

Lecture 8

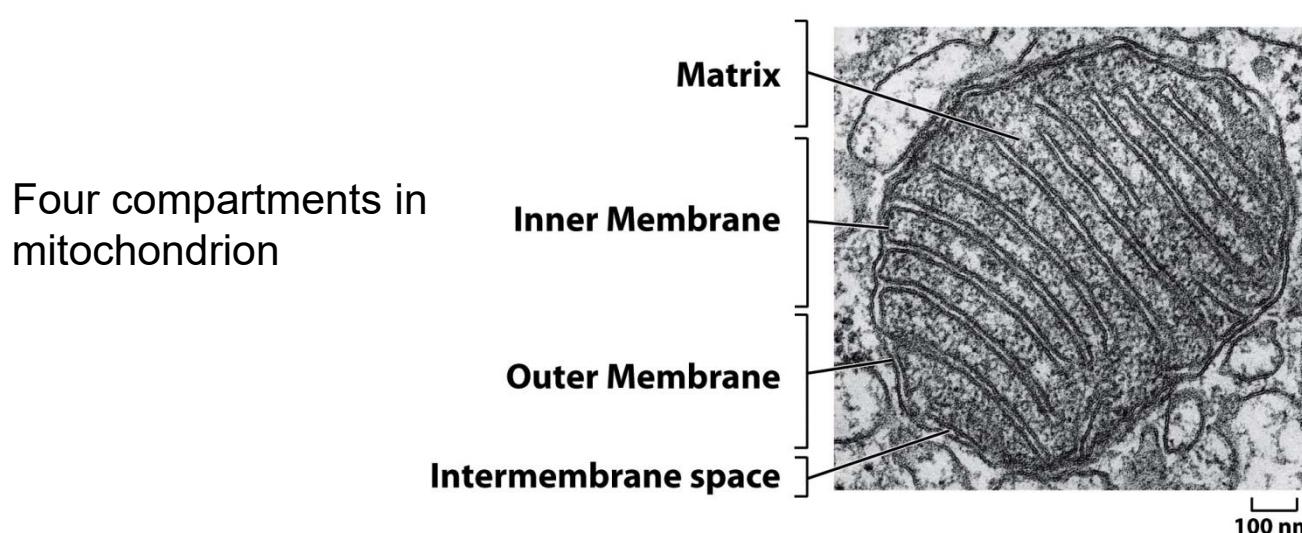
Mitochondrion and chloroplast

Outline

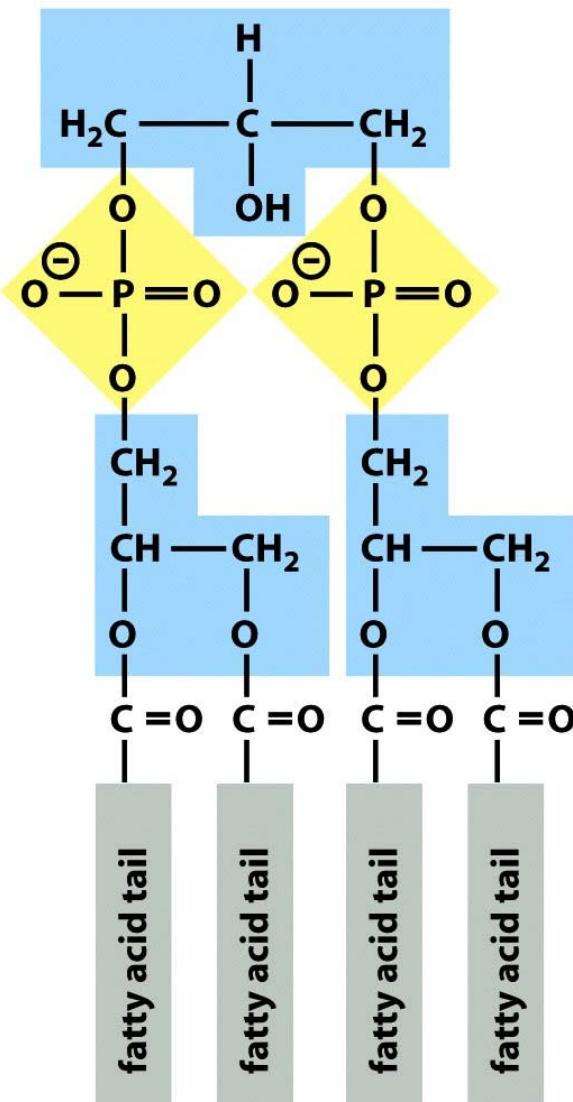
- I. Mitochondrion structure and its compartment
- II. Plastid and chloroplast structure/ function
- III. Genetic systems in mitochondria and chloroplasts
- IV. Energy conversion---chemiosmotic coupling

I. mitochondria

- **Outer membrane** contains porins and is highly permeable to molecules 5kd or less.
- **Inner membrane** is highly selective in permeability for molecules and contains the proteins for electron transport proteins and ATP synthase, transporters
- **Matrix** contains hundreds of enzymes for citric acid cycle, pyruvate oxidation, fatty acid oxidization, mitochondria DNA, tRNA, ribosomes.
- **Intermembrane space** contains several enzyme to use ATP to phosphorylate other nucleotides



Cardiolipin in the inner membrane (IM) makes it highly impermeable



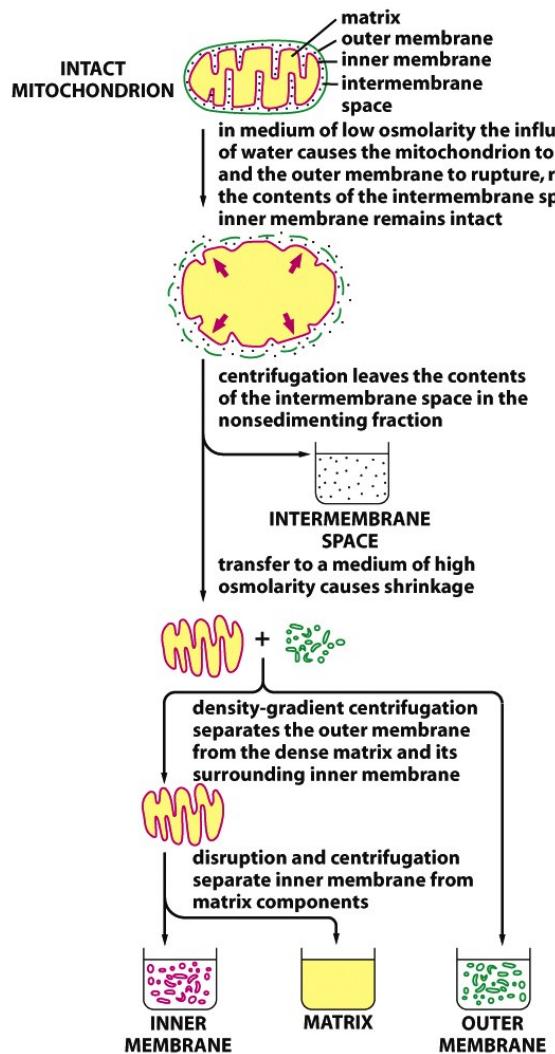
Double phospholipids contains 4 fatty acid chains

It constitutes 20% of total inner membrane.

It is synthesized from **imported phosphatidylglycerol** in the mitochondria.

Cardiolipin consists of **two** covalently linked phospholipid units, with a total of **four** rather than the usual two fatty acid chains

How to fractionate separate compartments for analysis in mitochondria?



Outer membrane (OM) is easy to disrupt, but inner membrane (IM) has many folded cristae surrounding the matrix. This allows swelling without membrane rupture in low osmotic solution.

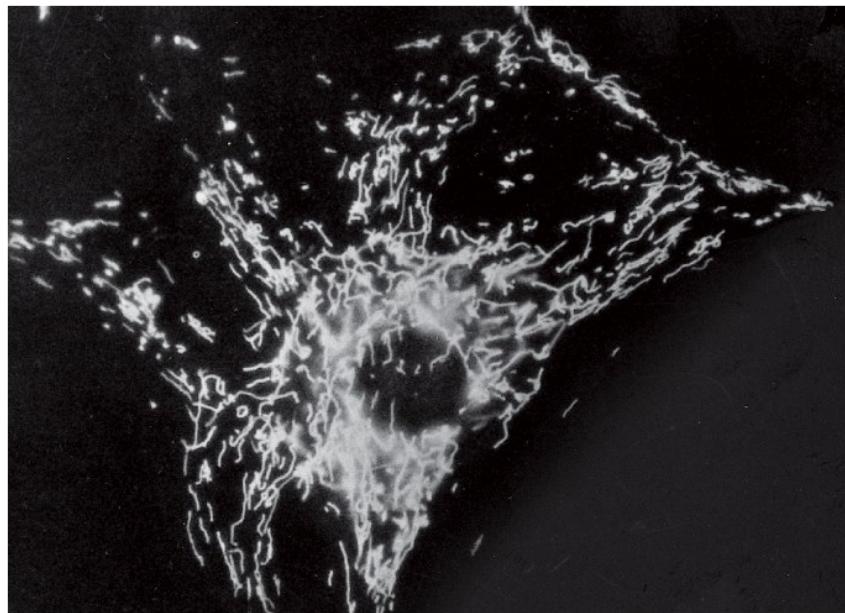
1st osmotic shock to disrupt the **outer membrane** and **releases content from the inner membrane space (IMS)**, this is **separated by centrifugation (soluble supernatant)**.

2nd osmotic shrinking to maintain the **inner membrane** and thus the matrix.

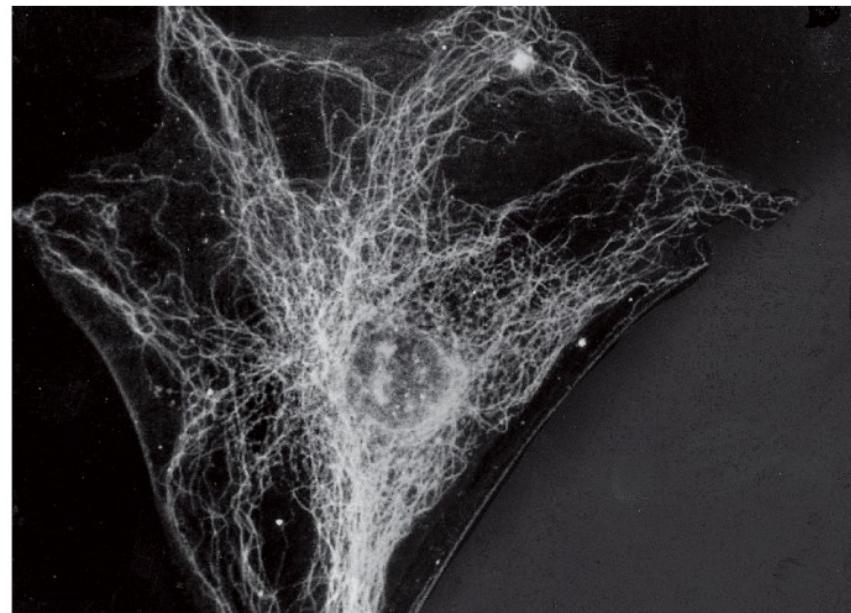
3rd separation by density-gradient centrifugation

4th extraction of the gradient fractions

Mitochondria often associate with microtubules in cells, form moving filaments or chains



(A)
stained mitochondria



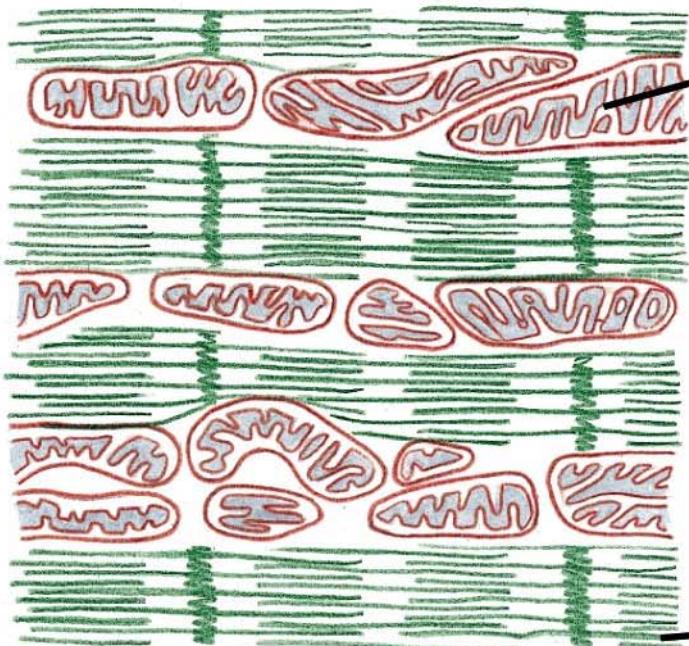
(B)
IF microtubules

10 μm

Microtubules are important for the orientation and mobility of mitochondria

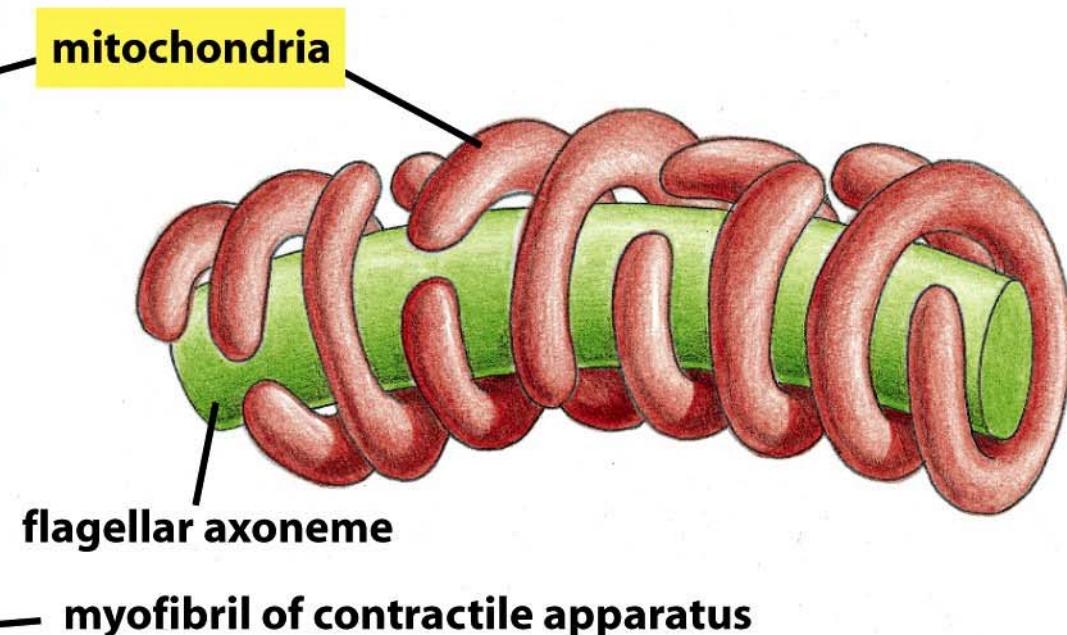
Mitochondria are mobile but remain fixed in energy requiring sites to supply energy

Two examples: cardiac muscle and flagella of sperm cells



(A) CARDIAC MUSCLE

Mitochondria align to the contractile myofibrils

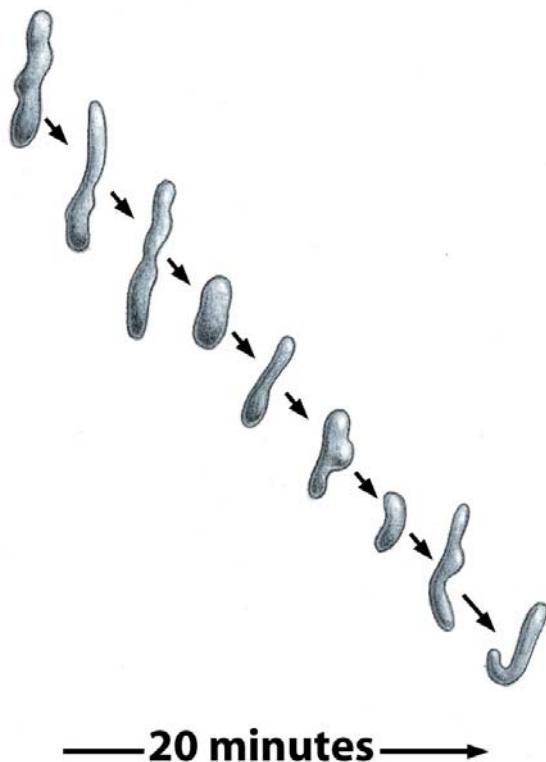


(B) SPERM TAIL

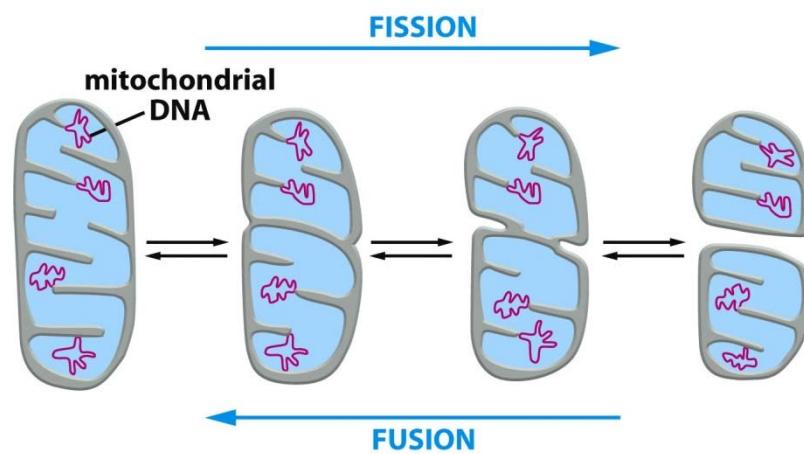
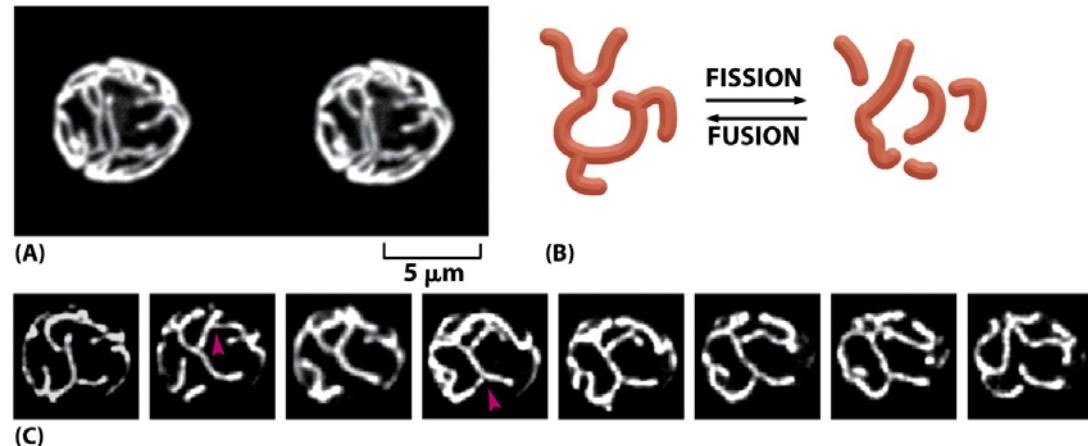
Mitochondria align to the flagellar axoneme (probably with help of microtubule)

Plasticity of mitochondria

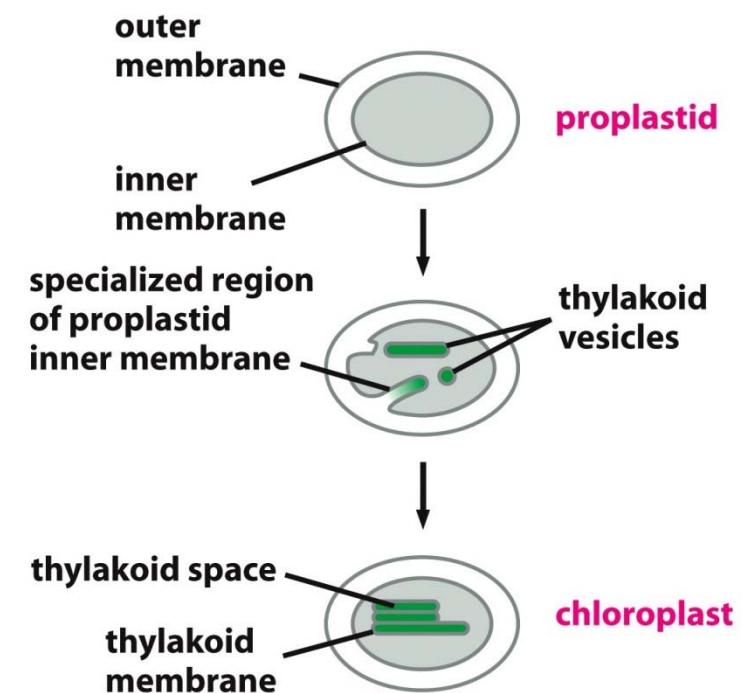
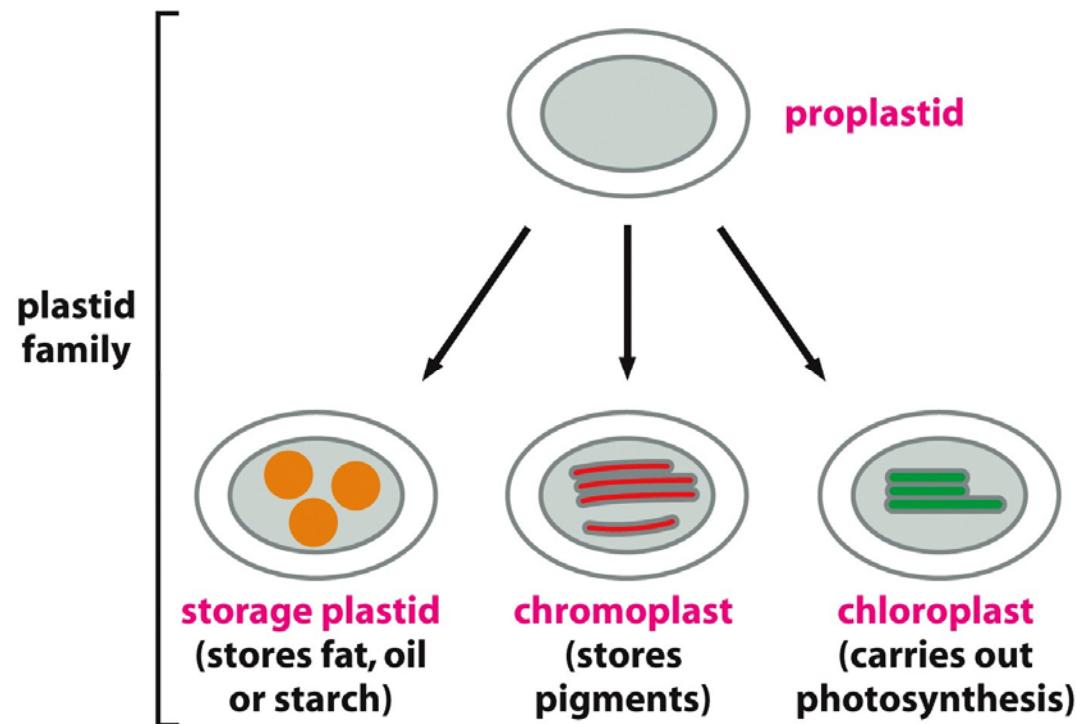
Shape change of a mitochondria
in a living cell during 20 minutes



Mitochondria are dynamic and
fuse and separate constantly



II. Plastids and evolution of chloroplast

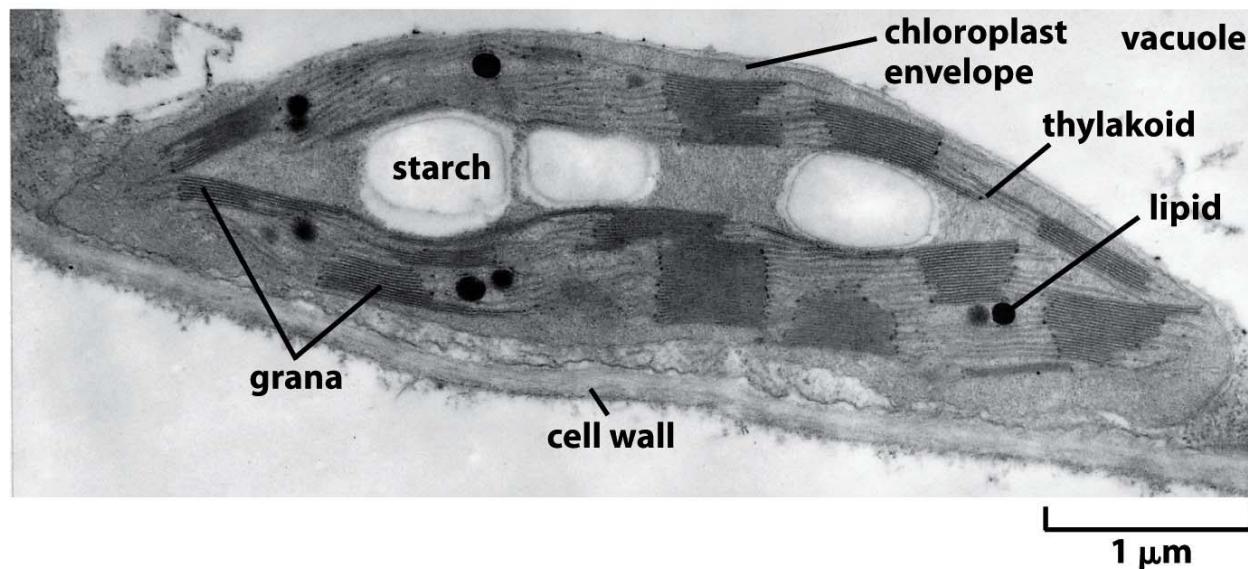
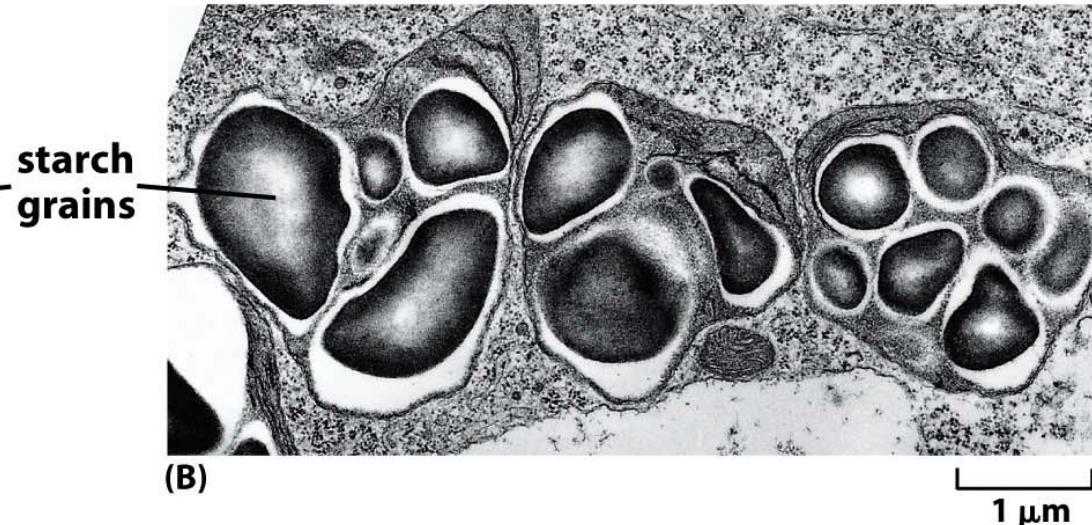
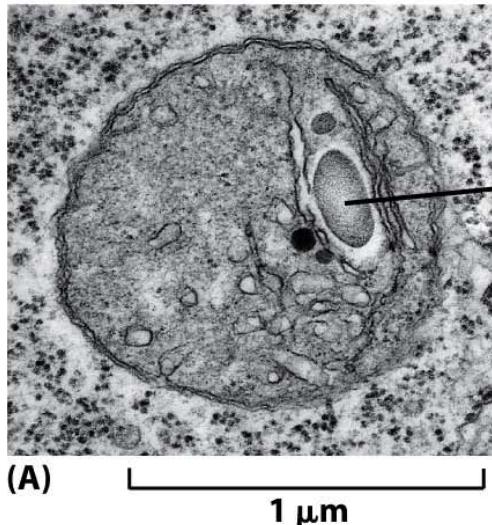


This is dependent on the different environment in differentiated cells

Plastid diversity

- In one plant species, all plastids share the same genome of their own.
- They are:
 1. most notable sites for photosynthesis
 2. storage of materials (oil, starch, proteins)
 3. provide compartments for synthesis of:
 - purine/pyrimidine
 - amino acids
 - fatty acids

Plastids, chloroplast



Chloroplast compartments

Outer membrane: highly permeable

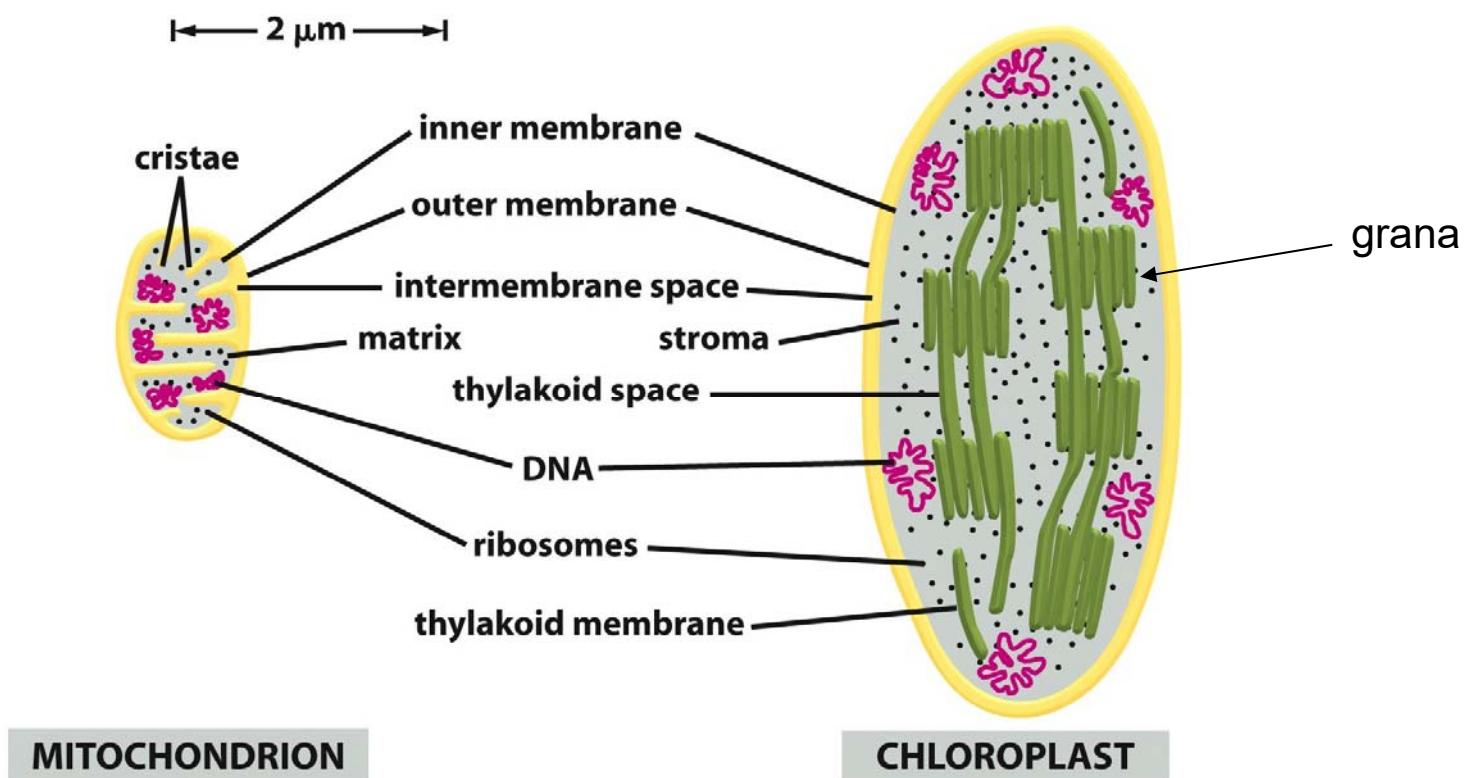
Inner membrane: much less permeable, localization of membrane transport proteins

Stroma: sites of metabolic enzymes, chloroplast ribosomes, RNAs, chloroplast DNA.

Thylakoid membrane: sites of electron-transport chains, photosynthetic light-capturing systems, ATP synthase

Thylakoid space: interconnected.

Both contains
multiple copies
of their genome
DNA molecules.



Mitochondria and chloroplast DNA in different cells

Table 14–2 Relative Amounts of Organelle DNA in Some Cells and Tissues

ORGANISM	TISSUE OR CELL TYPE	DNA MOLECULES PER ORGANELLE	ORGANELLES PER CELL	ORGANELLE DNA AS PERCENTAGE OF TOTAL CELLULAR DNA
MITOCHONDRIAL DNA				
Rat	liver	5–10	1000	1
Yeast*	vegetative	2–50	1–50	15
Frog	egg	5–10	10^7	99
CHLOROPLAST DNA				
<i>Chlamydomonas</i>	vegetative	80	1	7
Maize	leaves	0–300**	20–40	0–15**

*The large variation in the number and size of mitochondria per cell in yeasts is due to mitochondrial fusion and fission.

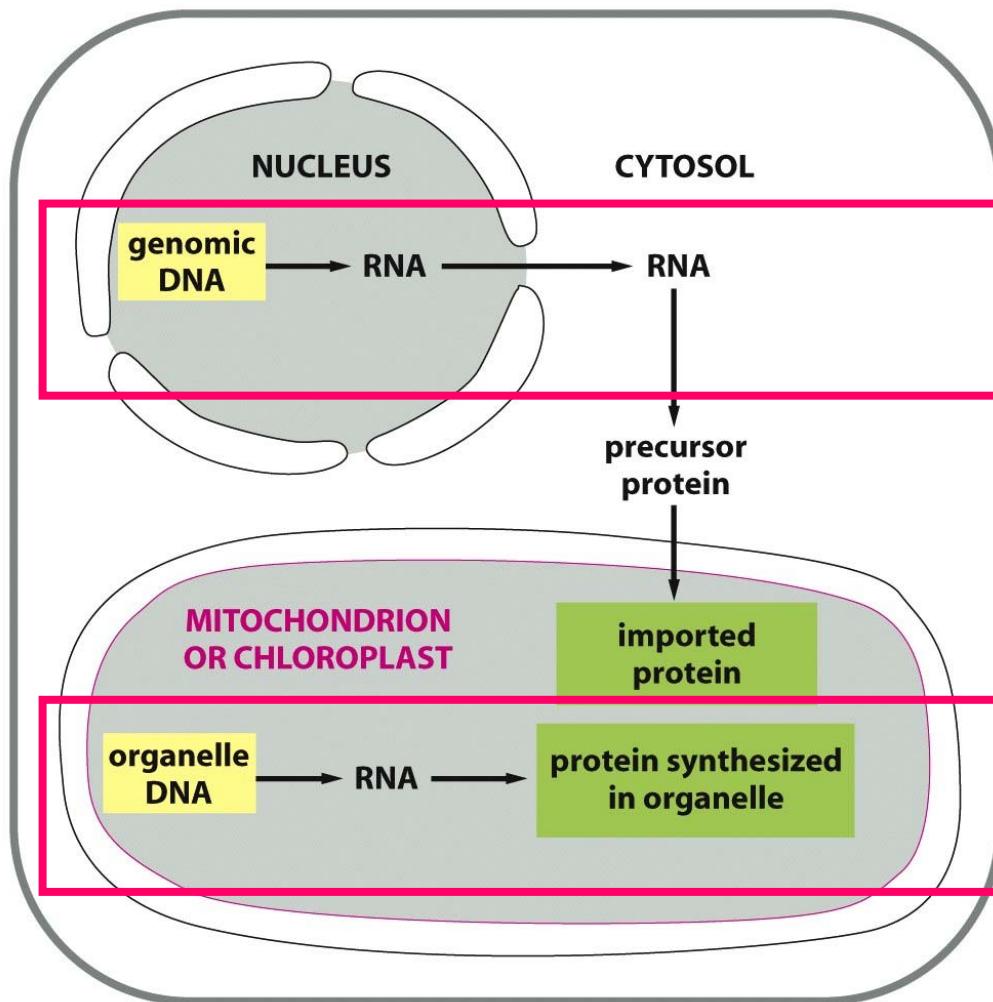
**In maize, the amount of chloroplast DNA drops precipitously in mature leaves, after cell division ceases: the chloroplast DNA is degraded and stable mRNAs persist to provide for protein synthesis.

The number of both chloroplast and mitochondrion can vary depending on physiological conditions and needs of the cells, they are dynamic.

III. Genetic systems of mitochondrion and plastid

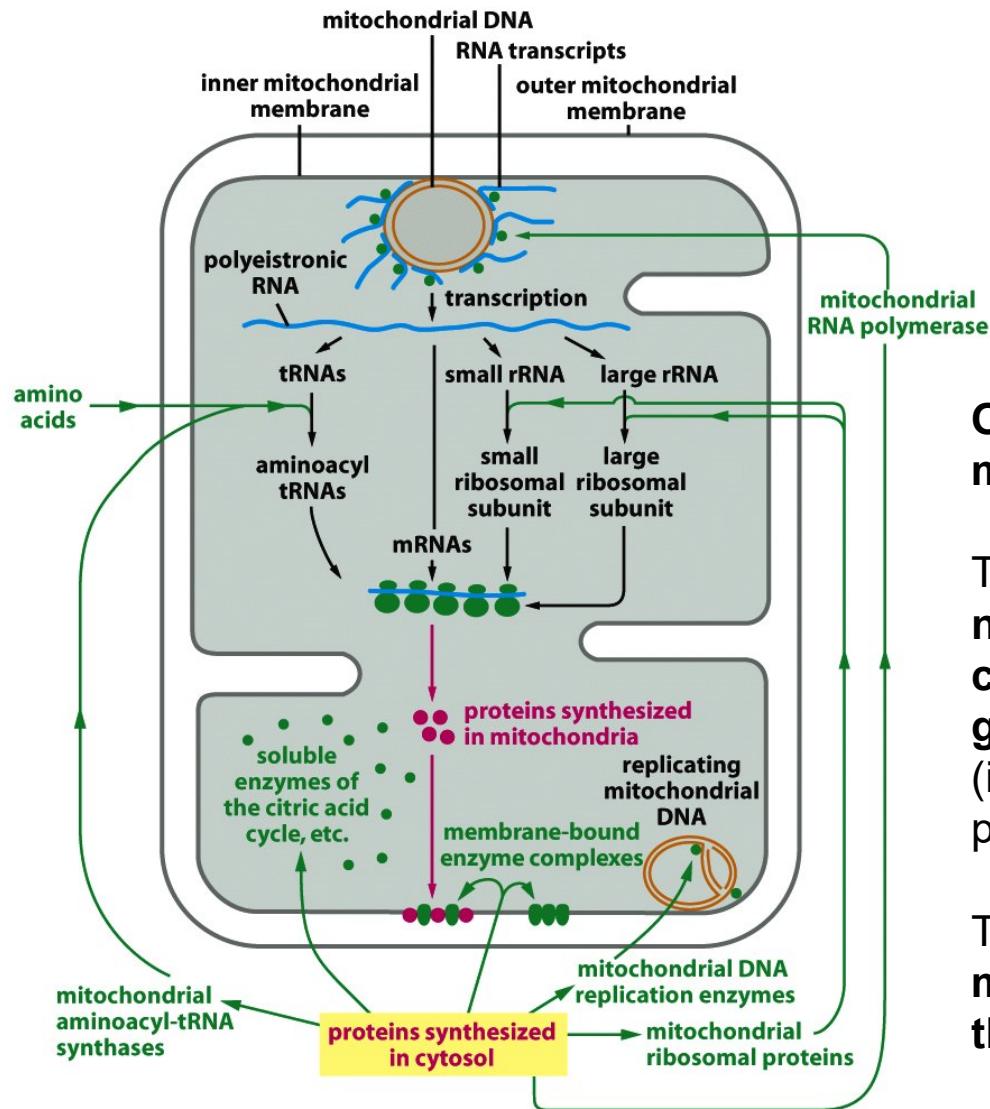
- Genetic system of mitochondria and chloroplasts
- Origin of mitochondria and chloroplast genomes
- Non-mendelian inheritance
- Diseases associated with mitochondria defects.

Mitochondrion and chloroplast contain their own complete genetic systems



Proteins in mitochondria and chloroplasts are produced by **two separate** genetic systems:
the nucleus
and
the mitochondrion/chloroplast

Coordinated protein synthesis between nucleus and mitochondria



Animal mitochondria contain the simplest genetic systems known

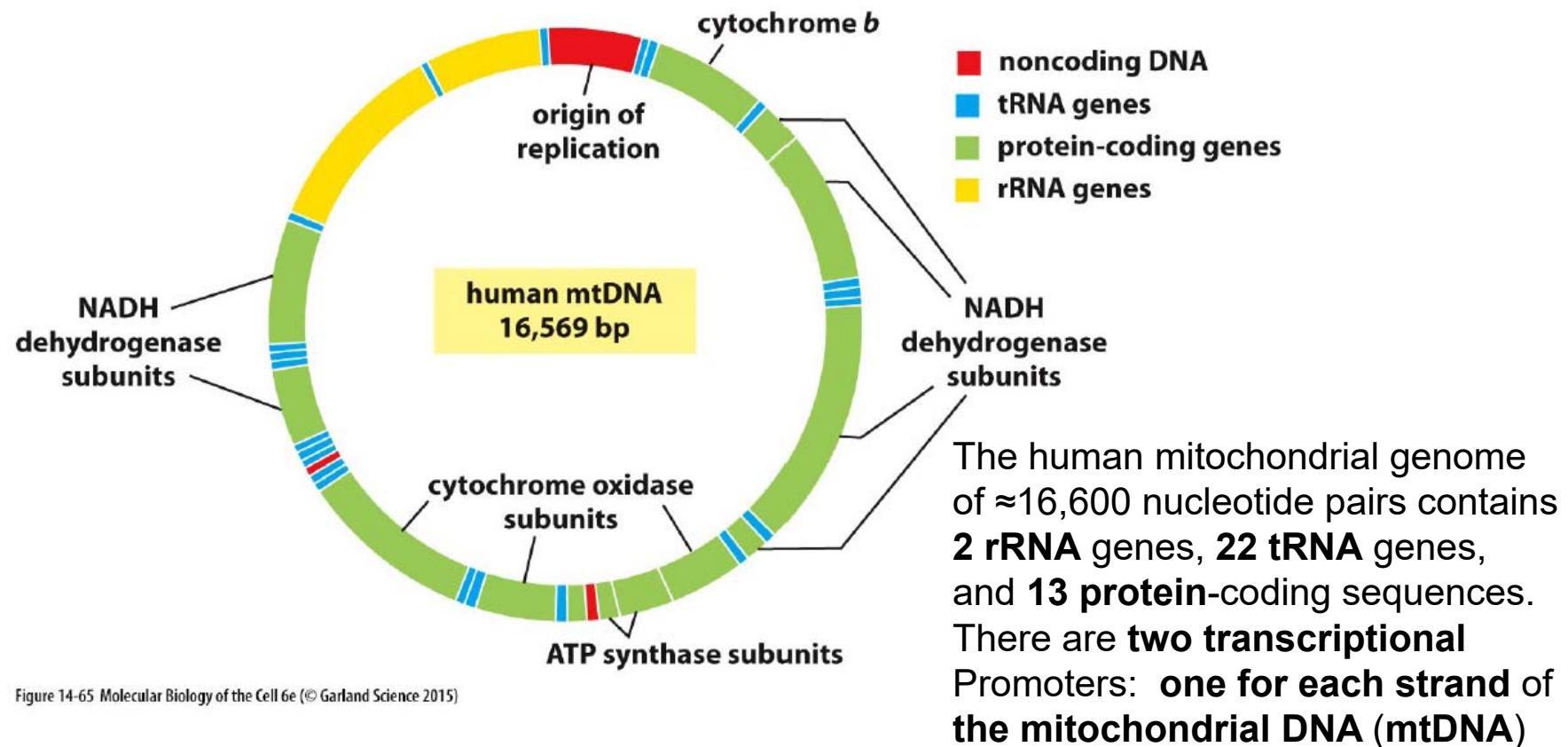
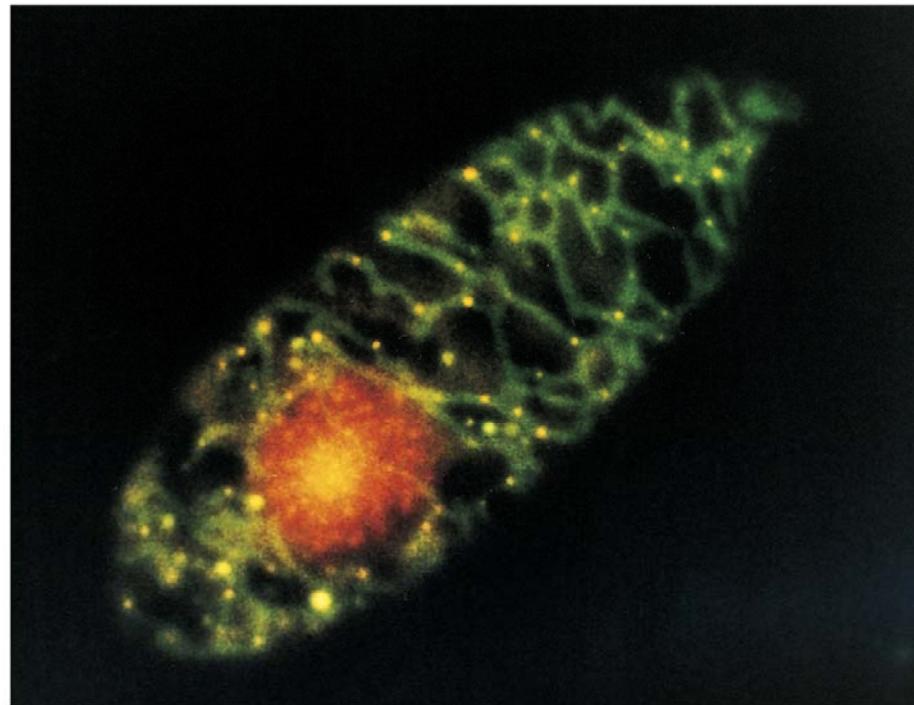


Figure 14-65 Molecular Biology of the Cell 6e (© Garland Science 2015)

Protein synthesis machinery from chloroplasts and mitochondria

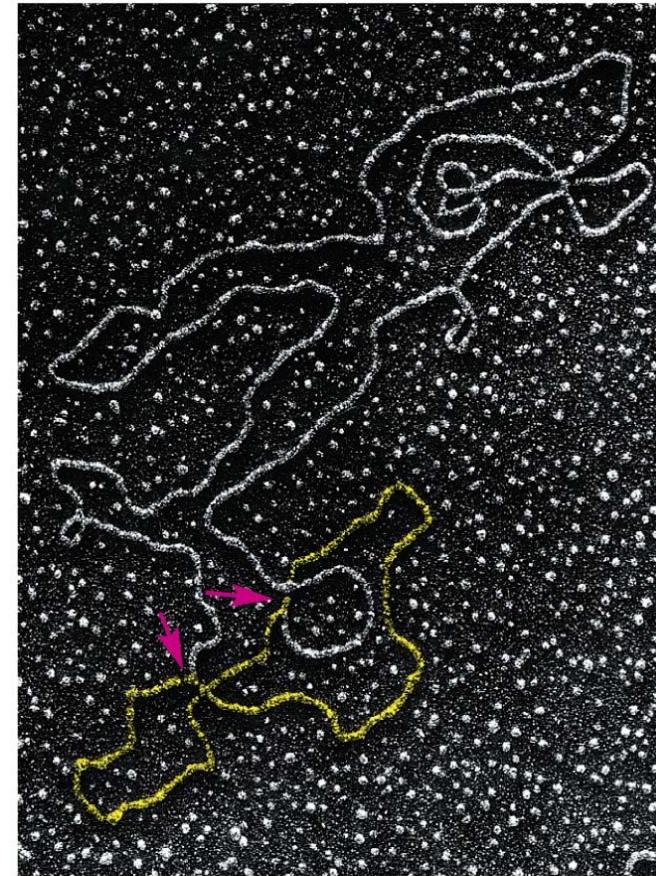
- **Chloroplast** more resembles **present-day bacteria** in its protein synthesis machinery
- Both chloroplast and mitochondrion use **N-formyl-Methionine (fMet)** as the **initiation amino acid** in protein synthesis.
- Protein synthesis in both chloroplast and mitochondrion can be **inhibited by antibiotics**.--- side effect of antibiotics in eukaryotic organisms?

Visualization of mitochondria DNA



DNA-EtBr- red
Mitochondrion matrix-green

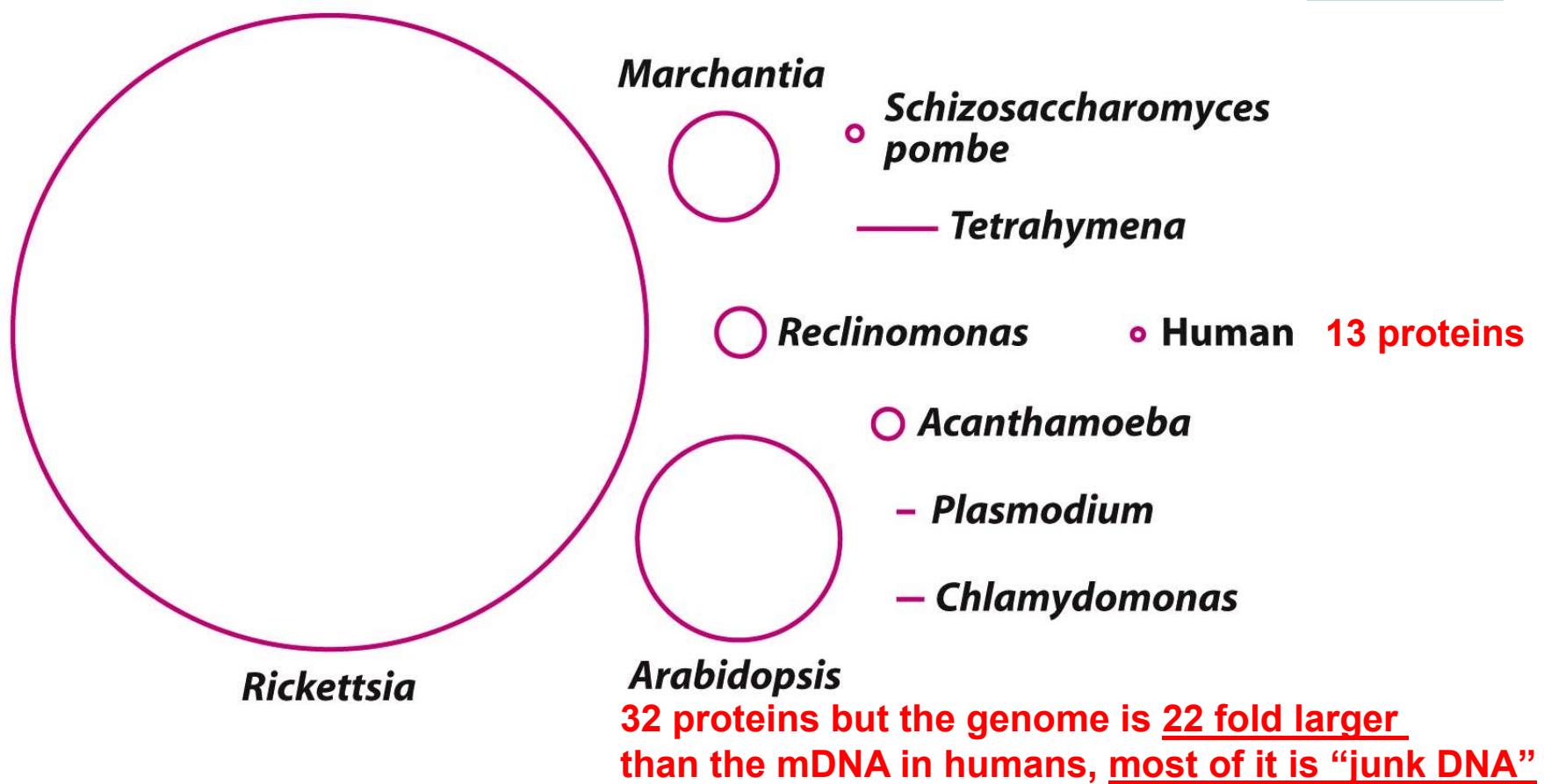
25 μm



1 μm

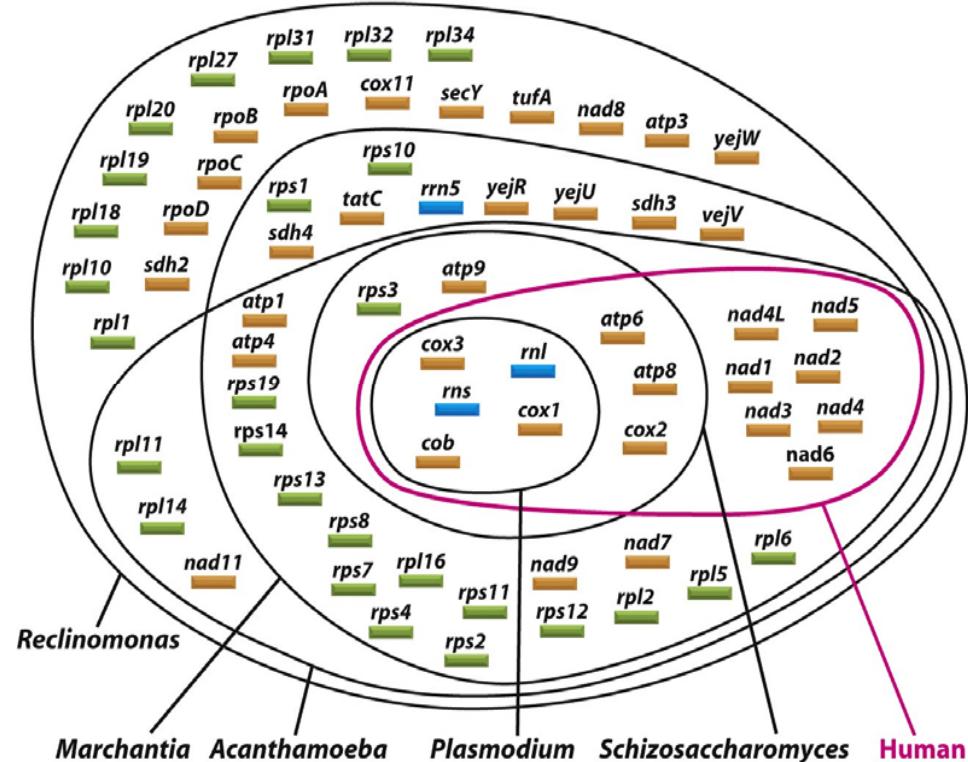
Circular mitochondria DNA is
Replicating, replication occurs
In a cell cycle dependent manner

Various sizes of mitochondria genomes



Generally range from 6000 bp to 300,000bp, for mammals, ~16500bp.
Some chloroplasts and mitochondria genomes are in linear form.

Gene transfer occurs after symbiosis events



Evidence to support this hypothesis:

1. Most of mitochondria and chloroplast proteins are encoded by nuclear genes.
2. Superoxide dismutase SOD in mitochondria more resembles that of bacteria than cytosolic SOD
3. Sequential reduction in mitochondria encoding genes during evolution

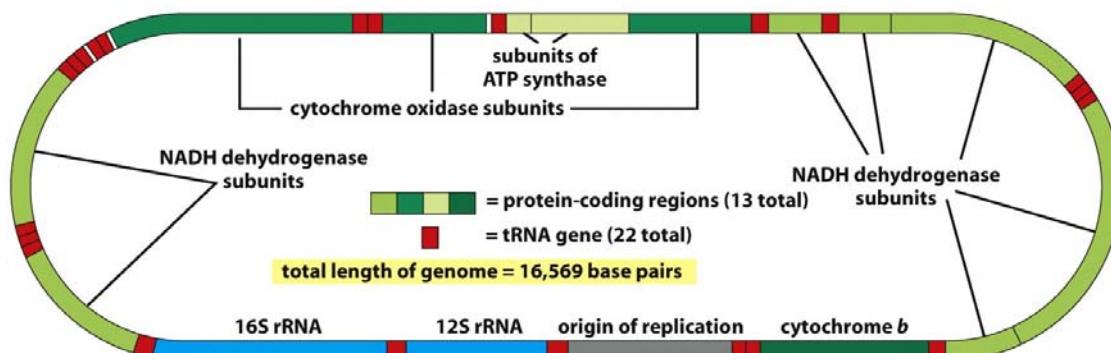
The **smallest and presumably most highly evolved mitochondrial genomes**, encode only a few hydrophobic inner-membrane proteins of the electron-transport chain, plus rRNAs and transfer RNAs (tRNAs).

Example humans: mtDNA encodes only 13 proteins but the nucleus encodes 1,000 mitochondrial proteins

Other genomes have **remained** more complex, and **contain this same subset of genes along with others**

Human mitochondria genome has its distinct features compared to other genomes

- Dense gene packing
(nearly all nucleotides are coding sequences)
- Relaxed codon usage
(22 tRNAs versus >30 tRNAs in cytosol, recognize **any one of the four nucleotides** in the **third (wobble) position**)
- Variant genetic code
(4 of the 64 codons have different “meanings”)



10X higher nucleotide substitution rate due to lower DNA replication fidelity and less efficient DNA repair.

Transcription in mitochondria is also special

- Occurs from **one promoter** but synthesis is in **both directions** with **both DNA strands** as templates.
- The **mRNA** has **no 5'-cap** but has **poly A tail**.
- Some plant and fungi mitochondria RNA has **introns**, as well as for some plant chloroplast, **introns are spliced out** in a **RNA-mediated catalysis** or facilitated by proteins.

Variant genetic code in mitochondria

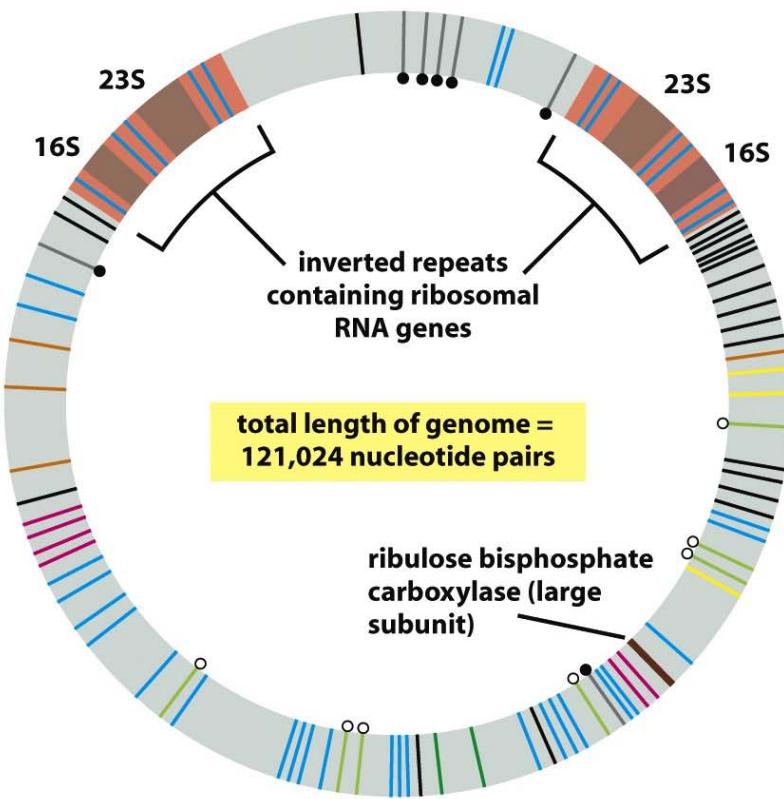
Table 14–3 Some Differences Between the “Universal” Code and Mitochondrial Genetic Codes*

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.

How to explain mitochondria genetic code is different from universal genetic code?

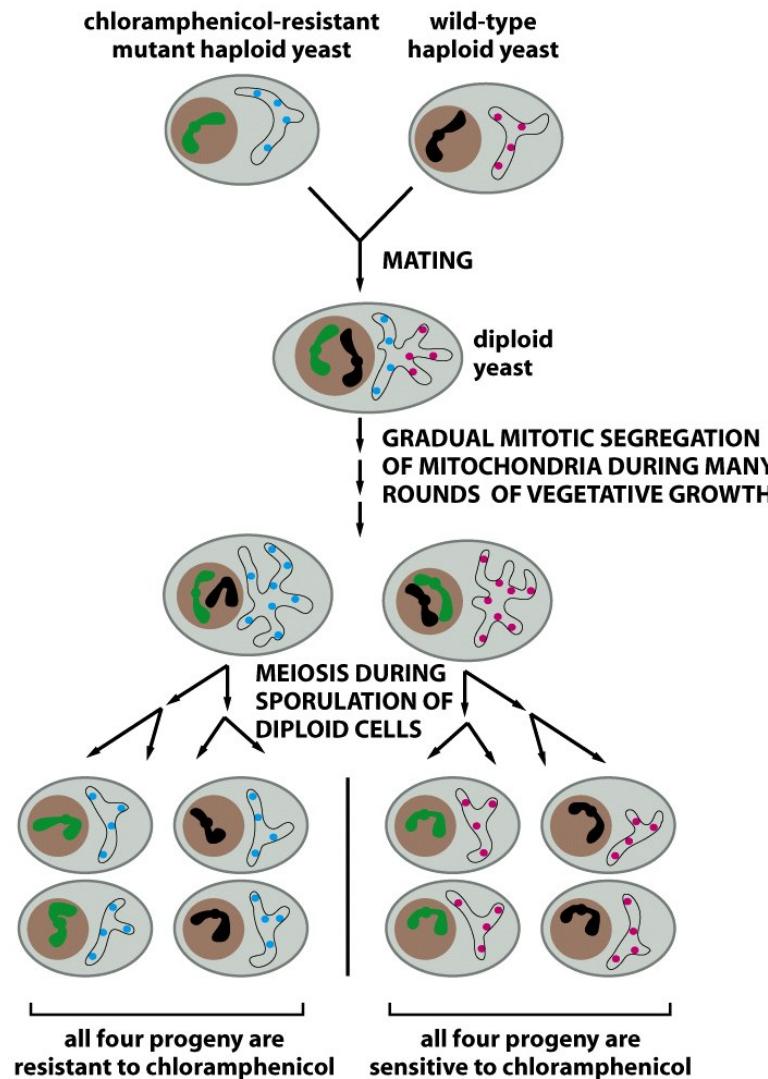
Genes in chloroplast genomes, all are highly similar



Higher plants have ~ 120 genes

- KEY:
- tRNA genes
 - ribosomal protein genes
 - photosystem I genes
 - photosystem II genes
 - ATP synthase genes
 - genes for *b*₆-*f* complex
 - RNA polymerase genes
 - genes for NADH dehydrogenase complex

Mitochondrial genes are inherited by a non-mendelian mechanism



Two haploid yeast cells mate, they are **equal** in size and **contribute equal amounts of mitochondrial DNA** to the **diploid zygote (biparental inheritance)**

During the course of the subsequent asexual, vegetative growth, the mitochondria become distributed more or less randomly to daughter cells.

After a few generations, the mitochondria of any given cell contain only the DNA from one or the other parent cell, because only a small sample of the mitochondrial DNA passes from the mother cell to the bud of the daughter cell. This process is known as *mitotic segregation*, and it gives rise to a distinct form of inheritance that is called **non-Mendelian, or cytoplasmic inheritance**

Non-mendelian inheritance--- cytoplasmic inheritance

- When diploid cells that have segregated their mitochondrial genomes in this way undergo meiosis to form four haploid daughter cells, each of the four daughters receives the same mitochondrial genes.
- The definition of mitotic segregation:
One daughter cell with more inheritance of one allele would enrich for this allele in the subsequent mitotic divisions.

Organelle genes are maternally inherited in many organisms

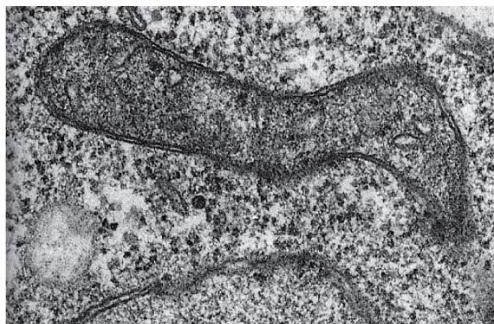
- Human zygote has most maternal mitochondria.
- 2/3 of higher plants have maternal chloroplasts.
- Inheritance in yeast is bi-parental.

Myoclonic epilepsy and ragged red fiber disease (MERRF)

- Mutation in one of the mitochondria tRNA gene, a defect in making electron transport protein which results in defects in ATP synthesis: most notably on muscle and nerve cells. Muscle weakness, epilepsy, etc.

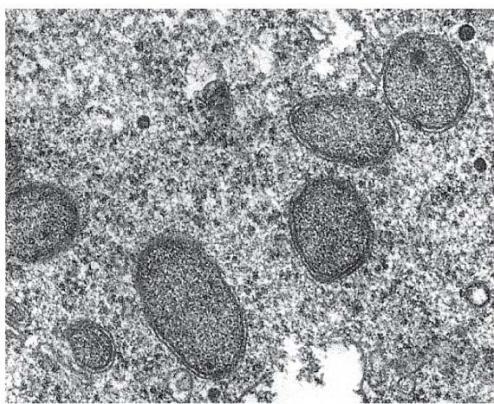
Petite mutants for mitochondria and chloroplasts

- Mutants with deletion of majority of mitochondria or chloroplasts DNA



(A)

These mutants have defect in synthesizing ATP and lack inner membrane cristae.



(B)

However, mitochondria has normal outer and inner membrane And can even perform DNA replication, etc.

Supporting that nucleus is sufficient to support mitochondria Biogenesis .

1 μm

Different lipid origins in mitochondria and chloroplast

- Mitochondria: most imported from ER
- Chloroplast: most synthesized on its own

Mitochondria and aging

- High rate of superoxide due to error in electron transfer to oxygen
- High percentage of damage on mitochondria and in turn on the cells

IV Energy Conversion

- Energy conversion in mitochondria
- Energy conversion in chloroplast

Electronic transfer in battery

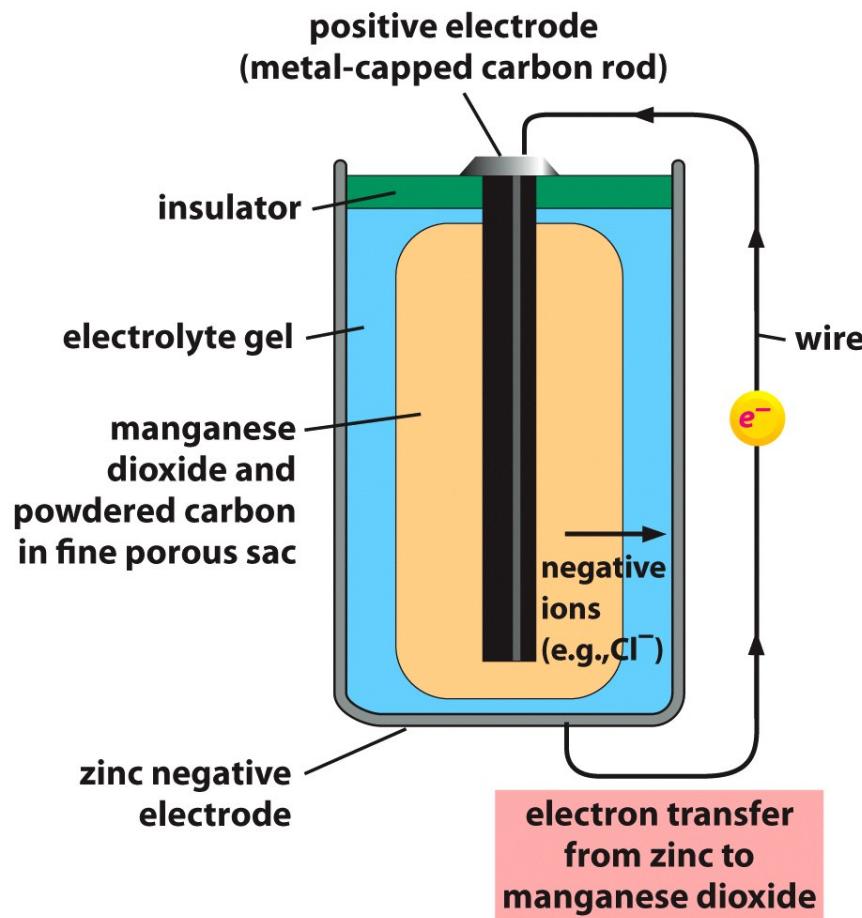


Figure 14-2a Essential Cell Biology 3/e (© Garland Science 2010)

Electronic energy can be consumed into heat

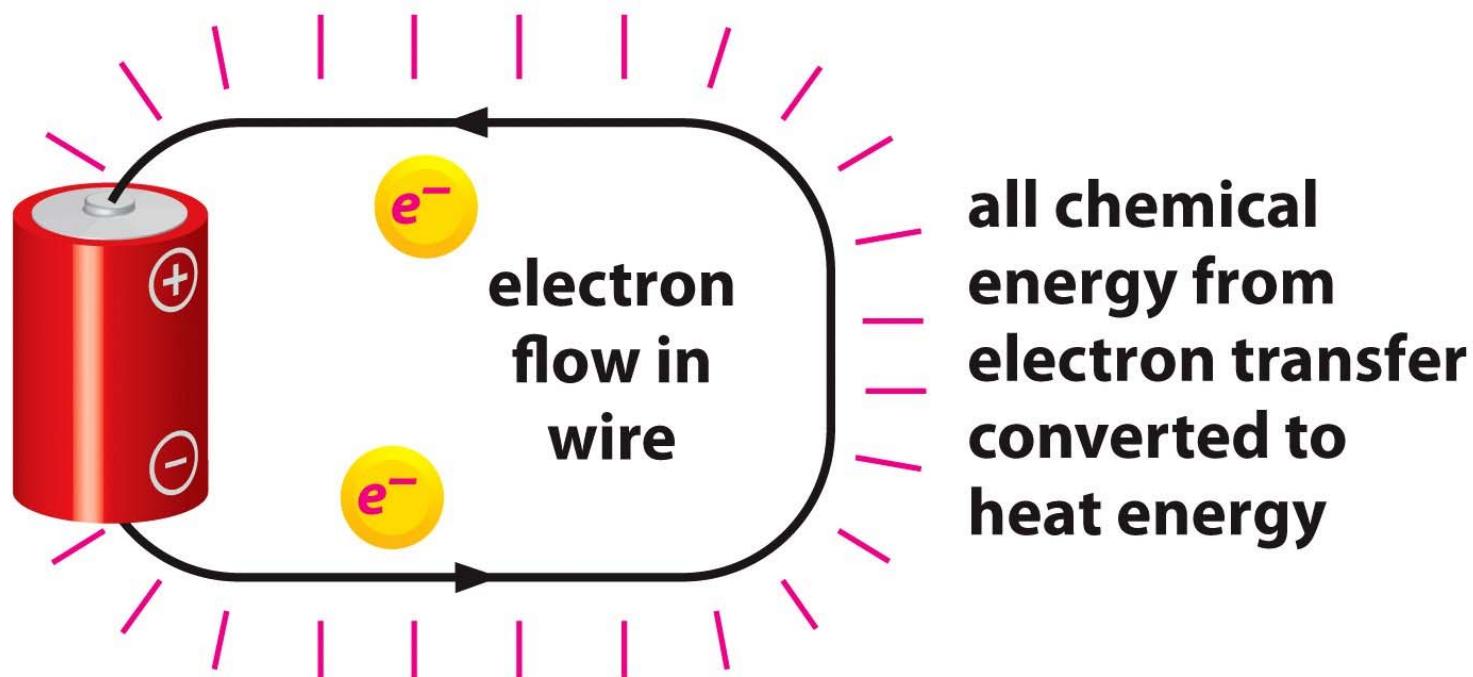
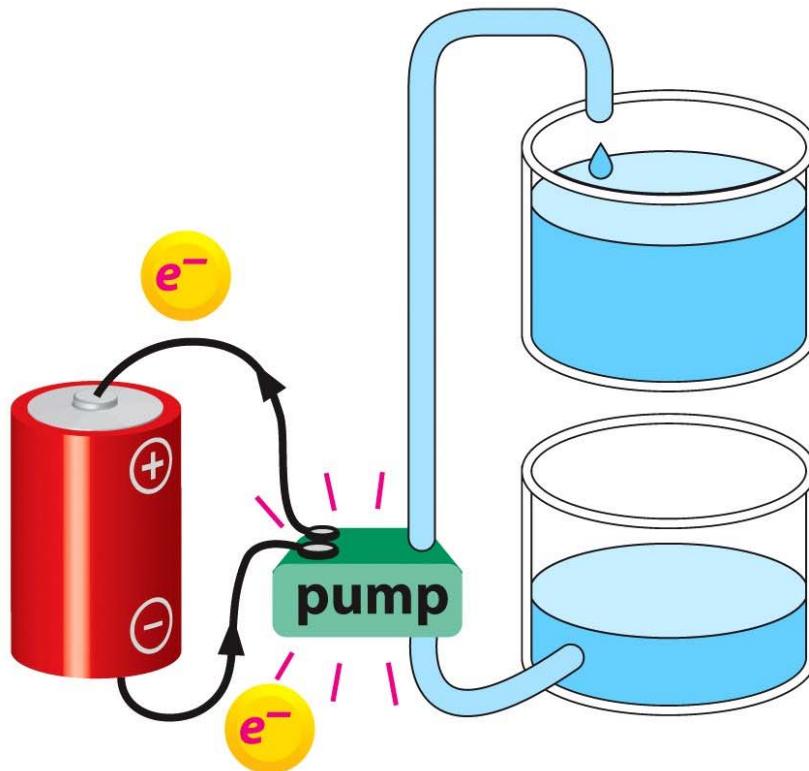


Figure 14-2b Essential Cell Biology 3/e (© Garland Science 2010)

Electronic flow converts into other forms of energy



chemical energy from electron transfer converted to the potential energy stored in a difference in water levels; less energy is therefore lost as heat energy

Figure 14-2c Essential Cell Biology 3/e (© Garland Science 2010)

Systems to provide energy for lives

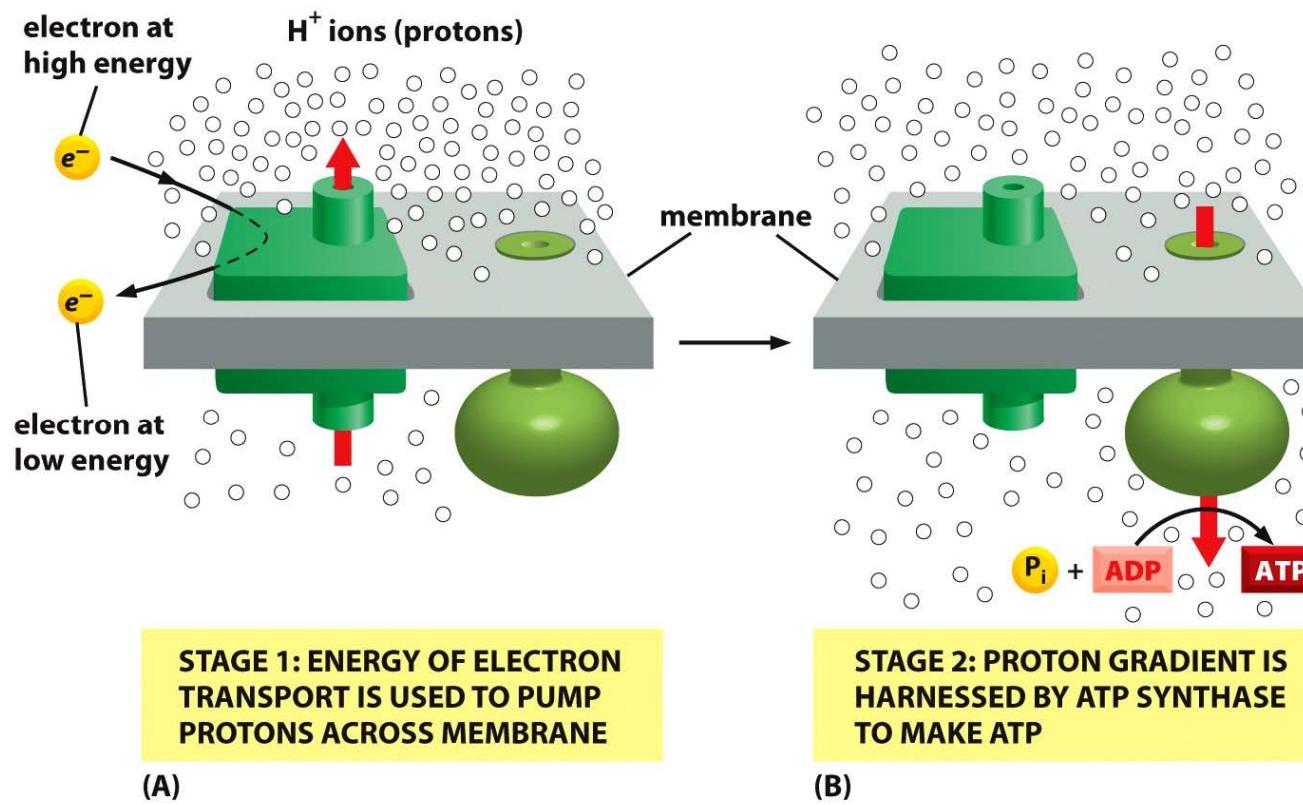


Figure 14-1 Essential Cell Biology 3/e (© Garland Science 2010)

How are the electrons generated? Release of a hydride ion!

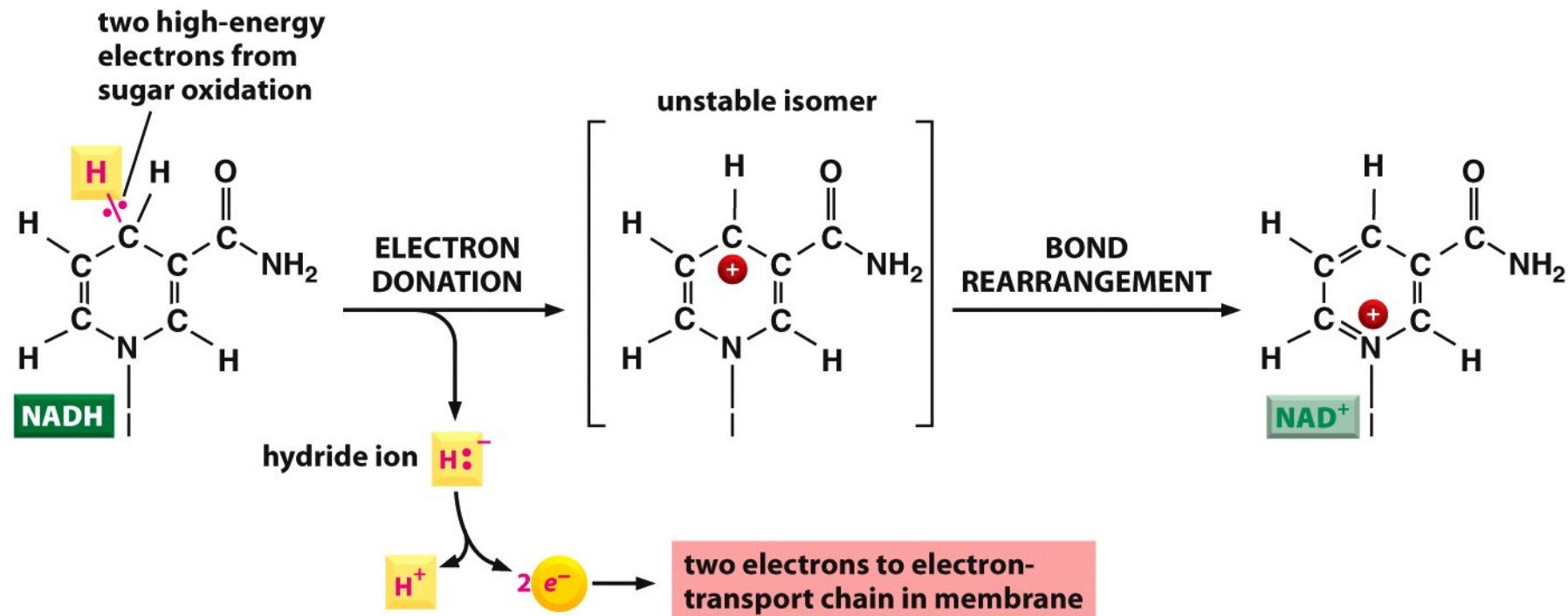
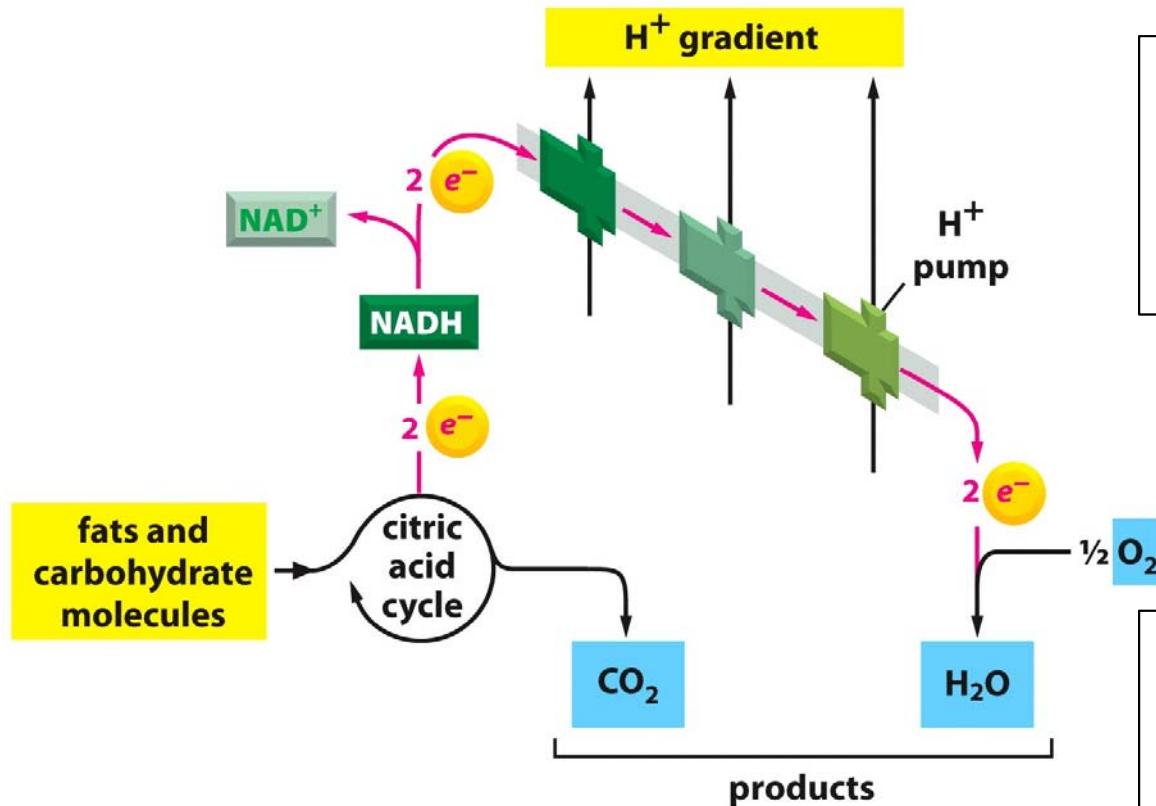


Figure 14-5 Essential Cell Biology 3/e (© Garland Science 2010)

NADH and FADH₂ carry high energy electrons

Electrons are transferred by the electron transfer chain (ETC)



The movement of electrons (e^-) is coupled to the pumping of Protons (H^+)

Electrons (e^-) are finally transferred to molecular oxygen to form H_2O

All happens in the inner membrane of mitochondria

Figure 14-6 Essential Cell Biology 3/e (© Garland Science 2010)

Three respiratory complexes receive high-energy electrons and pump protons into the IMS

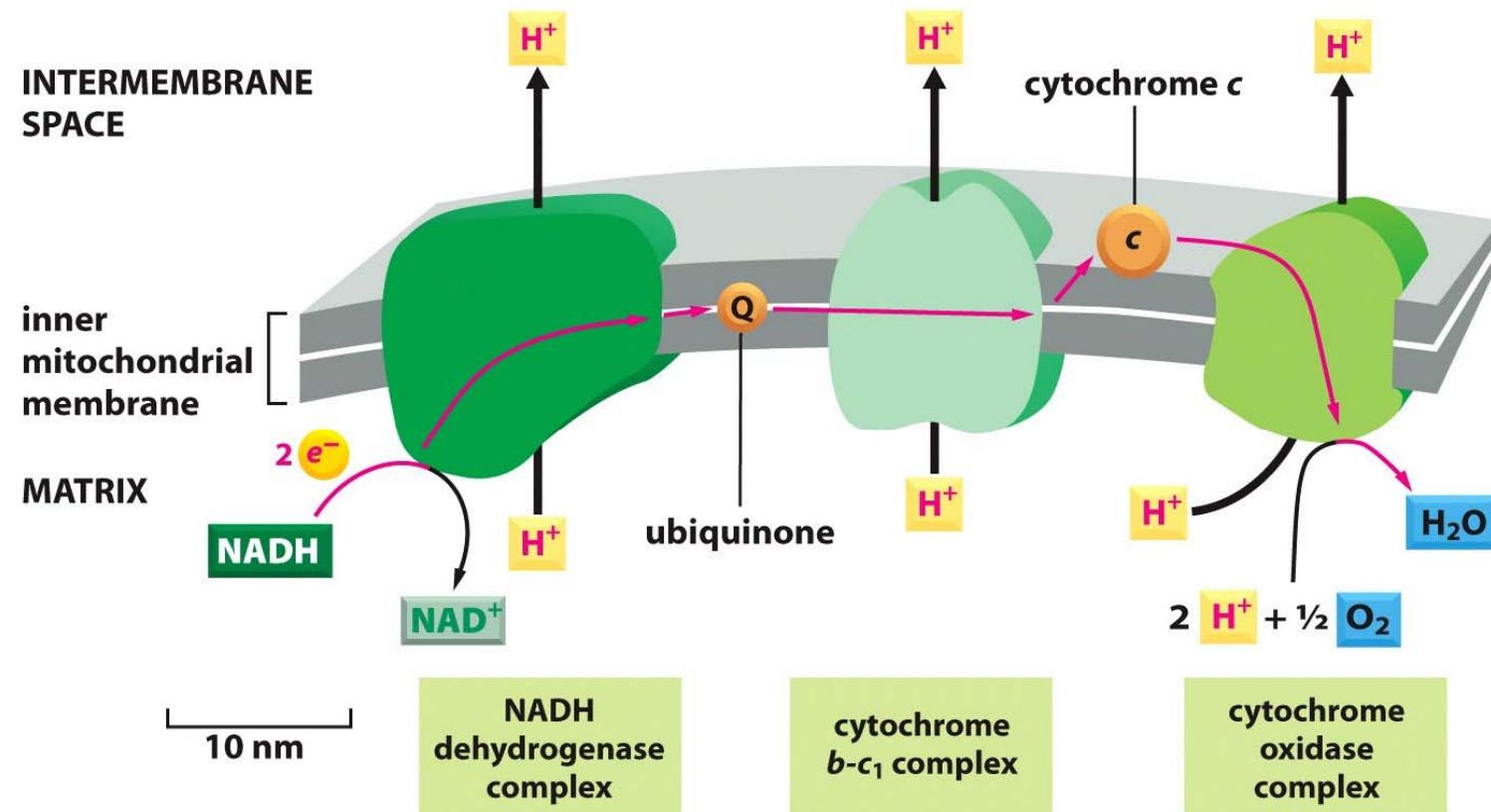


Figure 14-9 Essential Cell Biology 3/e (© Garland Science 2010)

Ubiquinone (Q) and cytochrome c (c) serve as mobile carriers that ferry electrons from one complex to the next.

The Q cycle

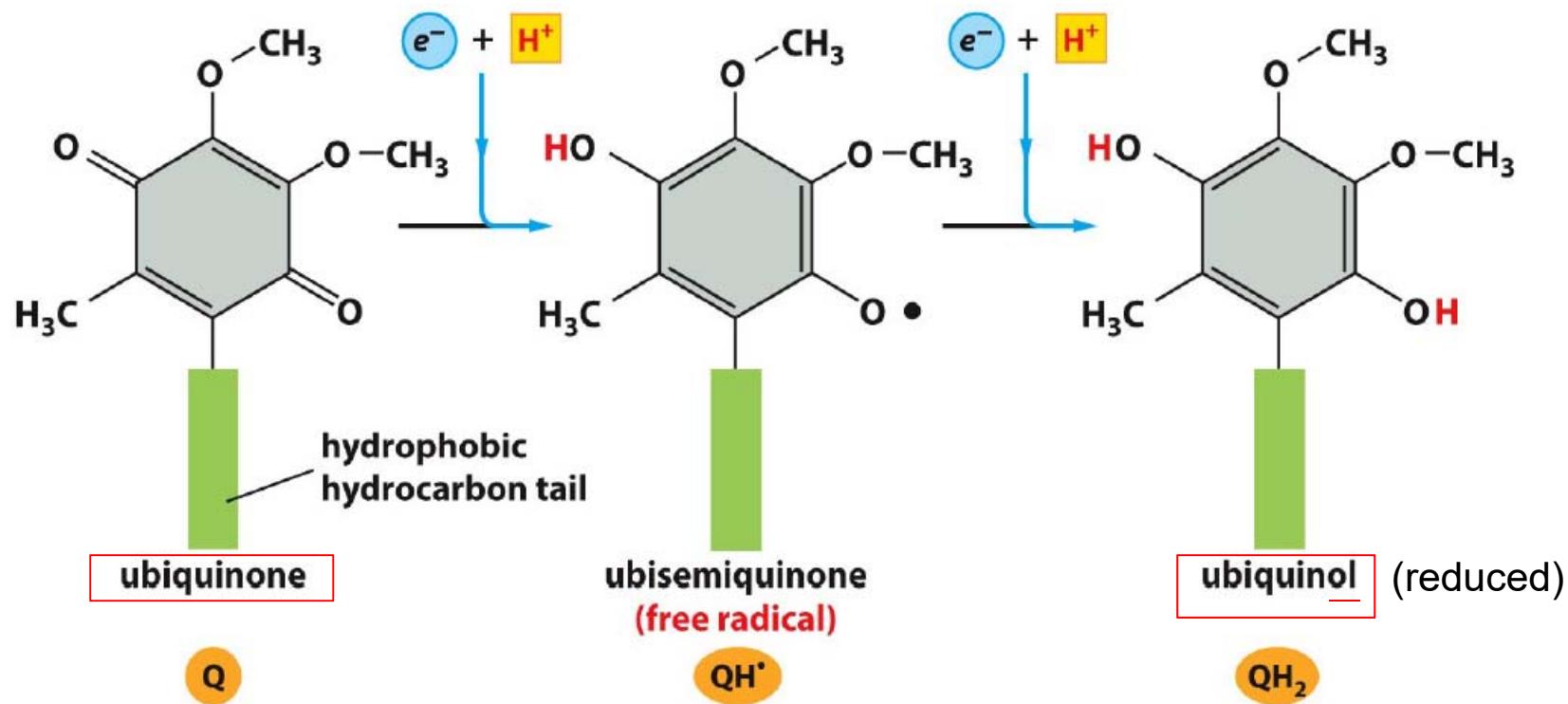


Figure 14-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

- New discovery revealed that the Q-cycle might not exist...

Proton is pumped back coupled with ATP synthesis

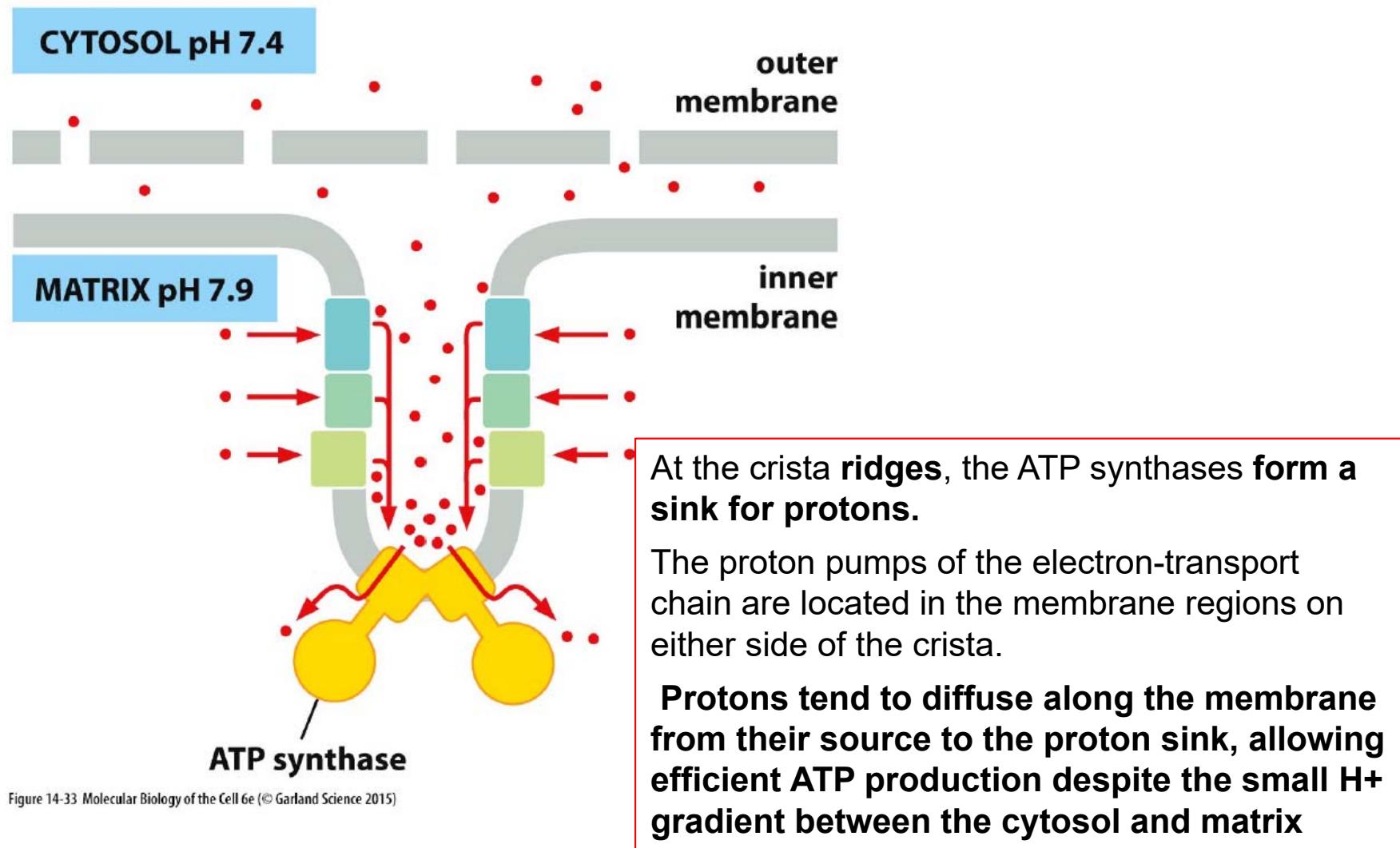
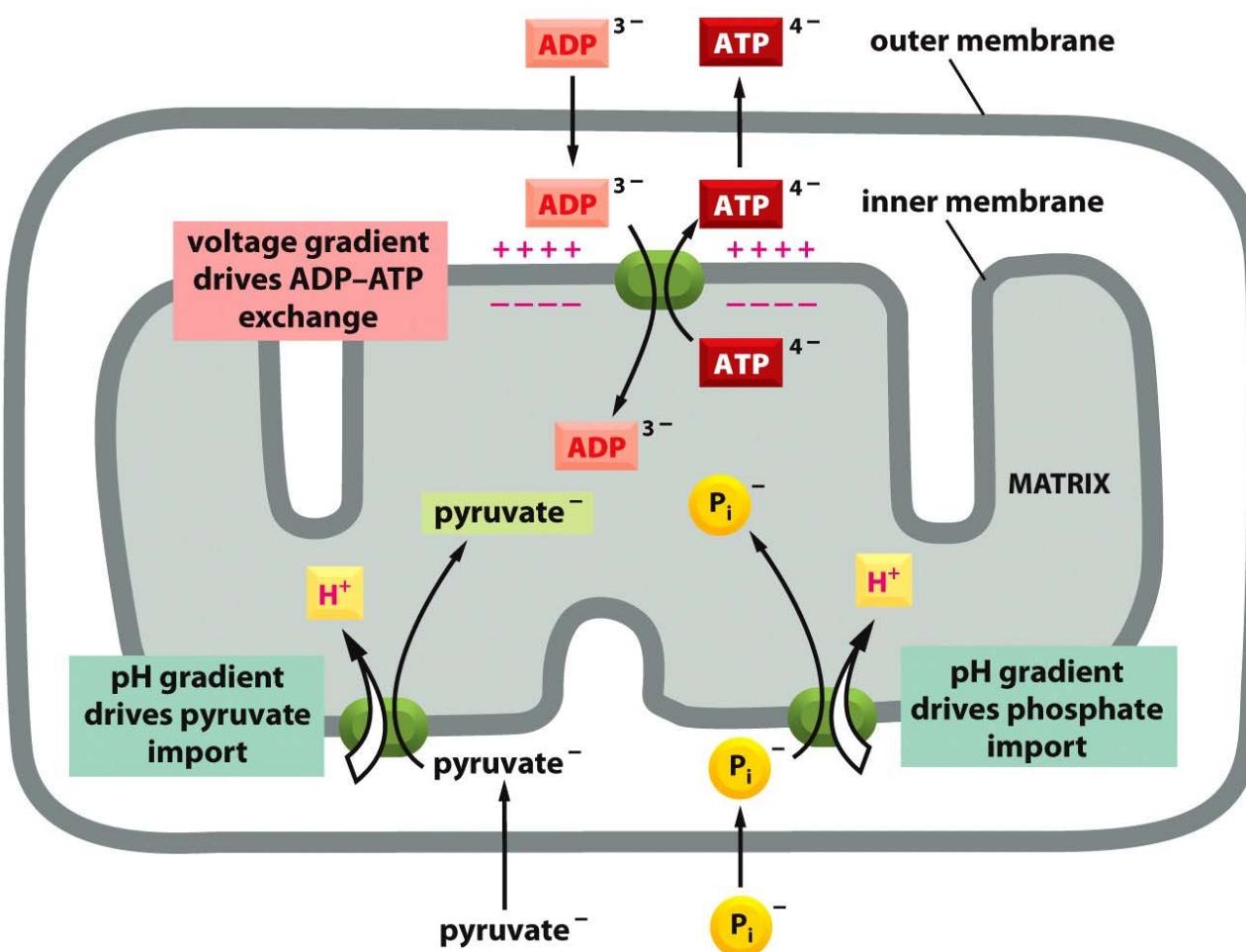


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ADP and Pi are transported in by electrochemical potential and proton gradient

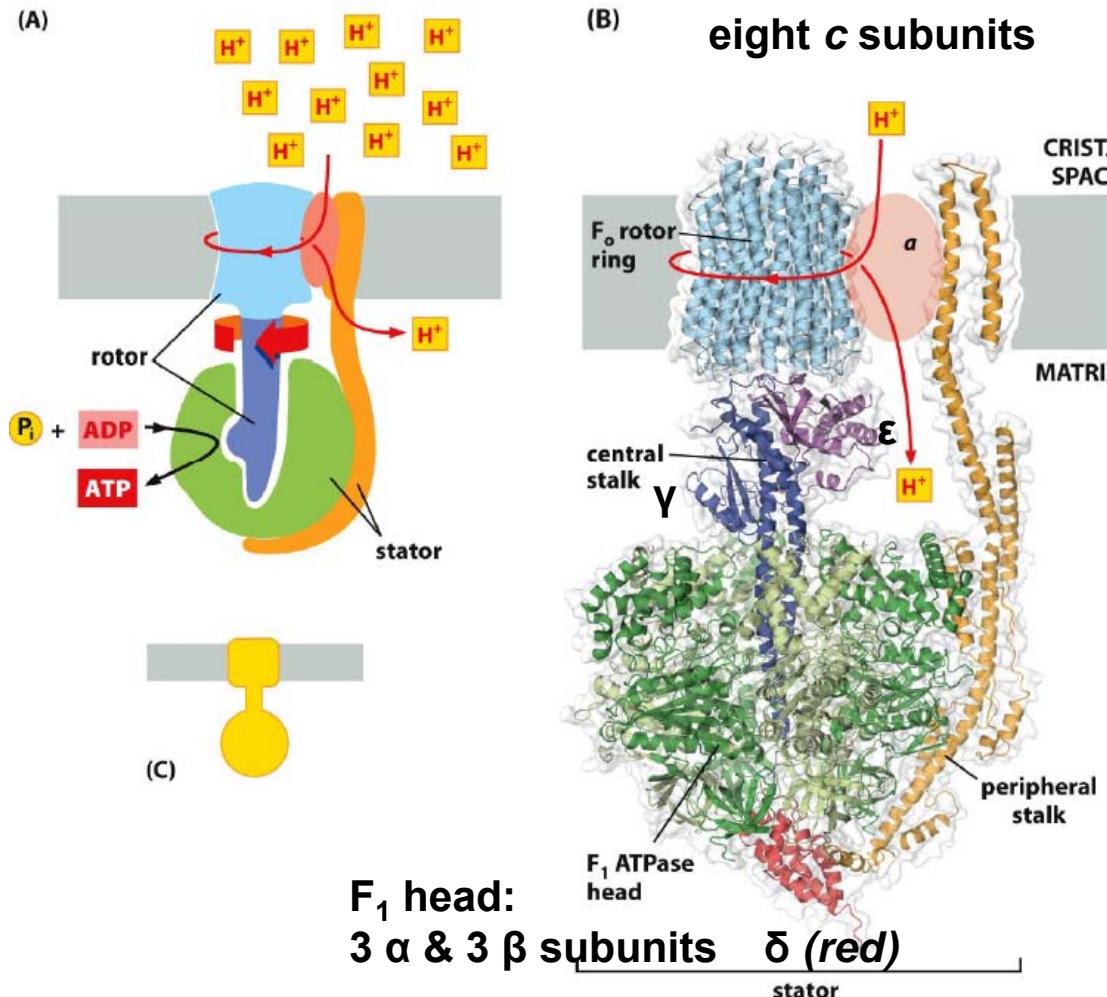
The electrochemical proton gradient across the inner mitochondrial membrane drives coupled transport processes



ADP is pumped into the matrix and ATP is pumped out by an antiport process that **uses the voltage gradient** across the membrane to drive transport

Pyruvate and inorganic phosphate (Pi) are moved **along with protons** down their electrochemical gradient

ATP synthesis by the F_1F_o ATP synthase (F-type ATPase)



Together with the c subunits of the ring rotating past it, the a subunit creates a path for protons through the membrane.

The protonmotive force across a membrane powers the rotation of the rotor (c) against the stator in a counterclockwise direction. The same enzyme complex can also pump protons against their electrochemical gradient by hydrolyzing ATP, which then drives the clockwise rotation of the rotor.

The torque produced by the ATP synthase during ATP hydrolysis is 60 times more powerful than a diesel engine of equal dimensions

Chloroplasts capture energy from sunlight to fix carbon for the synthesis of sugars, amino acids and fatty acids

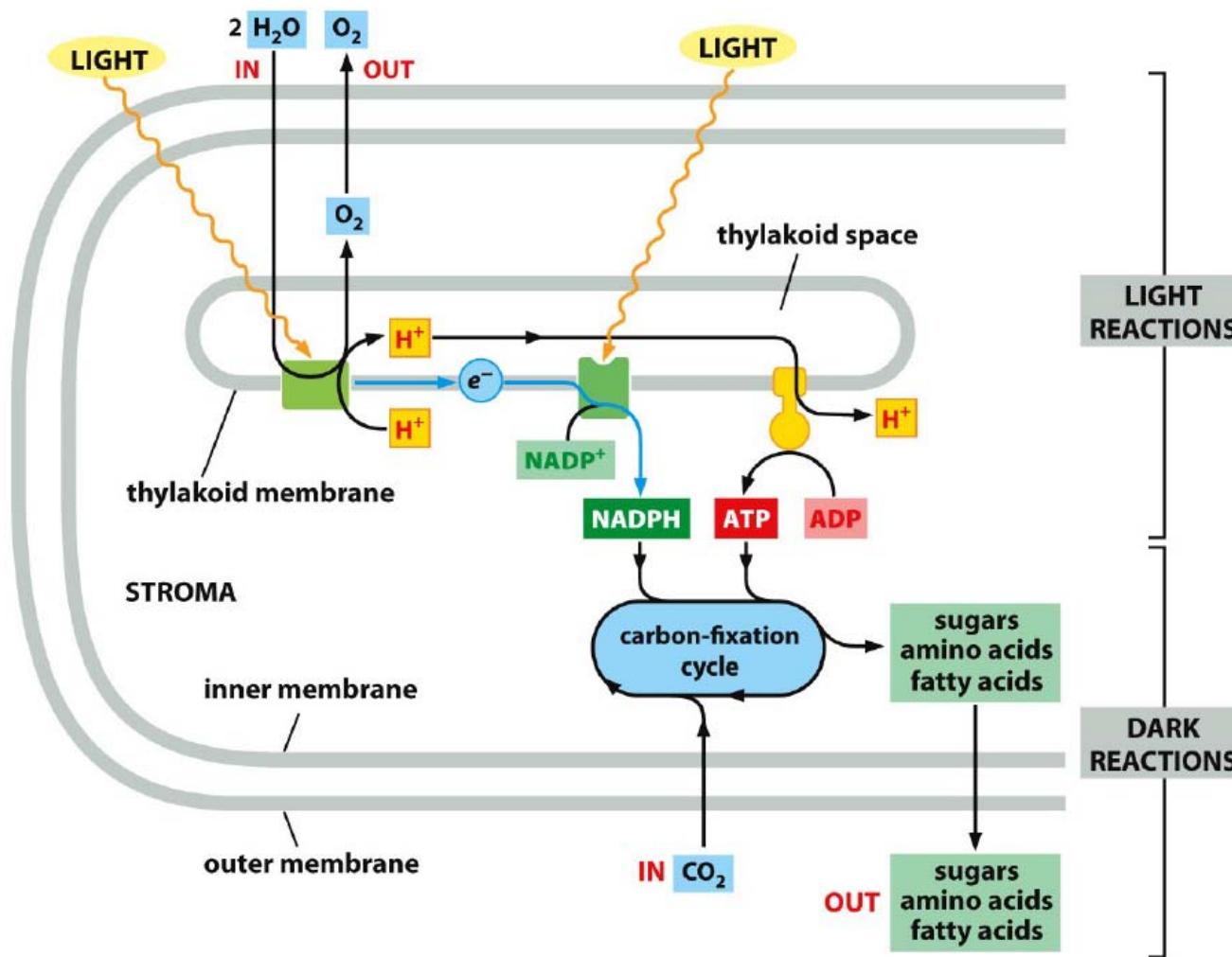


Figure 14-39 Molecular Biology of the Cell 6e (© Garland Science 2015)

Thylakoid membrane contains two different photosystems

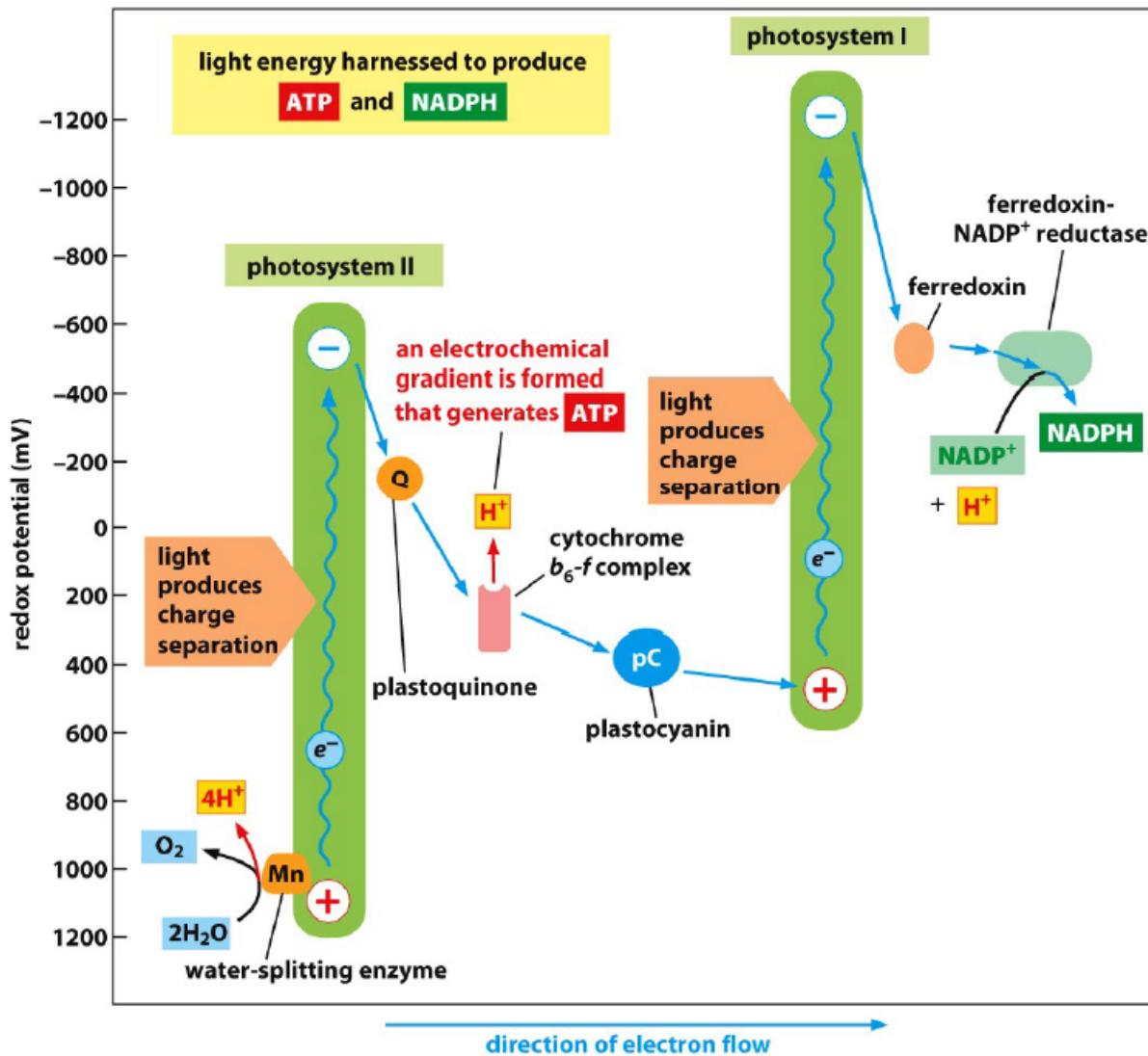
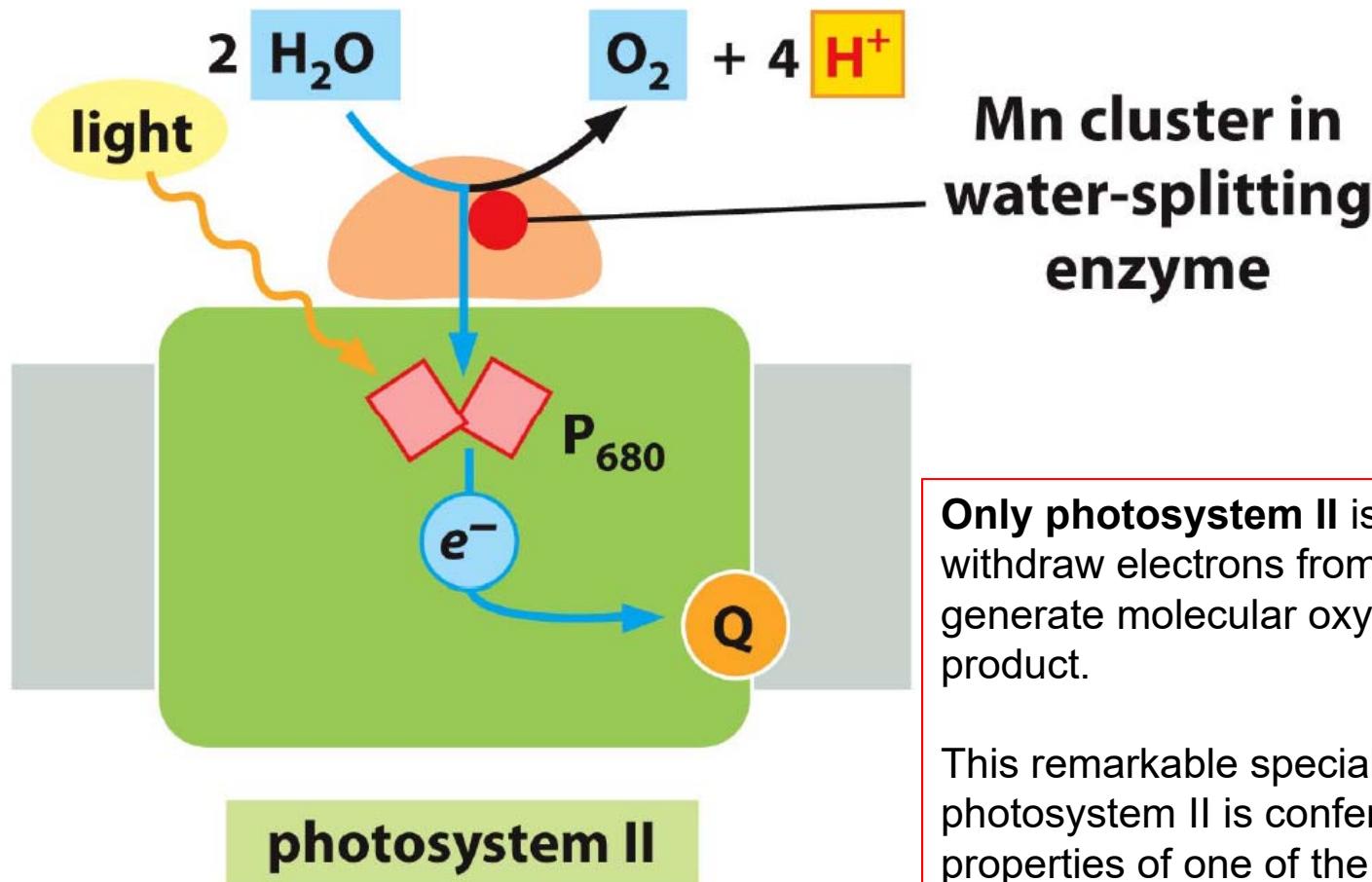


Figure 14-47 Molecular Biology of the Cell 6e (© Garland Science 2015)

PS II uses Manganese cluster to withdraw electrons from water



Only photosystem II is able to withdraw electrons from water and to generate molecular oxygen as waste product.

This remarkable specialization of photosystem II is conferred by the unique properties of one of the two chlorophyll molecules of its special pair and by a *manganese cluster* linked to the protein.

Cytochrome *b6-f* system connects PS II to PS I

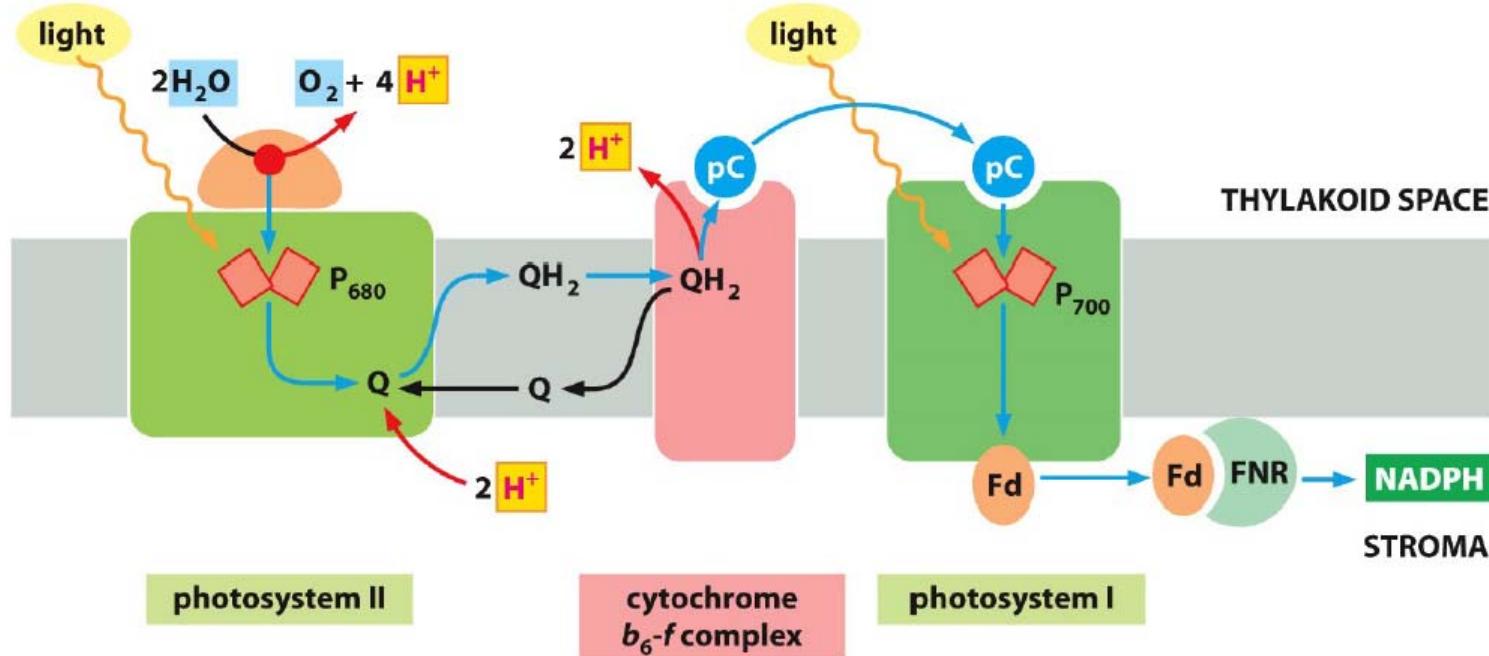


Figure 14-50 Molecular Biology of the Cell 6e (© Garland Science 2015)

The *b6-f* complex receives its electrons from a quinone and engages in a Q cycle that pumps two protons across the membrane . It hands its electrons to plastocyanin (pC), which diffuses along the membrane surface to photosystem I and transfers the electrons via ferredoxin (Fd) to the ferredoxin-NADP⁺ reductase (FNR), where they are utilized to produce NADPH

ATP and NADPH are used to convert CO₂ into sugar

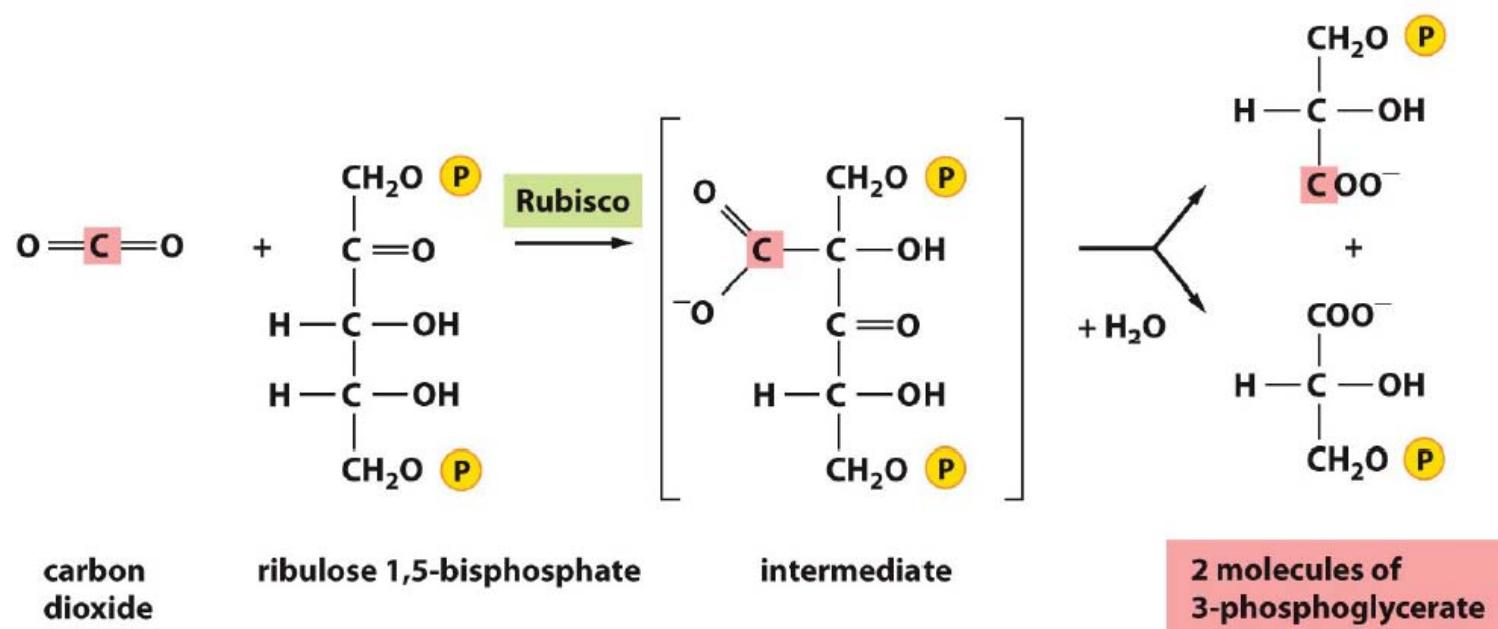


Figure 14-40 Molecular Biology of the Cell 6e (© Garland Science 2015)

ATP and NADPH are used to convert CO₂ into sugar

