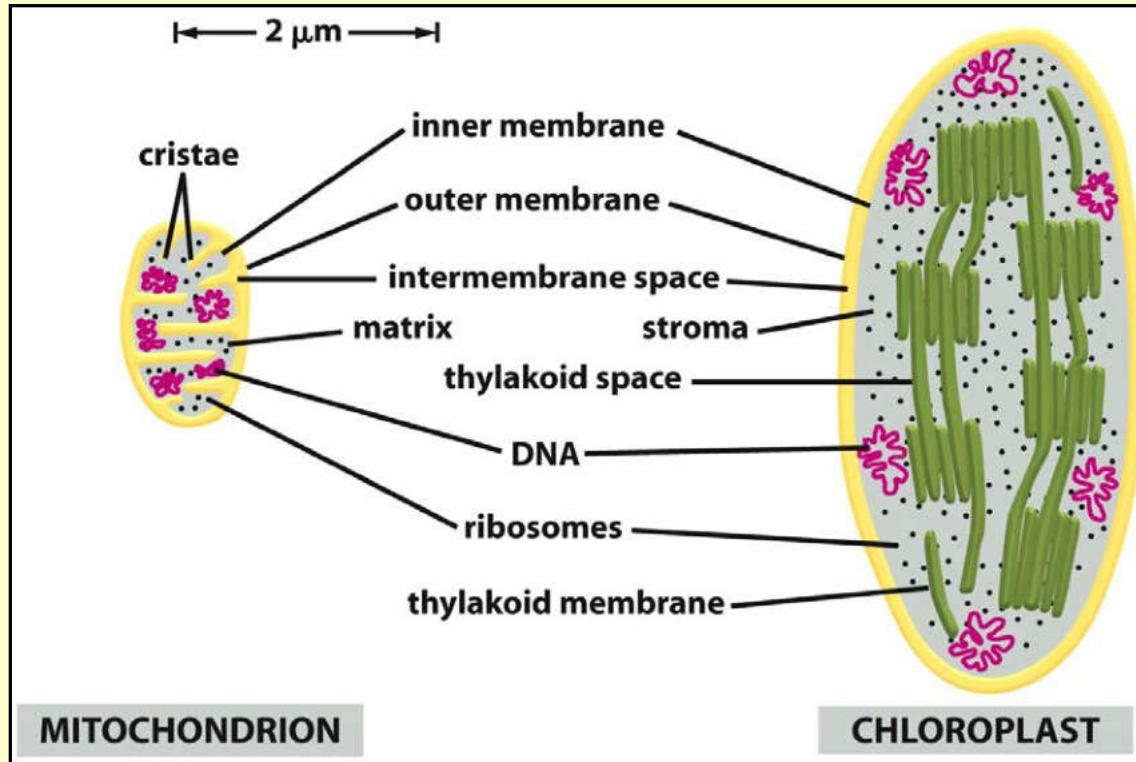
✓ 3 membranes → 3 different inner compartments:

1. intermembrane space
2. stroma
3. thylakoid lumen





# Mitochondrion vs. chloroplast



- ✓ **outer membrane** → similar to mitochondrial outer membrane (permeability, porins)
- ✓ **stroma** equivalent of matrix (genome, metabolic enzymes)
- ✓ **thylakoid membrane** → e<sup>-</sup> transport and ATP synthesis (inner mitochondrial membrane)
- ✓ **inner membrane** → not involved in photosynthesis

# Chemiosmotic generation of ATP in chloroplasts and mitochondria

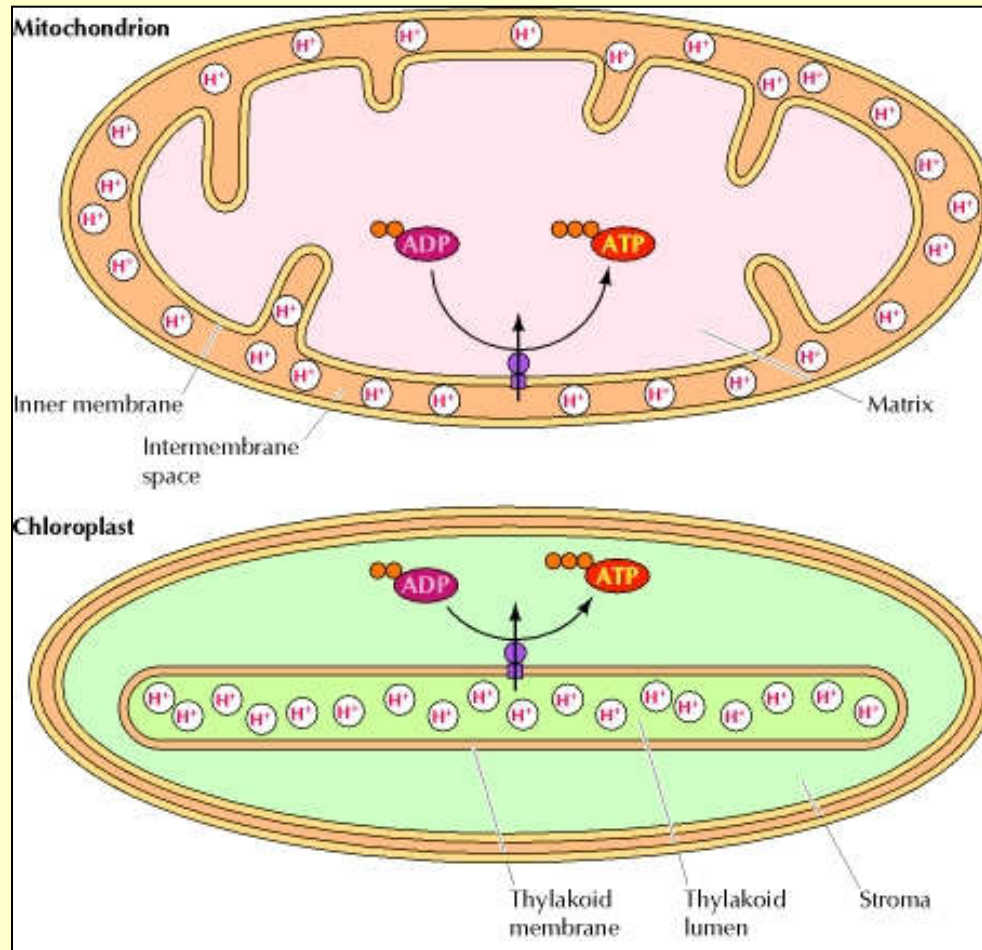


Figure 10.14. 2000. Cooper

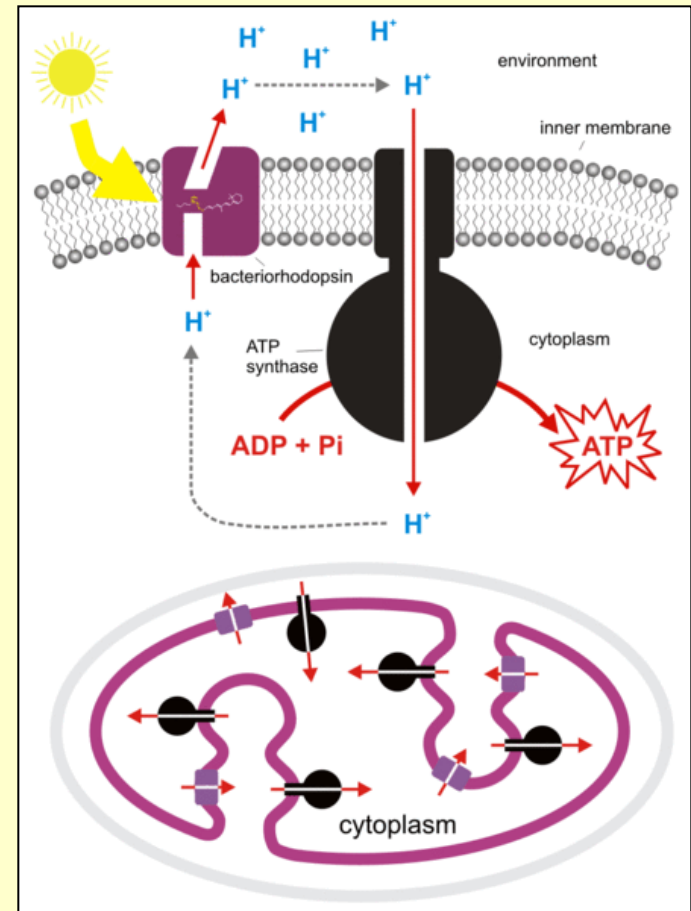
→ **mitochondria** -  $e^-$  transport generates a  $H^+$  gradient across the inner membrane, which is then used to drive ATP synthesis in the matrix

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

# Photosynthesis

## Algae, bacteria and archebacteria

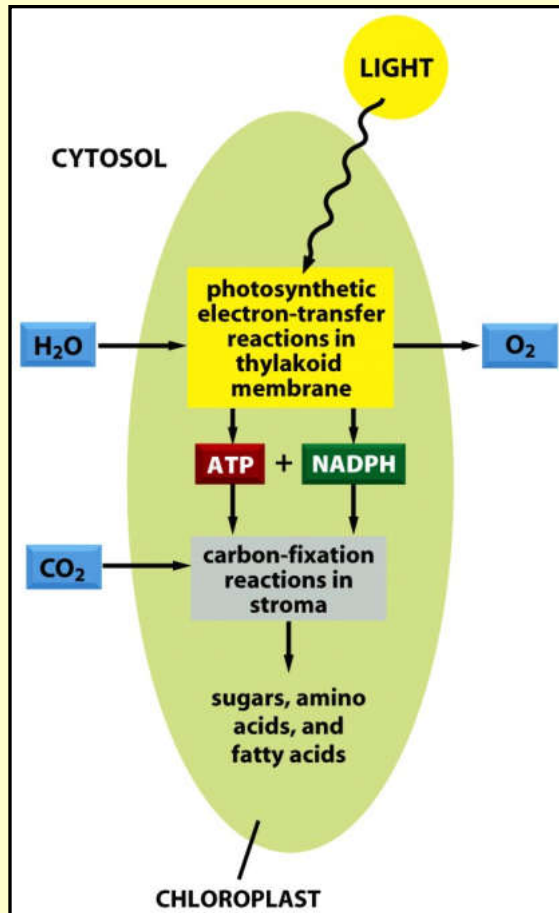
- photosynthesis on the plasma membrane
- eg. **bacteriorhodopsin**
- photosynthetic proton pump
- *Halobacterium* cell membrane



# Photosynthesis in chloroplasts

→ energy from sunlight is harvested and used to drive the synthesis of glucose from  $\text{CO}_2$  and  $\text{H}_2\text{O}$

→ the ultimate source of metabolic energy for all biological systems



→ takes place in two distinct stages

## 1. Light reactions

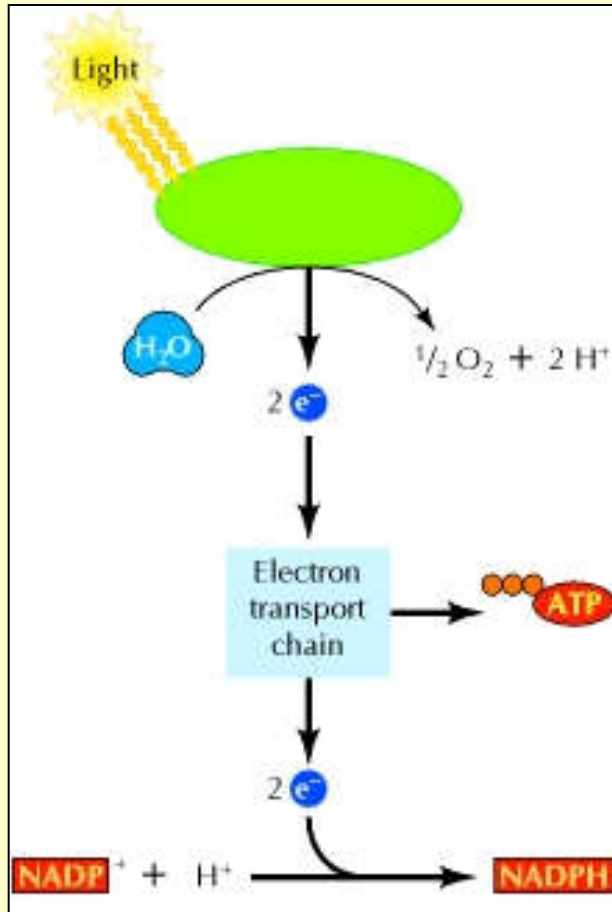
- ✓ energy from sunlight drives the synthesis of ATP and NADPH
- ✓ coupled to the formation of  $\text{O}_2$  from  $\text{H}_2\text{O}$
- ✓ thylakoid membranes

## 2. Calvin cycle

- ✓ ATP and NADPH produced by the light reactions drive glucose synthesis
- ✓ reduction of  $\text{CO}_2$
- ✓ stroma



# Light reactions



✓ energy from sunlight is used to split H<sub>2</sub>O to O<sub>2</sub>

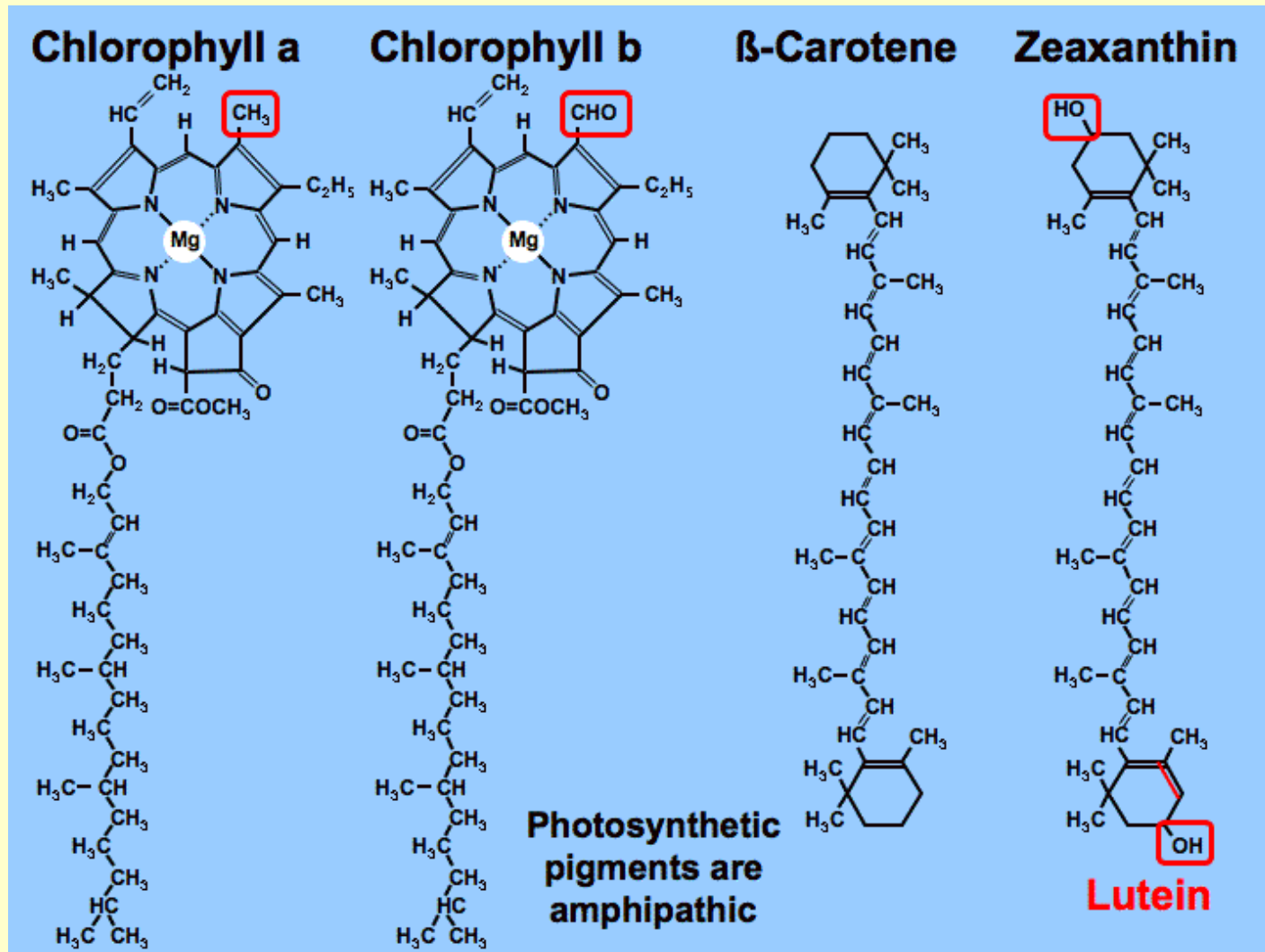
✓ high-energy e<sup>-</sup> derived from this process are then transported through a series of carriers and used to convert NADP<sup>+</sup> to NADPH

✓ energy derived from the e<sup>-</sup> transport reactions also drives the synthesis of ATP

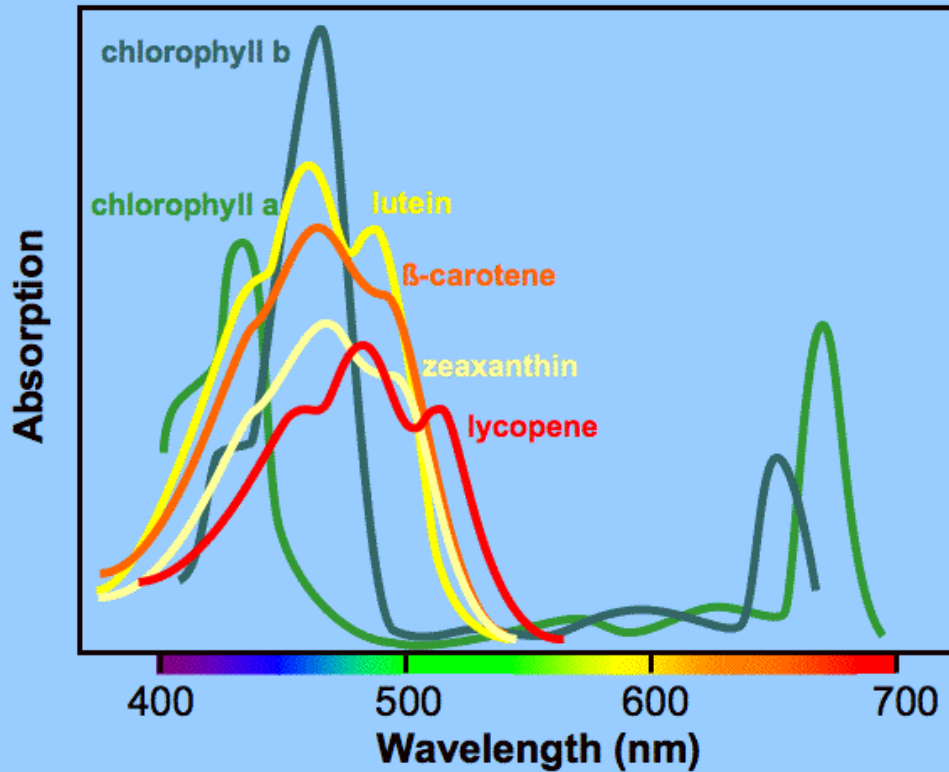
Figure 2.38. 2000. Cooper

# Absorption of sunlight

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

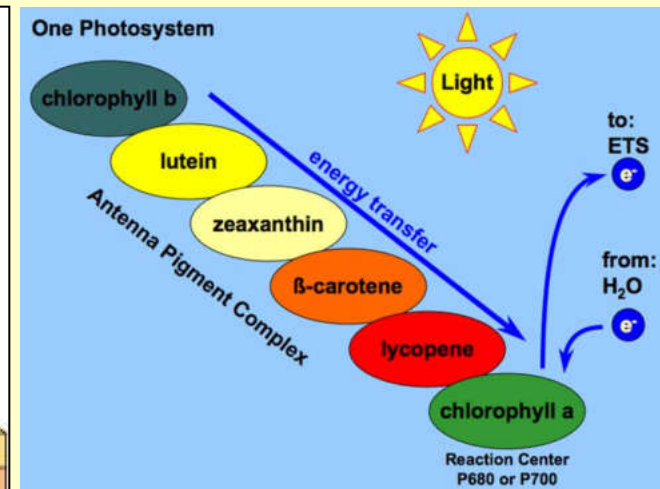
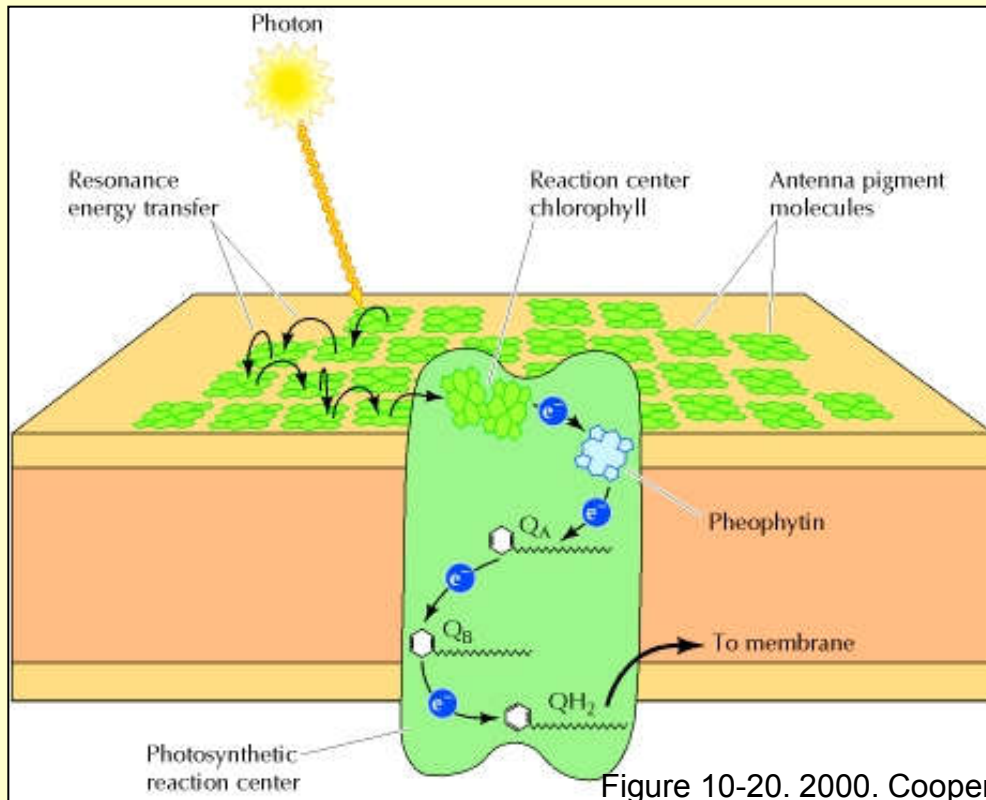


## The photosynthetic pigments absorb much of the spectrum



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

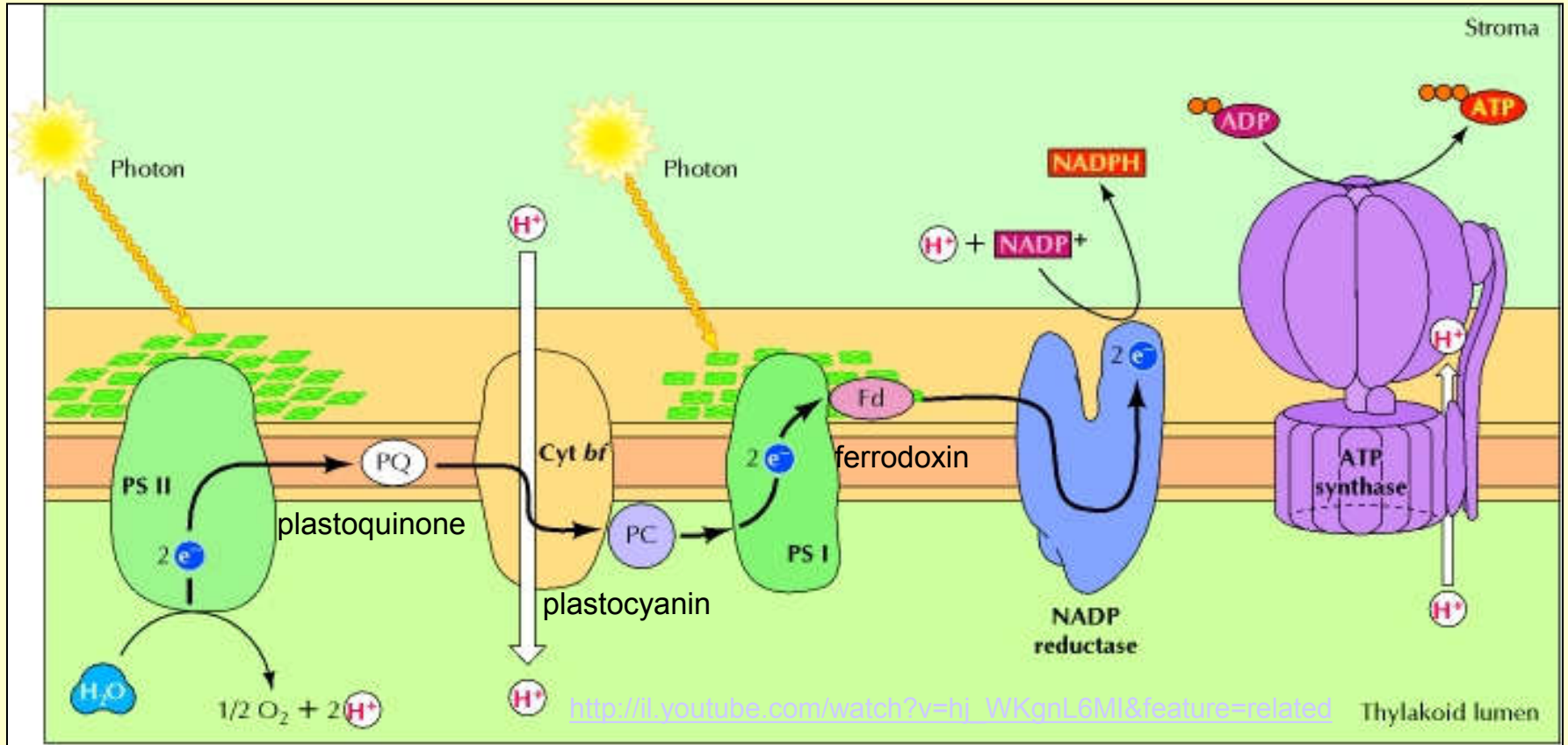
# Organization of a photocenter



[http://plantphys.info/plant\\_physiology/light.shtml](http://plantphys.info/plant_physiology/light.shtml)

- ✓ each photocenter  $\rightarrow$  hundreds of antenna pigment molecules  $\rightarrow$  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<sub>2</sub>O within the thylakoid lumen

## cytochrome *b<sub>6</sub>* complex

e<sup>-</sup> transferred to a lower energy state - H<sup>+</sup> are pumped into the thylakoid lumen

## photosystem I

energy derived from light absorption generates high energy e<sup>-</sup>

## NADP reductase

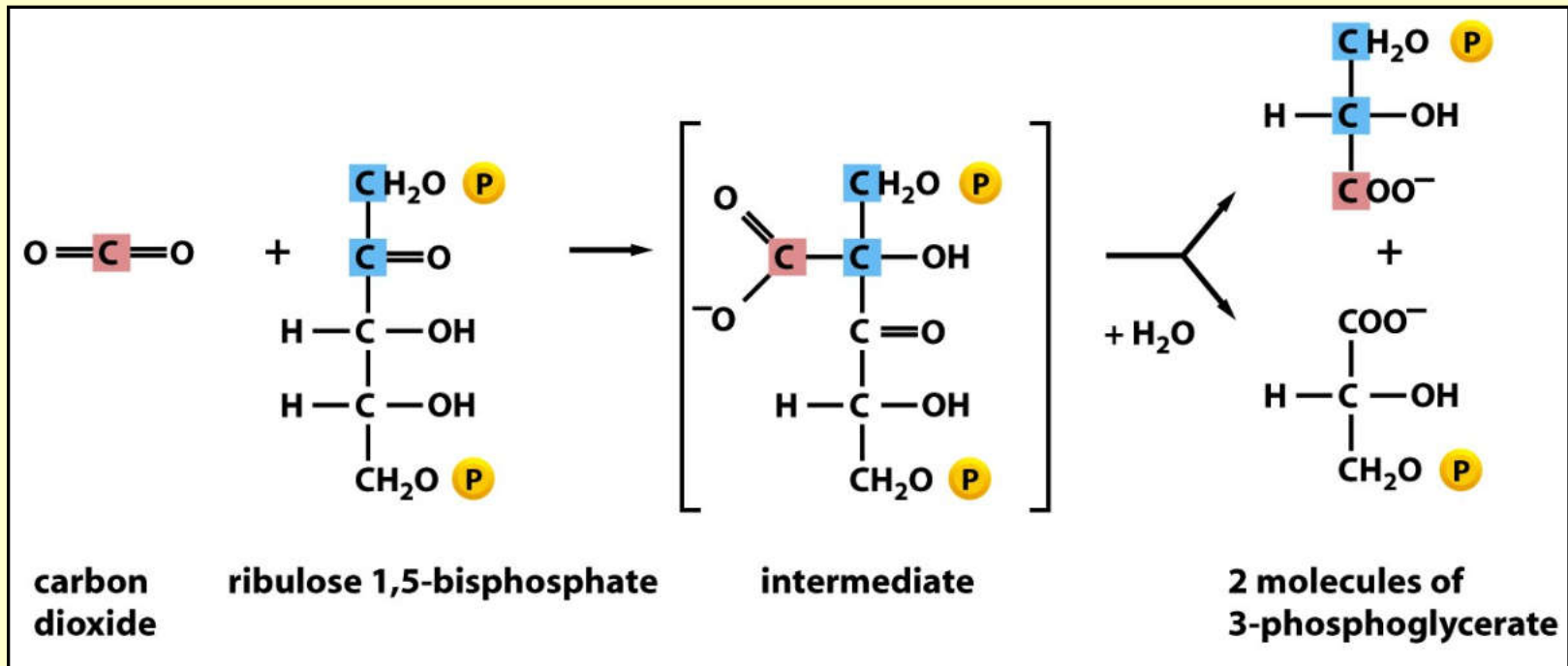
reduce NADP<sup>+</sup> to NADPH in the stroma

## ATP synthase

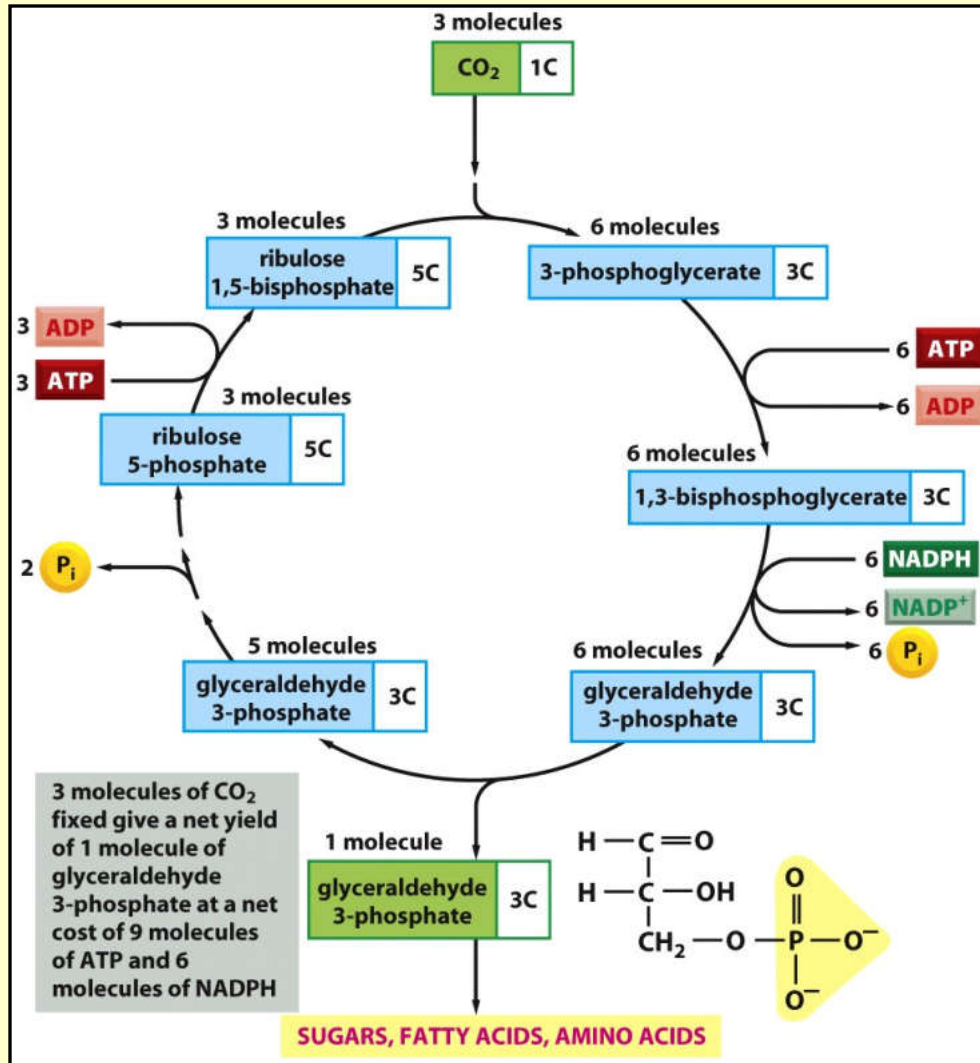
uses the energy stored in the proton gradient to convert ADP to ATP

# Calvin cycle

- ✓ ATP and NADPH produced from the light reactions drive the synthesis of carbohydrates from  $\text{CO}_2$  and  $\text{H}_2\text{O}$
- ✓ 1  $\text{CO}_2$  at a time is added to a cycle of **reactions** – Calvin cycle
- ✓ enzyme **Rubisco** (Ribulose Bisphosphate Carboxylase)  
→ adds  $\text{CO}_2$  to ribulose-1,5-bisphosphate



# Calvin cycle



→ synthesis of 1 glyceraldehyde-3-phosphate from 3 CO<sub>2</sub>

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

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



# Chloroplast genome

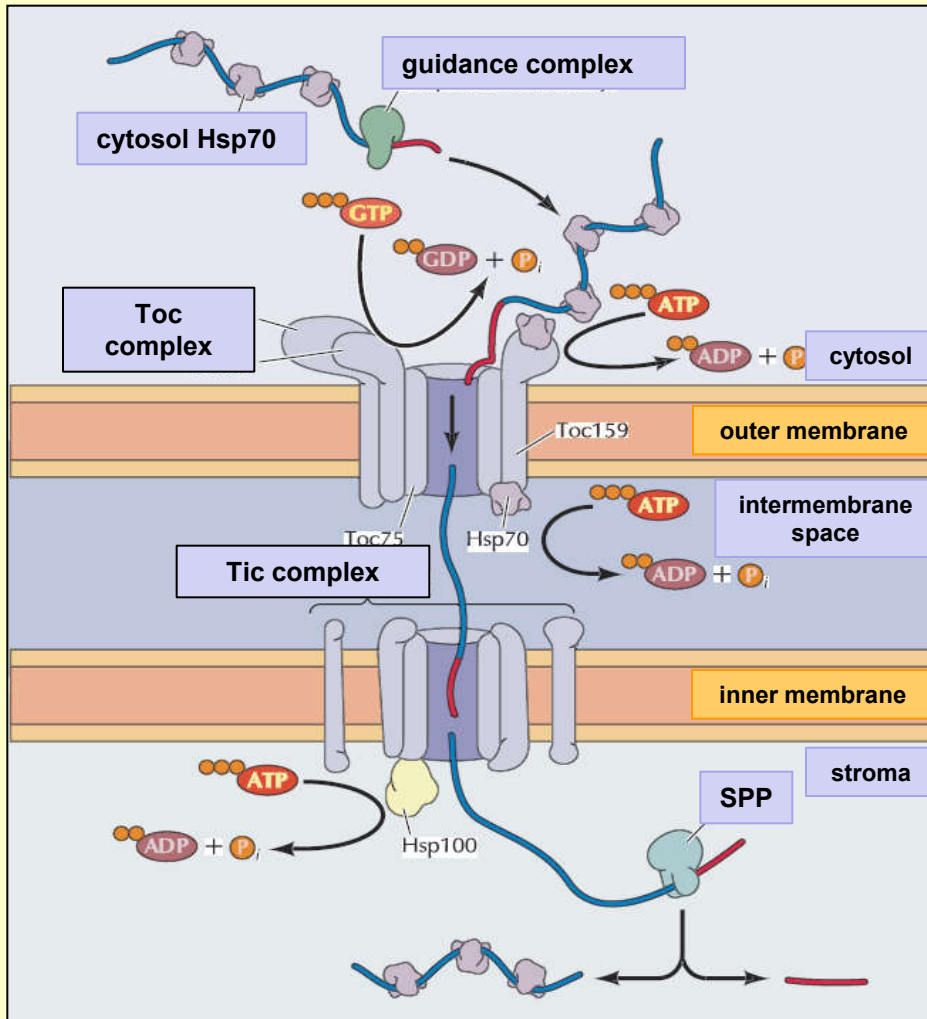
- ✓ circular DNA (like mitochondria)
- ✓ more copies per organelle (like mitochondria)
- ✓ more complex than mitochondrial (120 - 150 kb; cca 150 genes)
- ✓ encodes:
  - **RNA**: 4 rRNAs and 30 tRNAs (universal genetic code)
  - **20 ribosomal proteins**
  - **RNA polymerase subunits**
  - **30 photosynthesis proteins**
  - **30 proteins which need to be identified**

Table 10.2 Genes Encoded by Chloroplast DNA

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



# Import of chloroplast proteins



- ✓ ATP- and GTP-driven
- ✓ **N-terminal presequence** (30 – 100 aa)
- ✓ **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

Figure 10-15. 2004. Cooper and Hausman

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

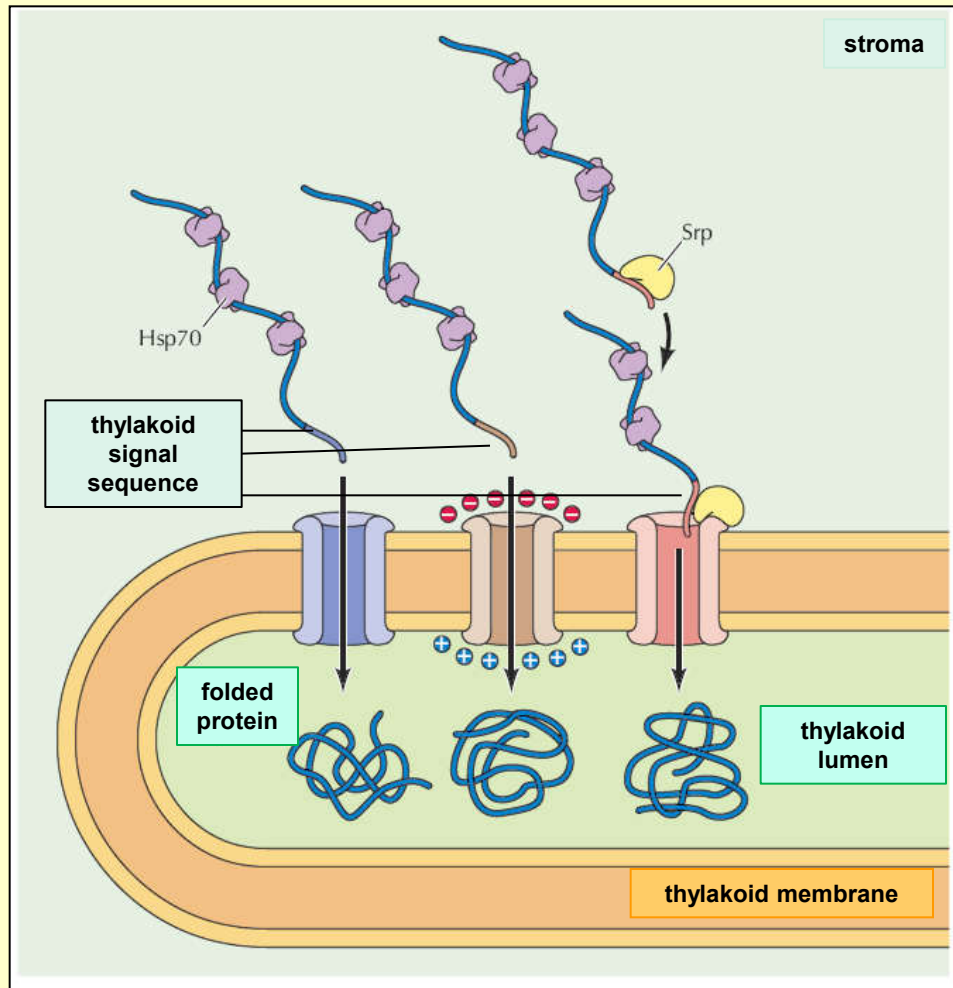
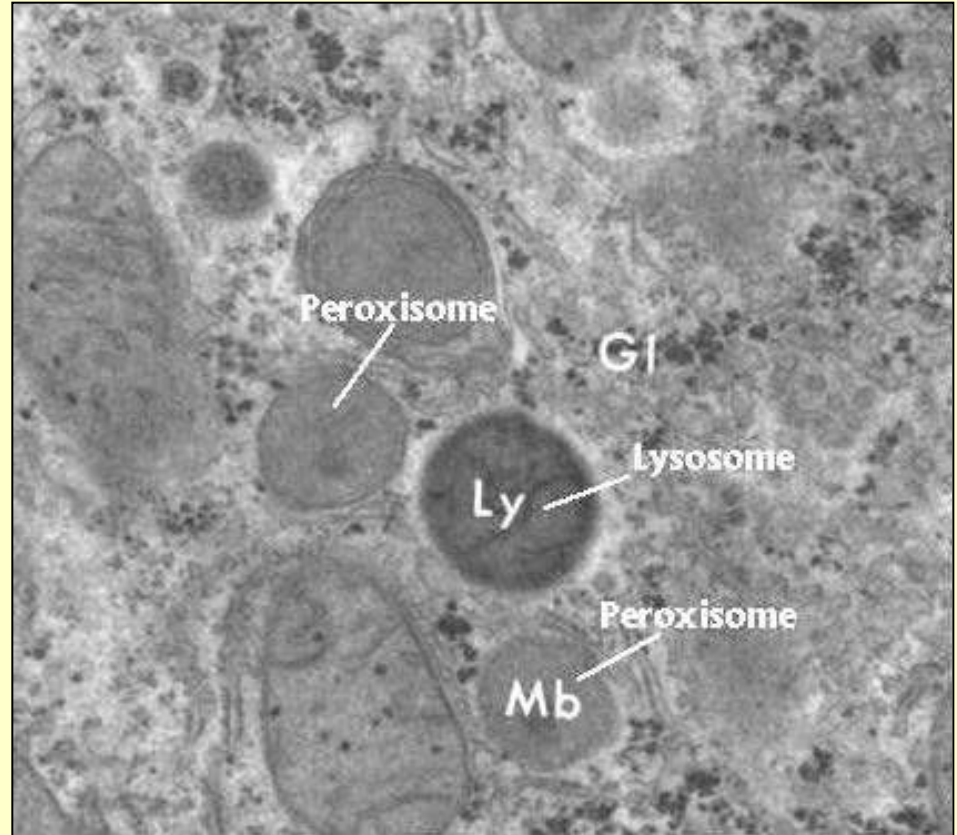


Figure 10-16. 2004. Cooper and Hausman

# 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  $\text{H}_2\text{O}_2$  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

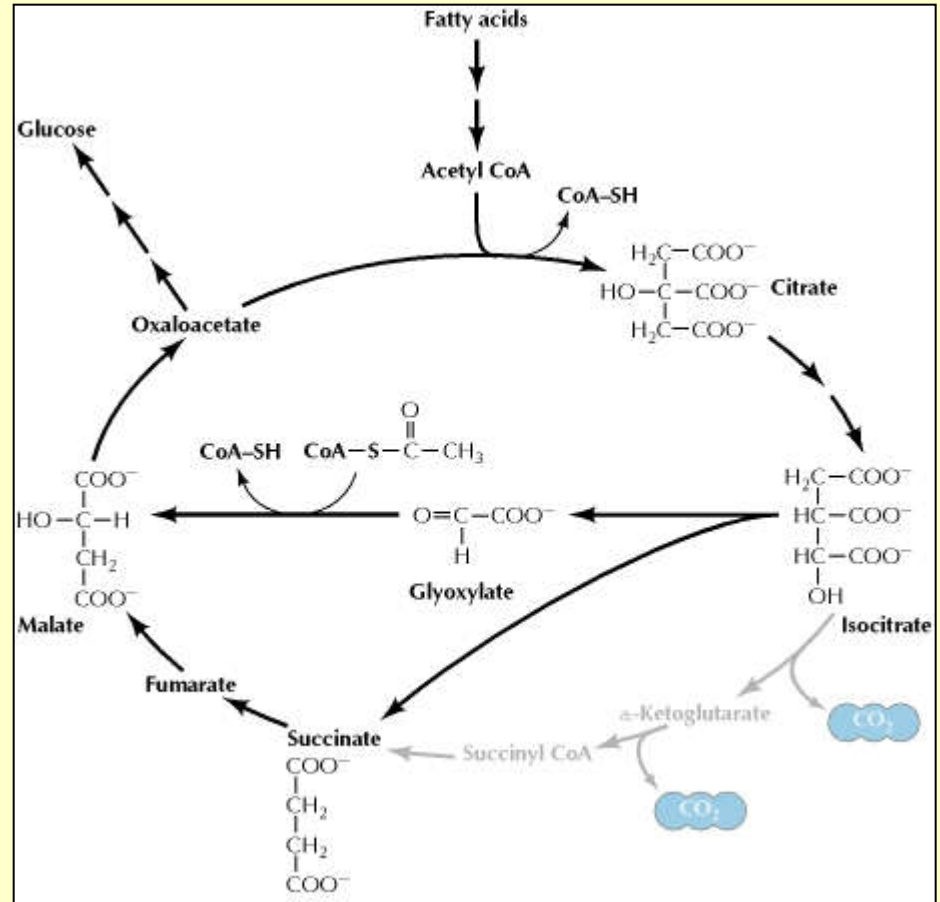


Figure 10-27. 2000. Cooper

## 2. In leaves

- ✓ **photorespiration** → **adaptation on hot and dry environment**
- ✓ serves to metabolize a side product formed during photosynthesis
- ✓ Rubisco adds  $\text{CO}_2$  = **photosynthesis**
- ✓ Rubisco sometimes catalyzes **the addition of  $\text{O}_2$**  instead of  $\text{CO}_2$ 
  - result - one 3-phosphoglycerate and one **phosphoglycolate**
  - a side reaction, and **phosphoglycolate** is not a useful metabolite!

# Role of peroxisomes in photorespiration

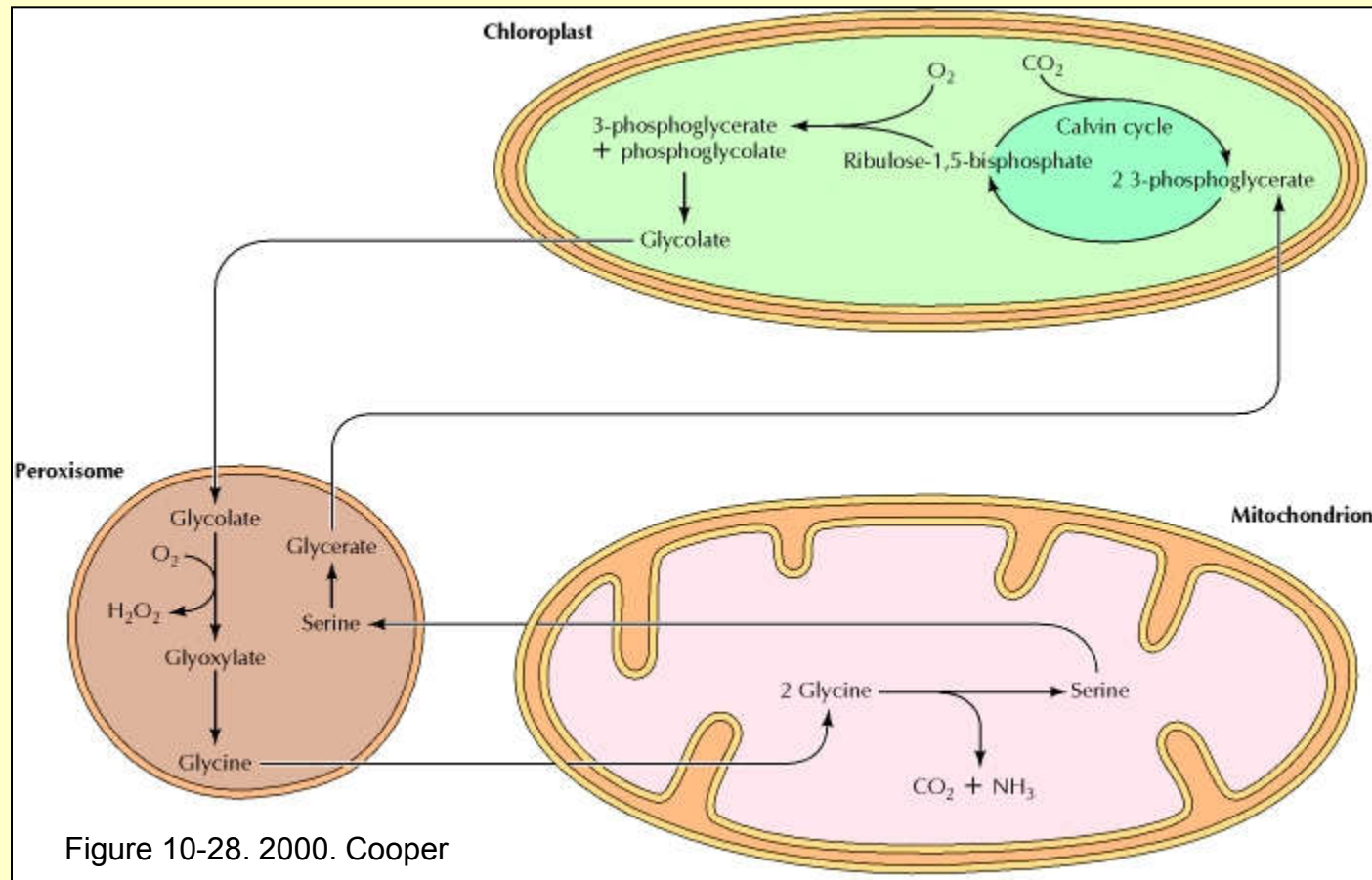


Figure 10-28. 2000. Cooper

- ✓ phosphoglycolate is converted to glycolate
- ✓ transfer to peroxisomes → oxidation and conversion 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

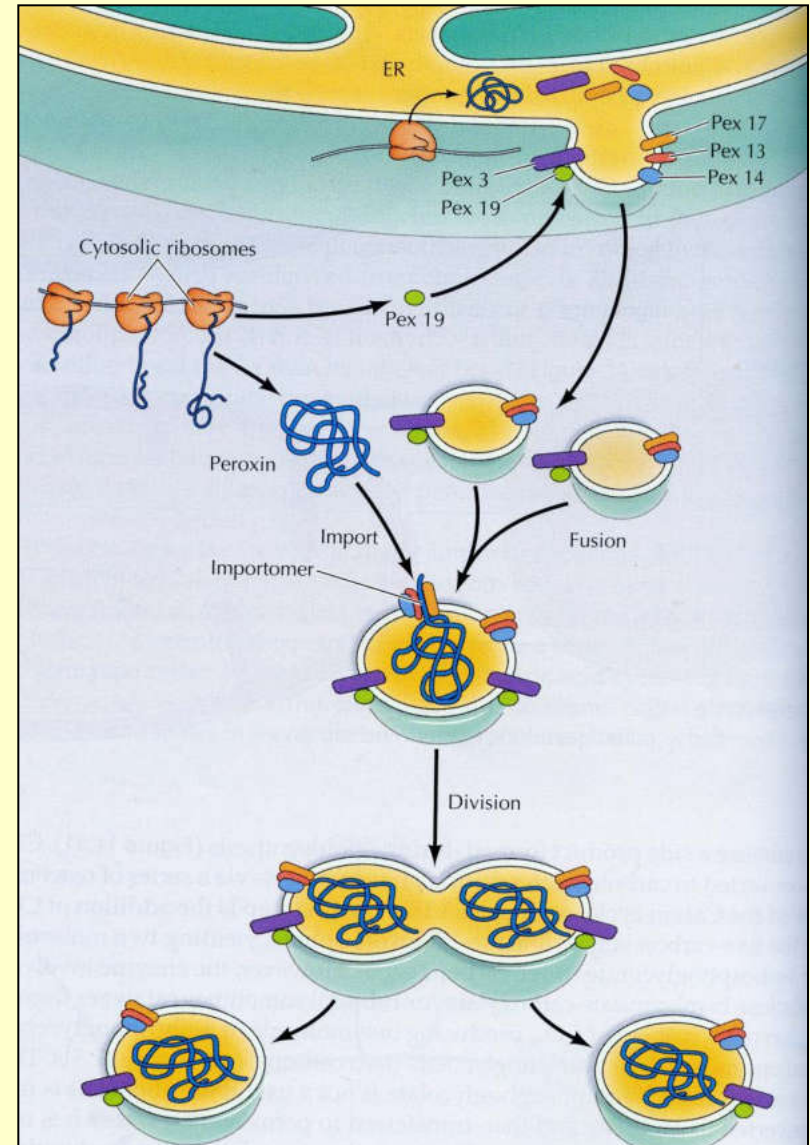


Figure 11-32. 2013. Cooper