Lecture 8

Mitochondria & chloroplasts

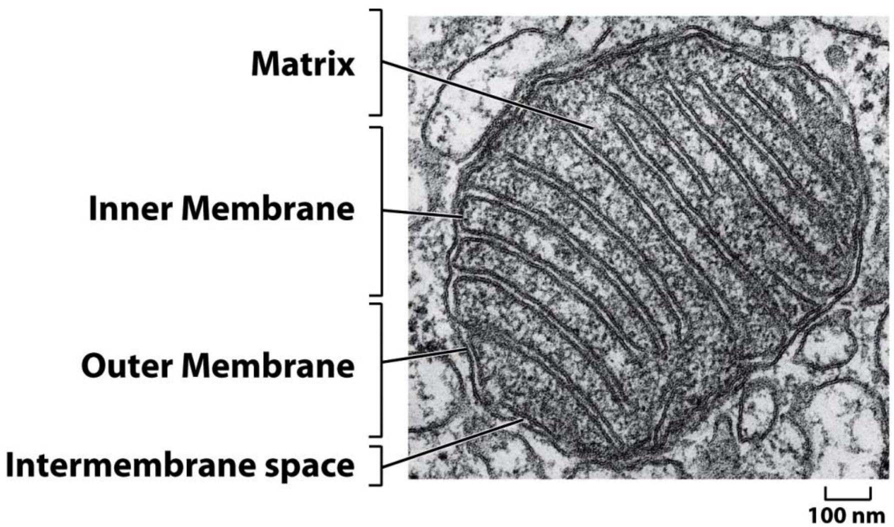
Outline

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

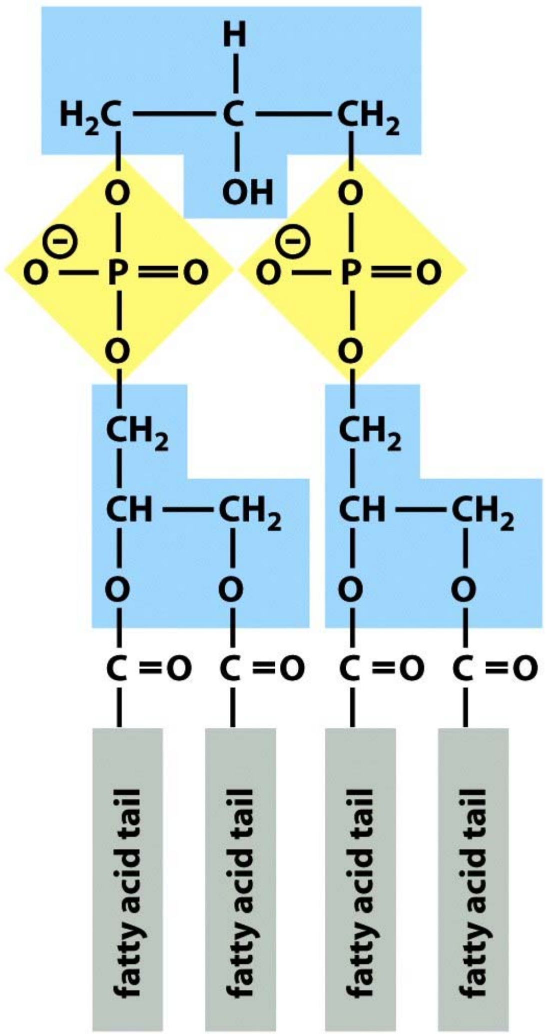
I. Mitochondria

- **Outer membrane** contains porins and is highly permeable to molecules 5kDa 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

Four compartments in mitochondrion



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



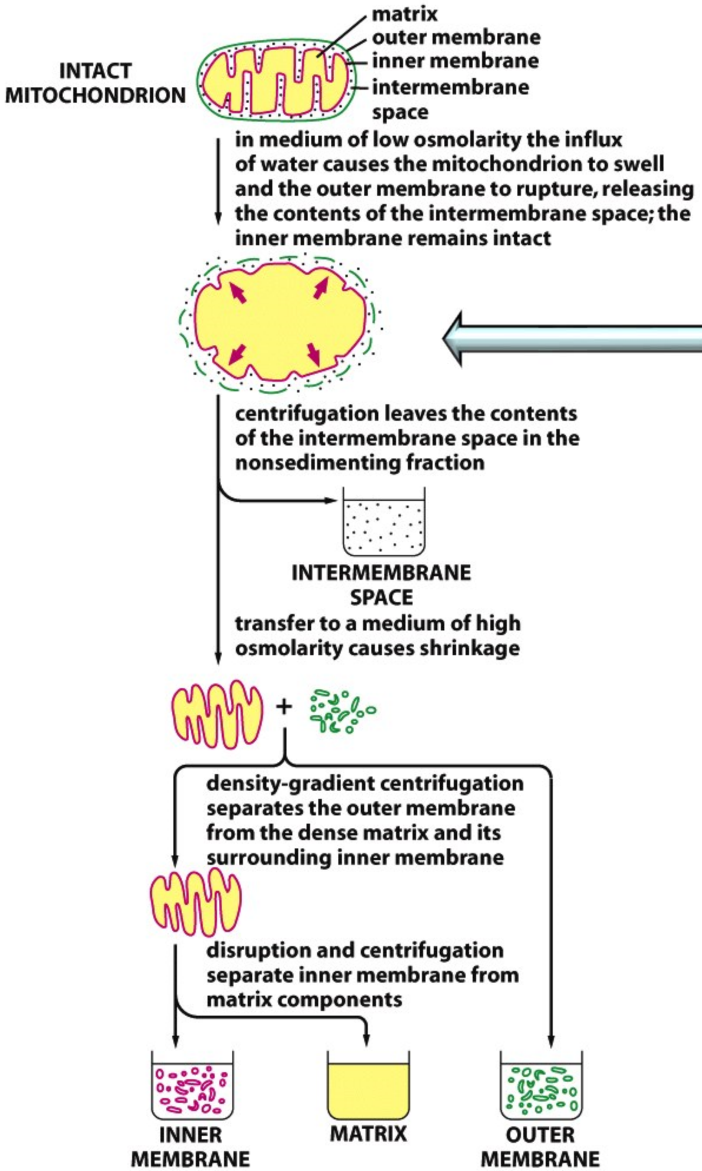
Double phospholipids contains 4 fatty acid chains

It constitutes 20% of total inner membrane.

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

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

Separation of mitochondrial compartments for analysis



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

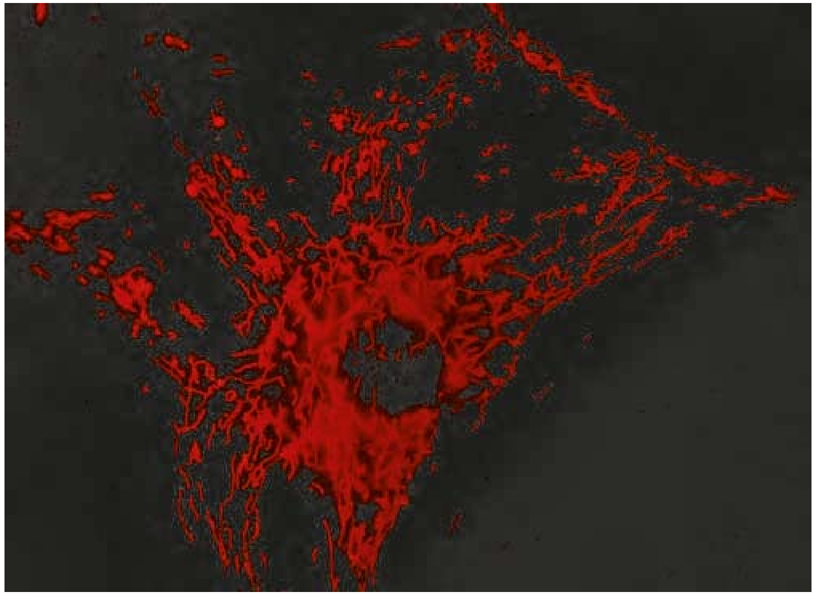
1st **osmotic shock** to disrupt the **outer membrane** and **releases content from the inner membrane space (IMS)**, this is **separated by centrifugation (soluble supernatant)**.

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

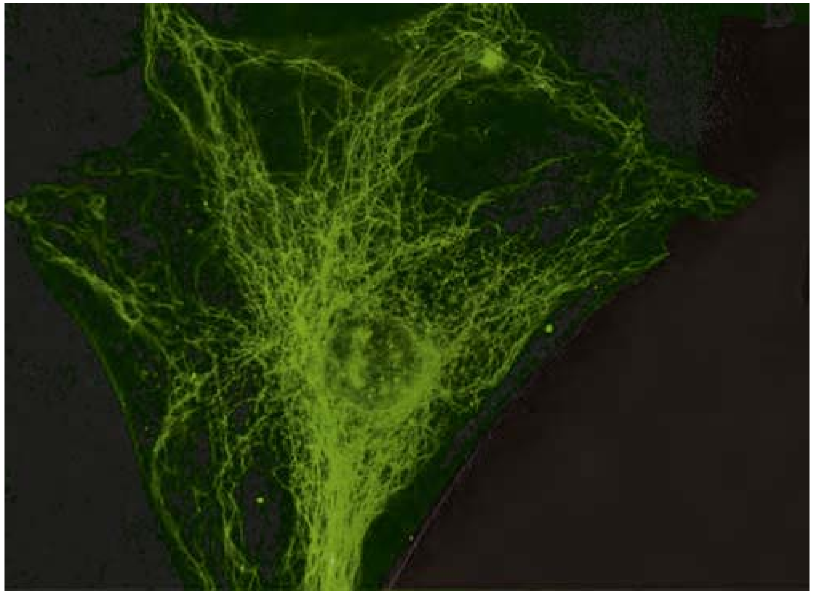
3rd separation by **density-gradient centrifugation**

4th **extraction** of the gradient fractions

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



stained **mitochondria**

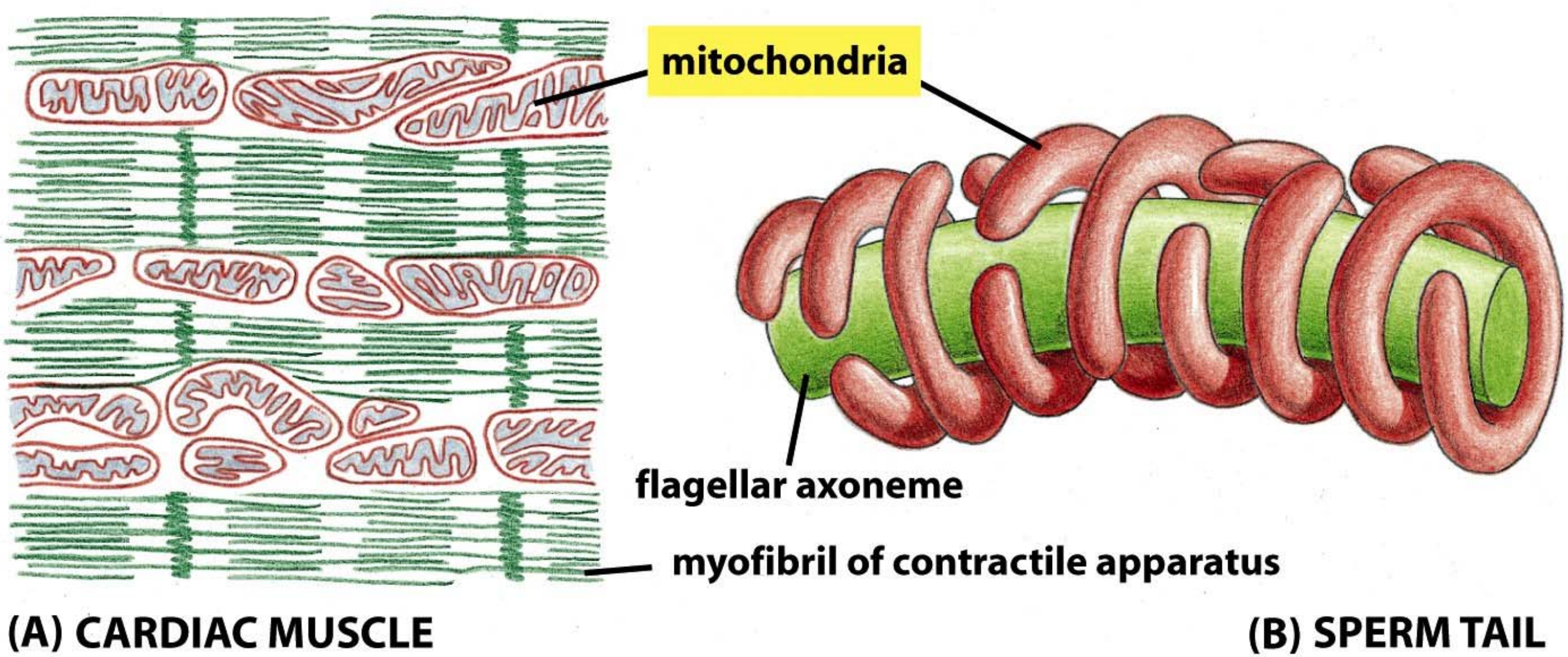


IF **microtubules**

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



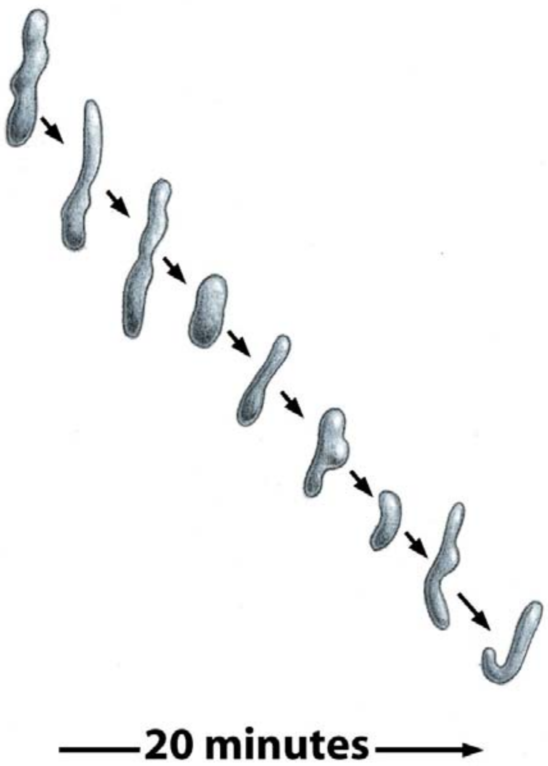
Mitochondria align to the contractile myofibrils

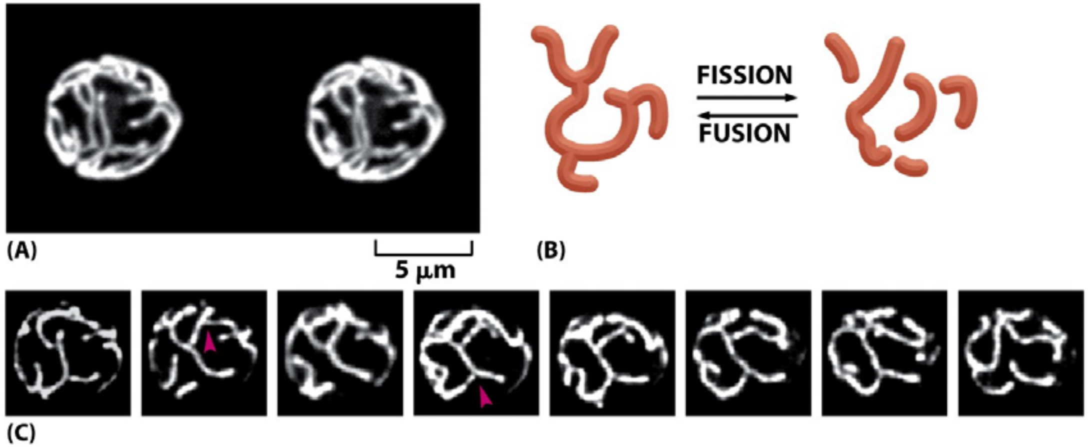
Mitochondria align to the flagellar axoneme (probably with help of microtubule)

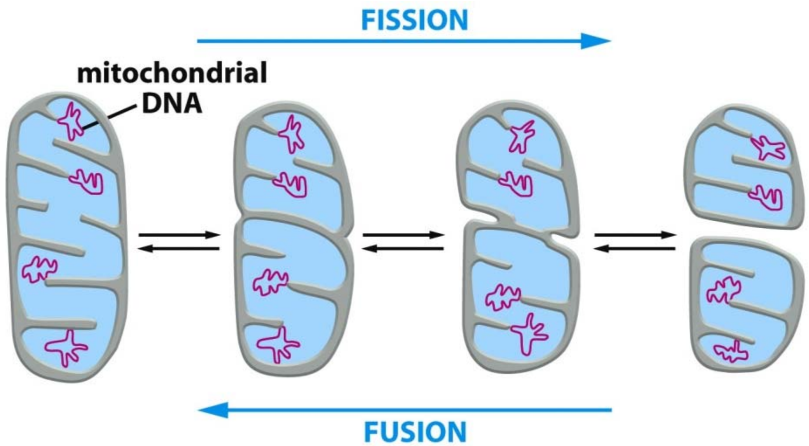
Plasticity of mitochondria

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

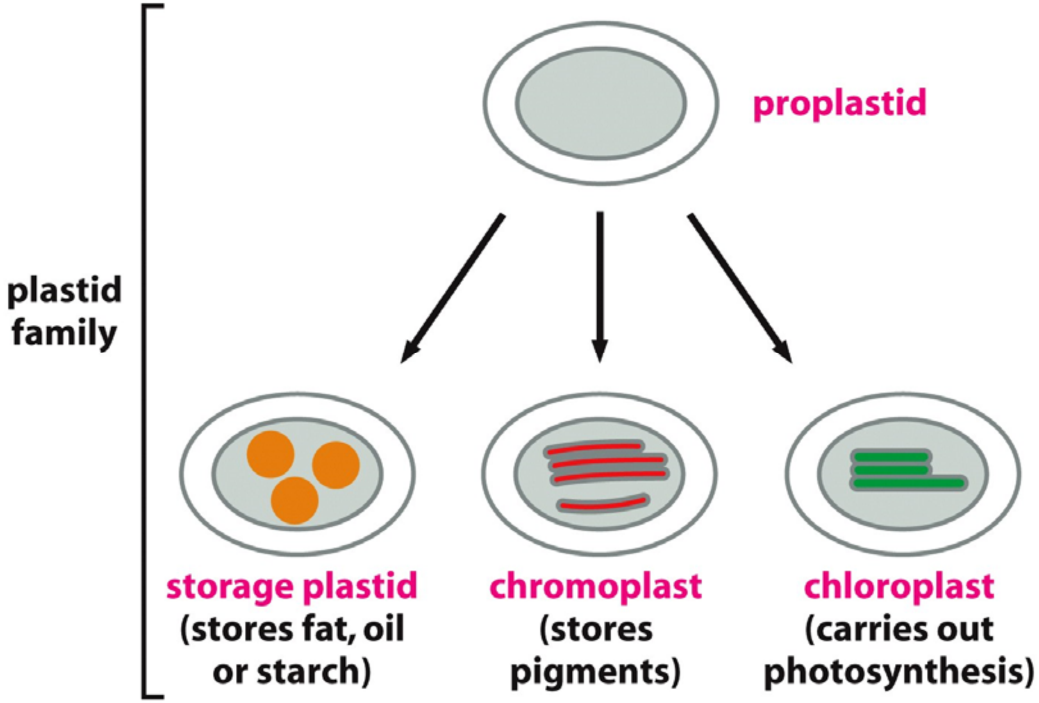
Mitochondria are dynamic and fuse and separate constantly

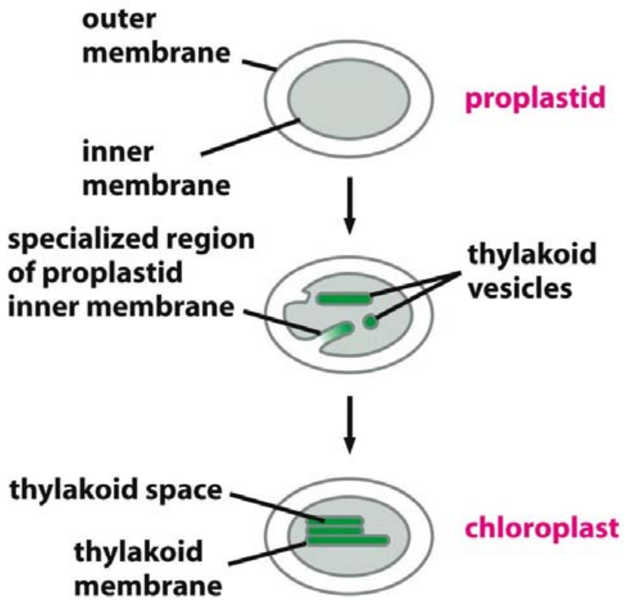






II. Plastids and evolution of chloroplast





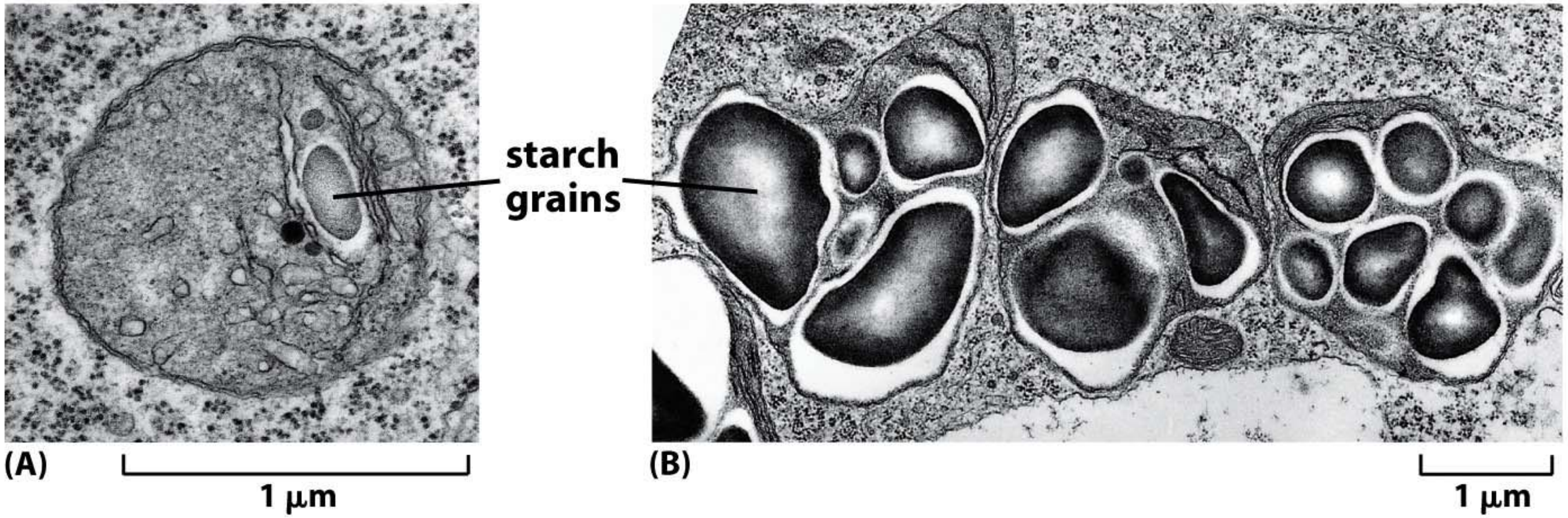
This depends on environment and the physiology of differentiated cells

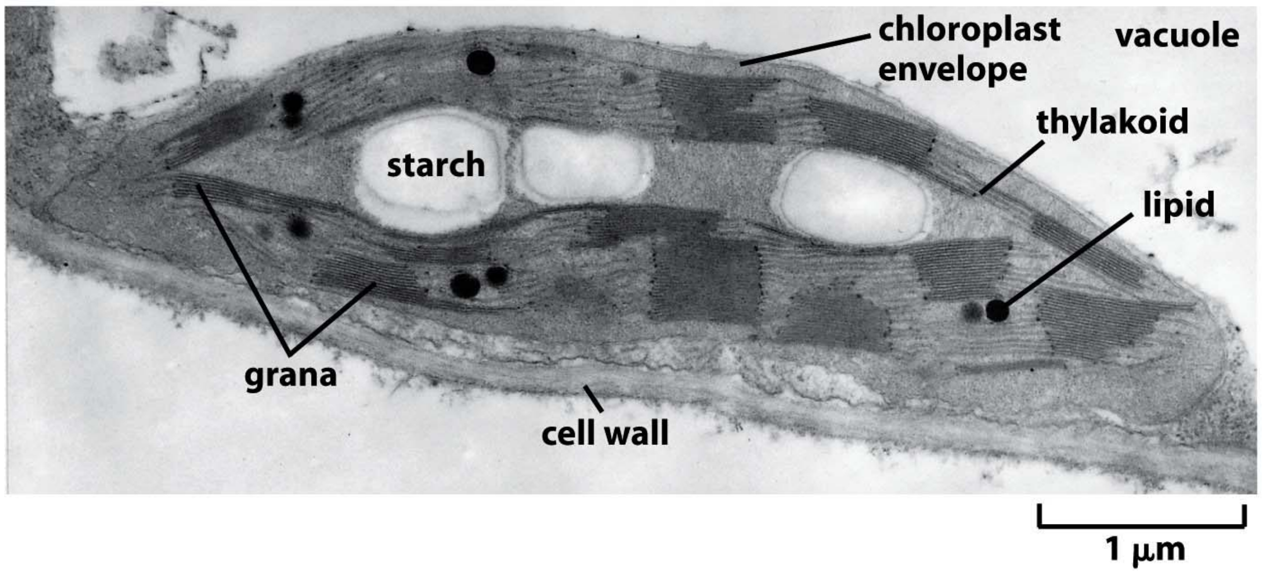
Plastid diversity

- 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

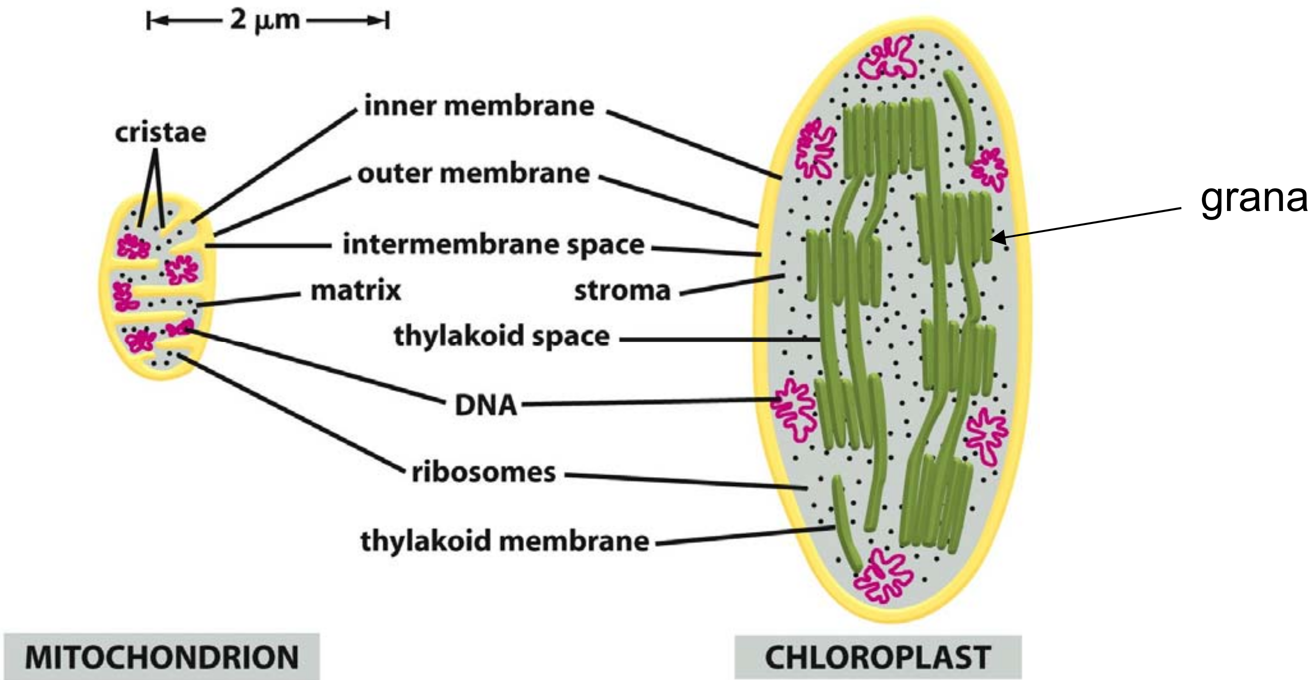




Chloroplast compartments

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 contain 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				
Chlamydomonas	vegetative	80	1	7
Maize	leaves	0–300**	20–40	0–15**

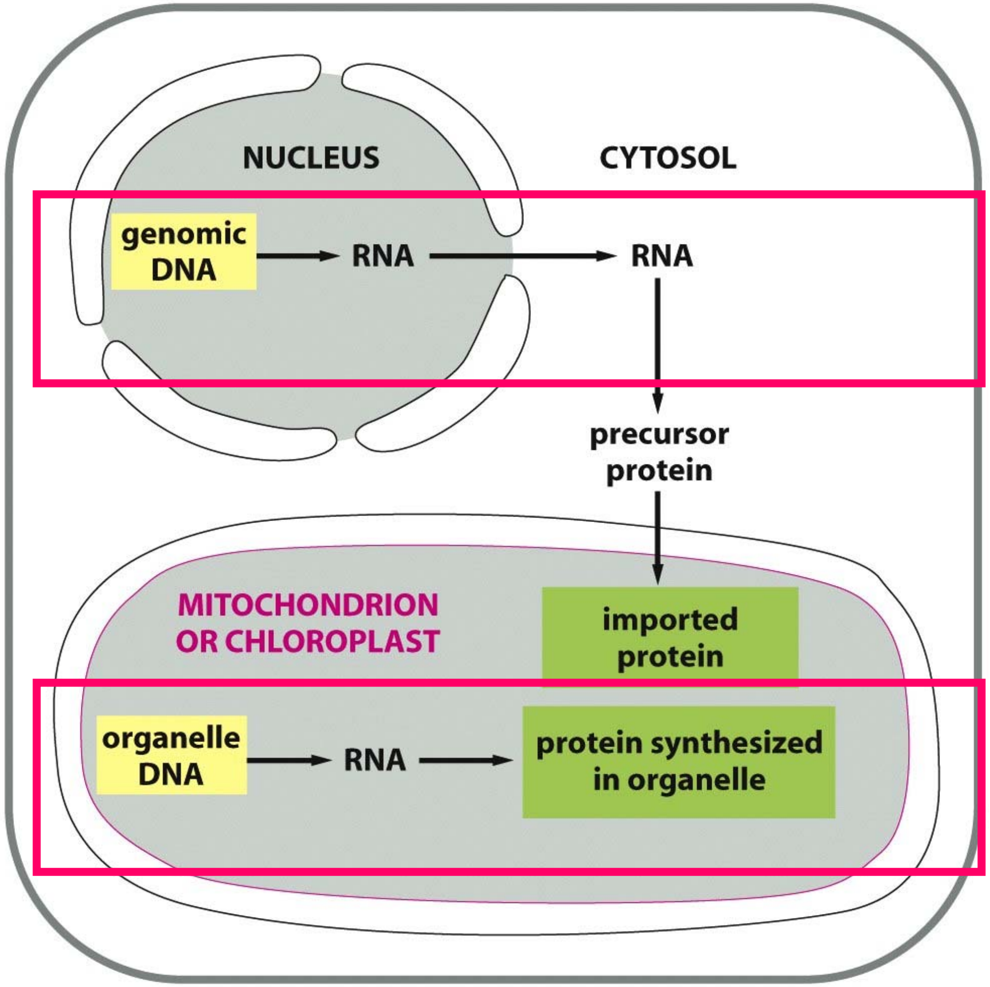
*The large variation in the number and size of mitochondria per cell in yeasts is due to mitochondrial fusion and fission.
**In maize, the amount of chloroplast DNA drops precipitously in mature leaves, after cell division ceases: the chloroplast DNA is degraded and stable mRNAs persist to provide for protein synthesis.

The **number** of both **chloroplast** and **mitochondrion varies** 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.

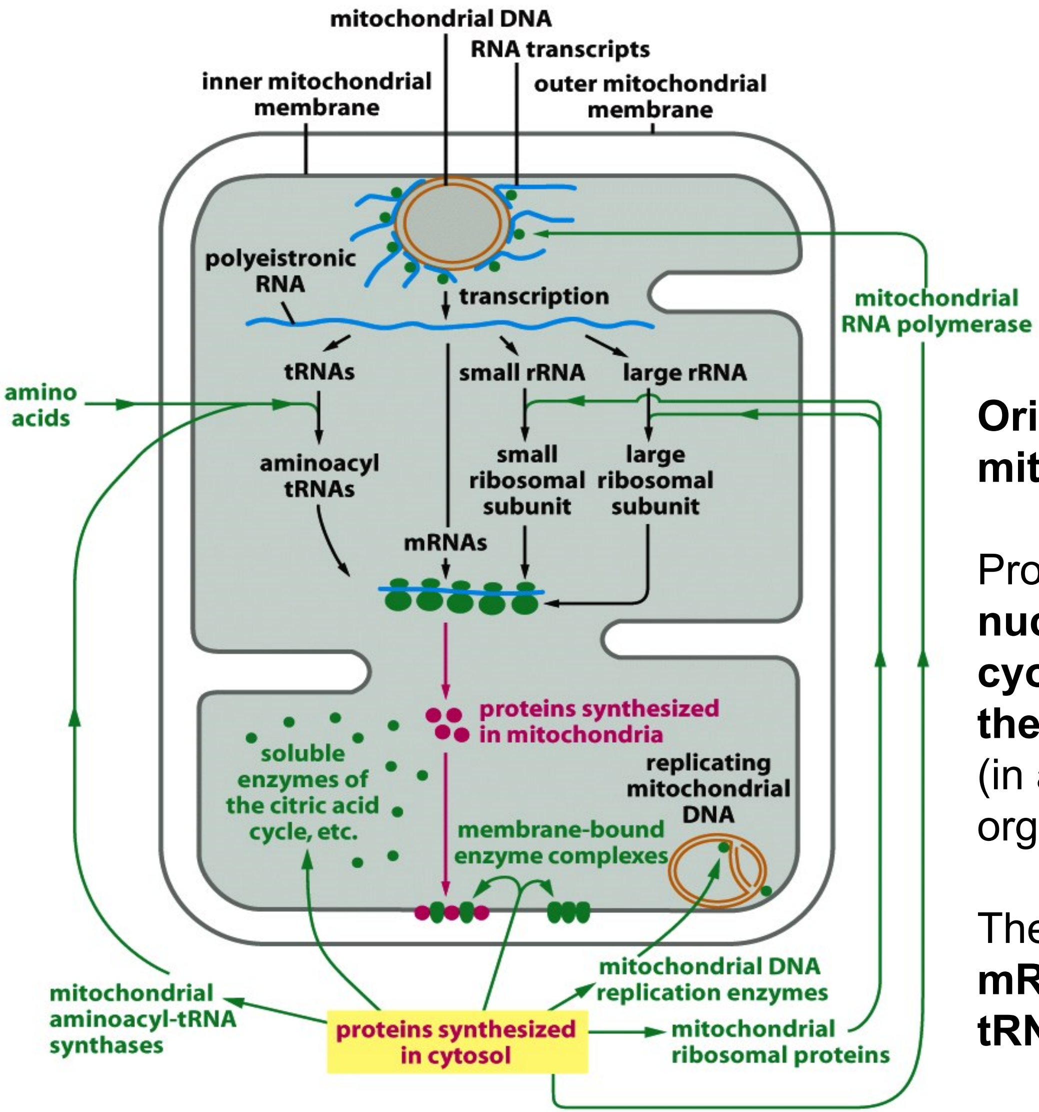
Mitochondrion and chloroplast contain their own complete genetic systems



<u>Proteins</u> in **mitochondria** <u>and</u> **chloroplasts** are produced by **two separate** genetic systems:

1. nucleus
2. mitochondrion/chloroplast

Coordinated protein synthesis between nucleus and mitochondria



Origins of mitochondrial RNAs and proteins:

Proteins that are encoded in the **nucleus** and are **imported** from the **cyosol** play a **major role in creating the genetic system** of mitochondria (in addition to contributing to the organellar proteins)

The mitochondrion contributes only **mRNAs, rRNAs and in some cases tRNAs to it's own genetic system**

Animal mitochondria contain the simplest genetic systems known

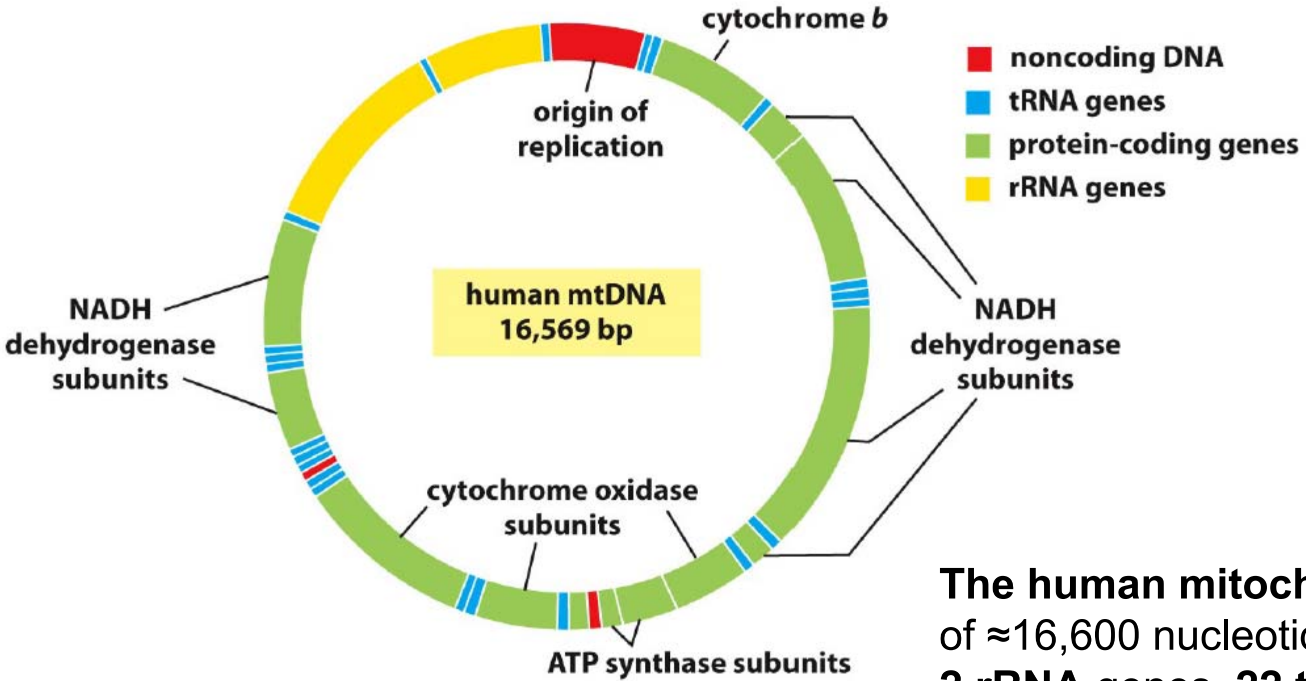


Figure 14-65 Molecular Biology of the Cell 6e (© Garland Science 2015)

The human mitochondrial genome of ≈16,600 nucleotide pairs contains: **2 rRNA** genes, **22 tRNA** genes, and **13 protein**-coding sequences.

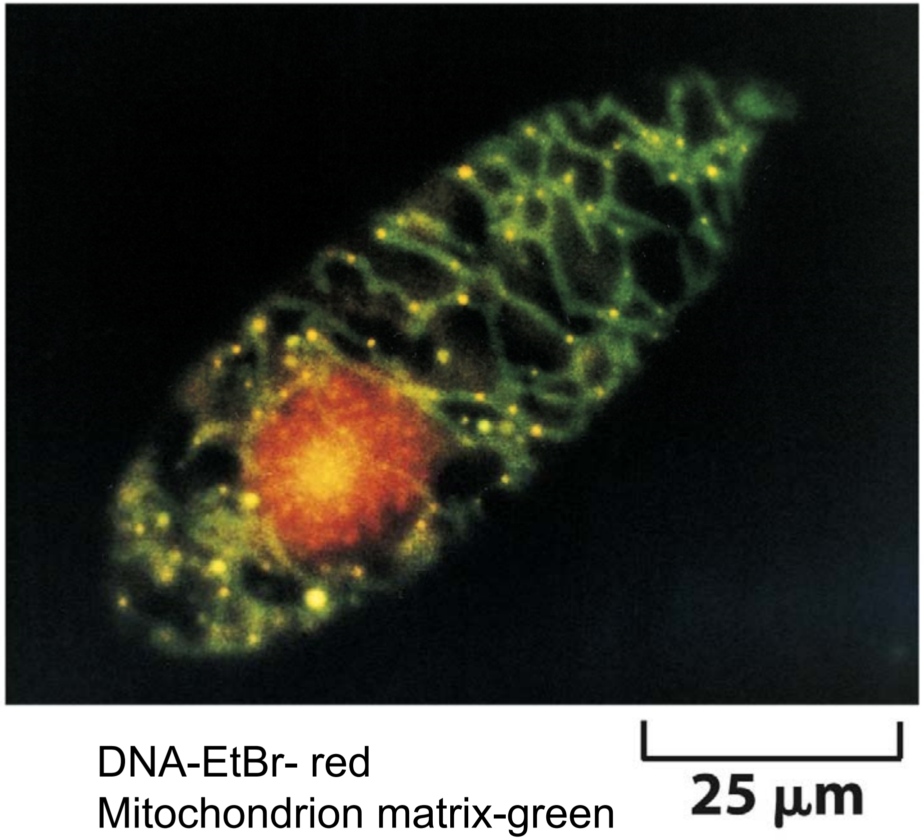
There are **two transcriptional** promoters: **one for each strand** of **the mitochondrial DNA** (**mtDNA**)

Protein synthesis machinery from chloroplasts and mitochondria

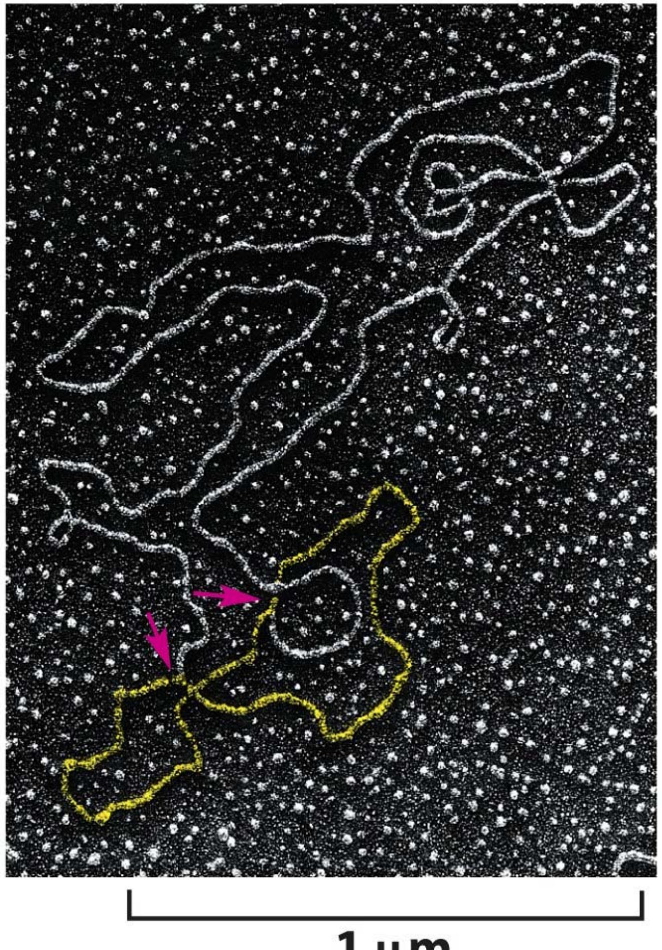
- **Chloroplast** more resembles **present-day bacteria** in its protein synthesis machinery
- Both chloroplast and mitochondrion use **N-formyl-Methionine (fMet)** as the **initiation amino acid** in protein synthesis.
- Protein synthesis in both chloroplast and mitochondrion can be **inhibited by antibiotics**.

Think about side effects of antibiotics in eukaryotic organisms?

Visualization of mitochondria DNA

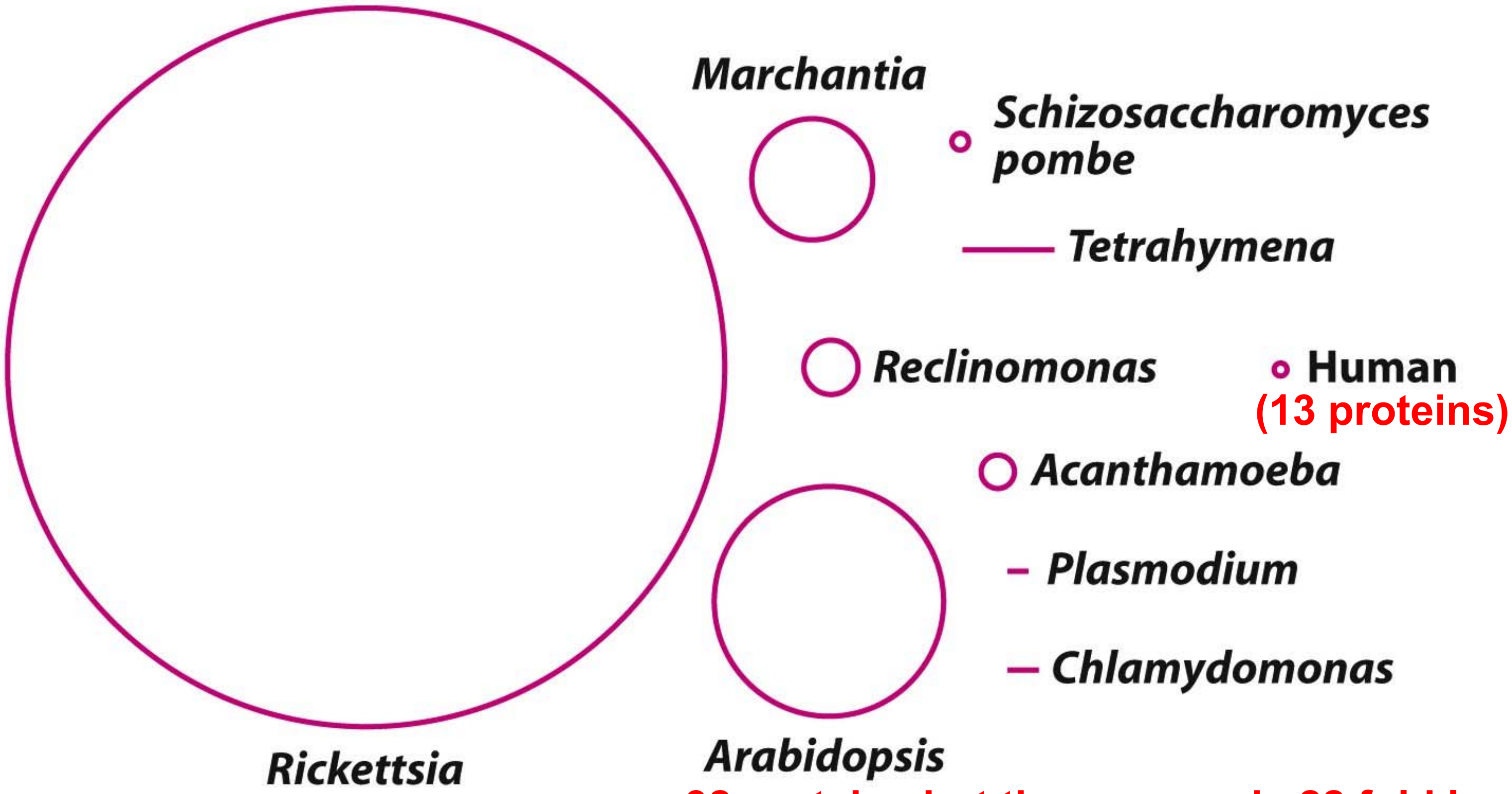


DNA-EtBr- red
Mitochondrion matrix-green



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

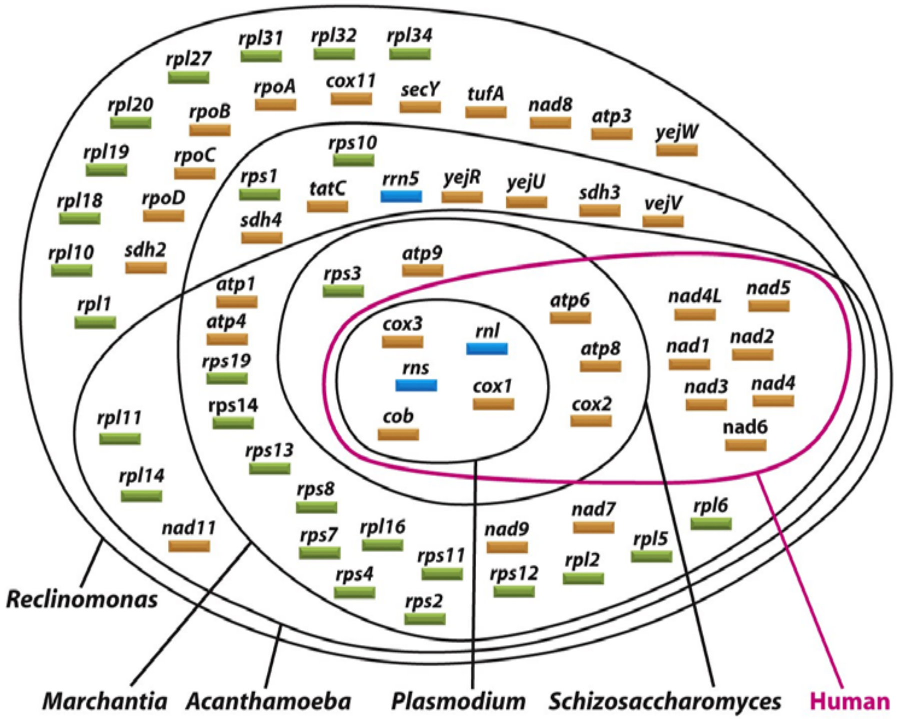
Various sizes of mitochondria genomes



32 proteins but the genome is 22 fold larger than the mDNA in humans, most of it is "junk DNA"

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

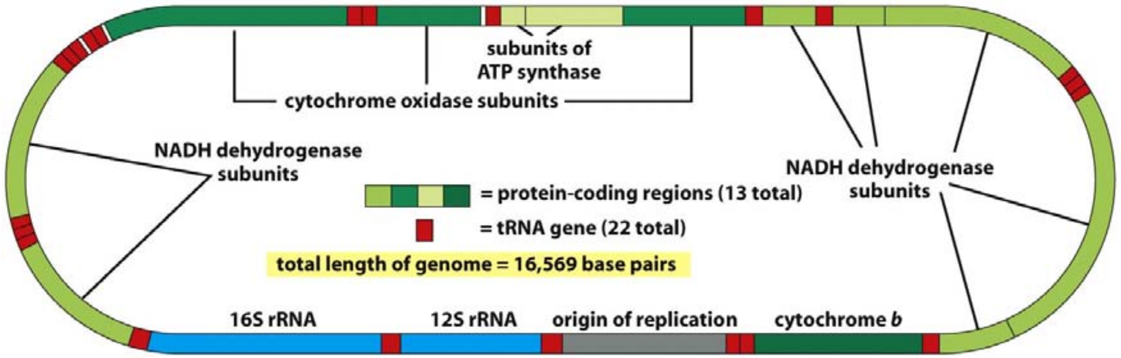
3. Sequential reduction in mitochondria encoding genes during evolution

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 tRNAs.

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

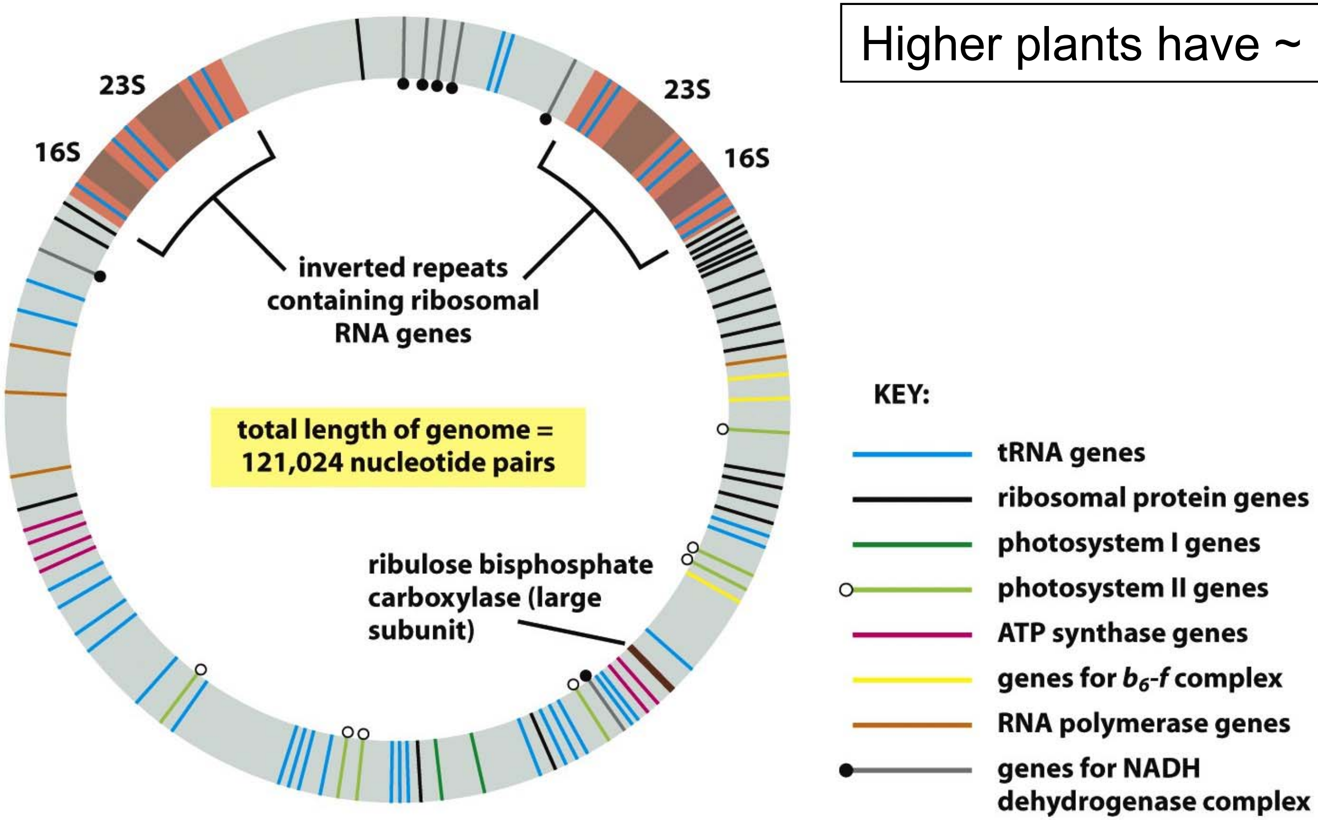
		MITOCHONDRIAL CODES			
CODON	"UNIVERSAL" CODE	MAMMALS	INVERTEBRATES	YEASTS	PLANTS
UGA	STOP	*Trp*	*Trp*	*Trp*	STOP
AUA	Ile	*Met*	*Met*	*Met*	Ile
CUA	Leu	Leu	Leu	*Thr*	Leu
AGA, AGG	Arg	*STOP*	*Ser*	Arg	Arg

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

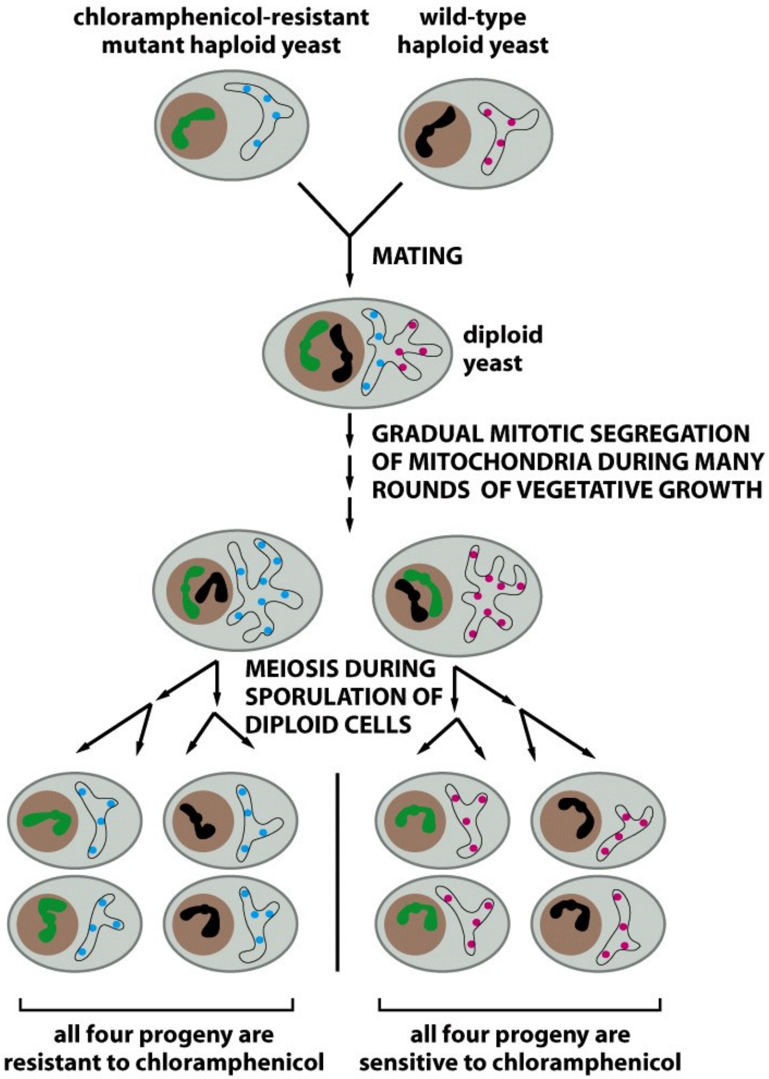
How to explain that the mitochondrial genetic code is different from universal genetic code?

Genes in chloroplast genomes, all are highly similar

Higher plants have ~ 120 genes



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

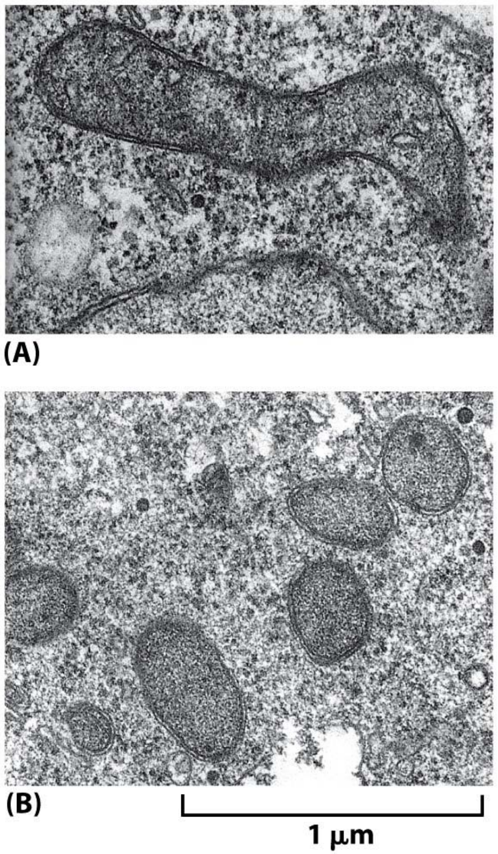
- Human zygote has most maternal mitochondria.
- 2/3 of higher plants have maternal chloroplasts.
- Inheritance in yeast is bi-parental.

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



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

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

Supporting that nucleus is sufficent to support mitochondria
Biogenesis .

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

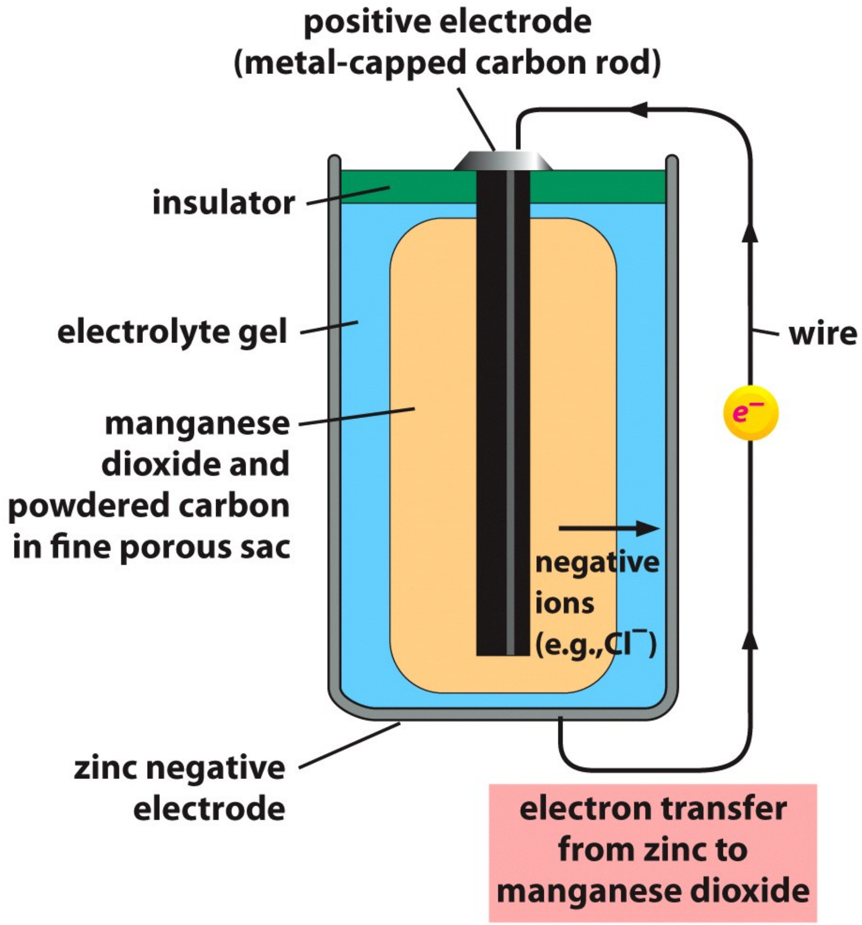
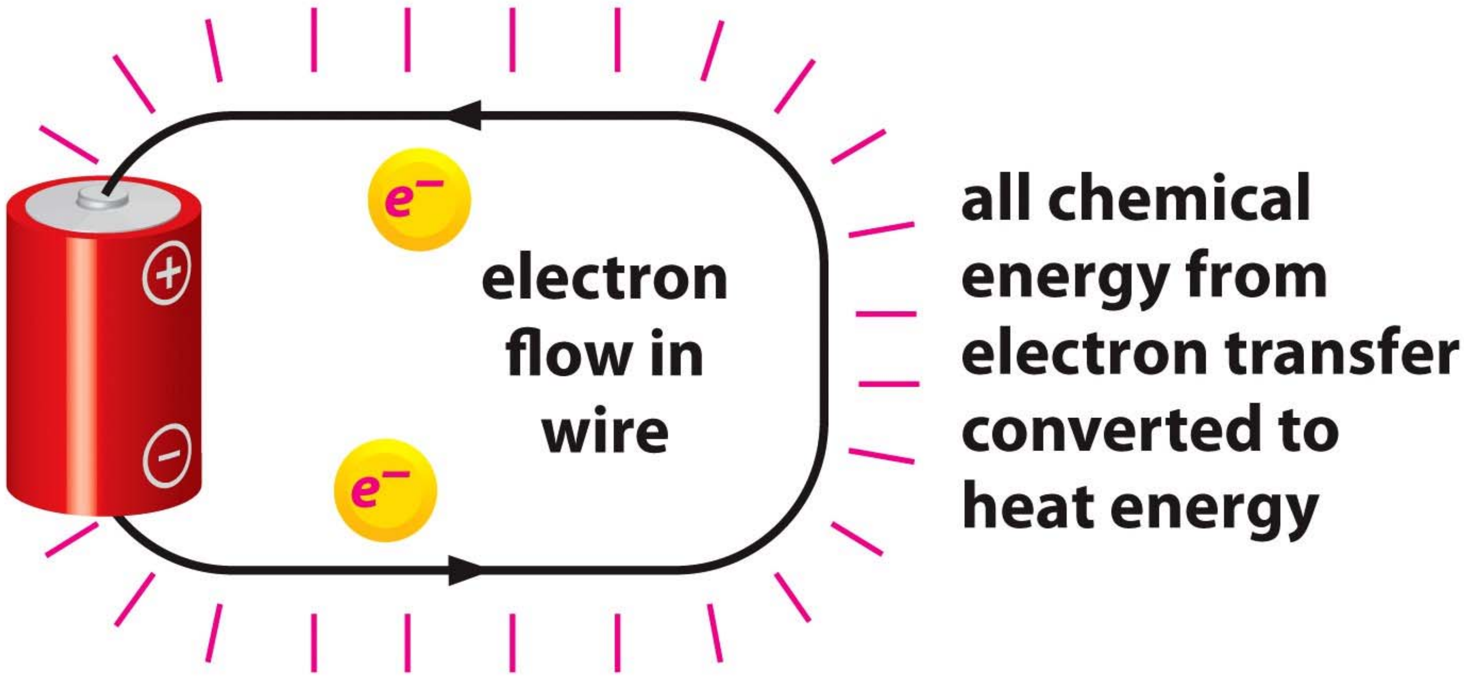


Figure 14-2a Essential Cell Biology 3/e (© Garland Science 2010)

Electronic energy can be consumed into heat



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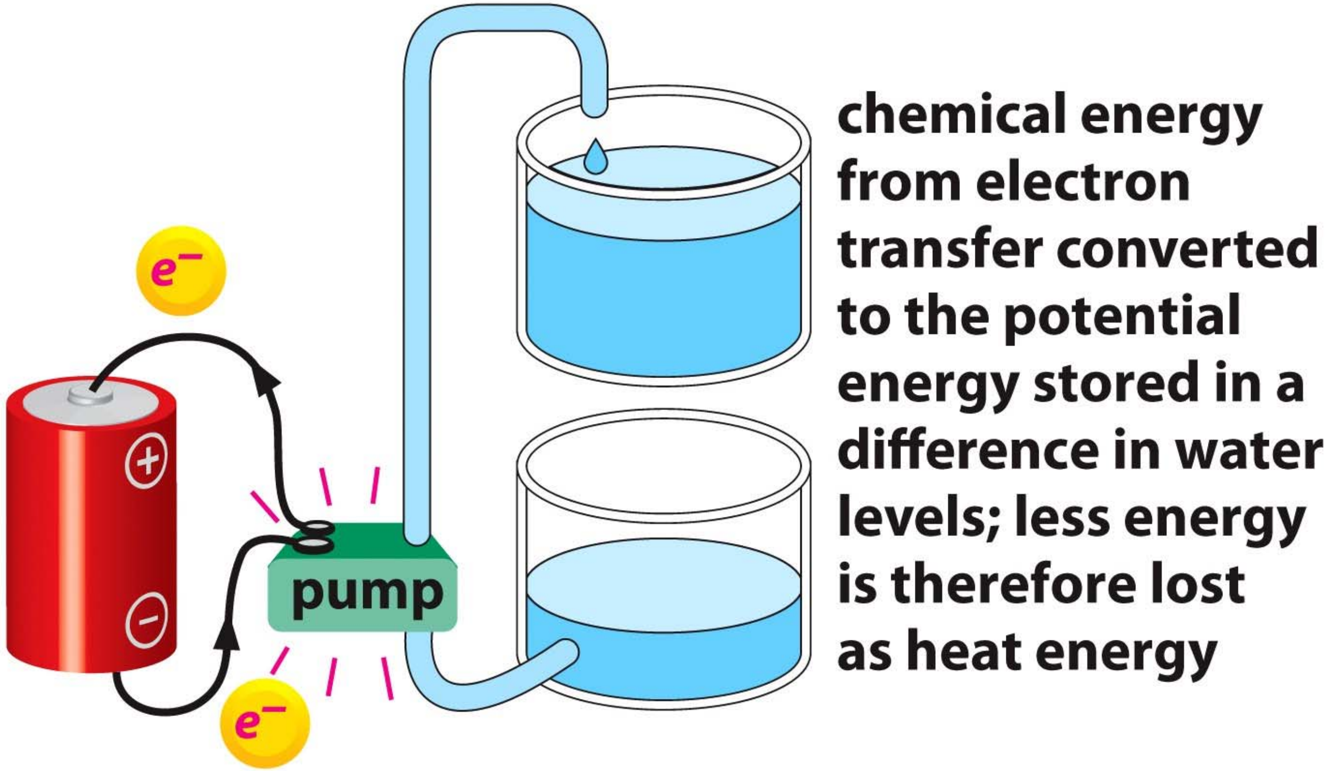
Electronic flow converts into other forms of energy



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Systems to provide energy for lives

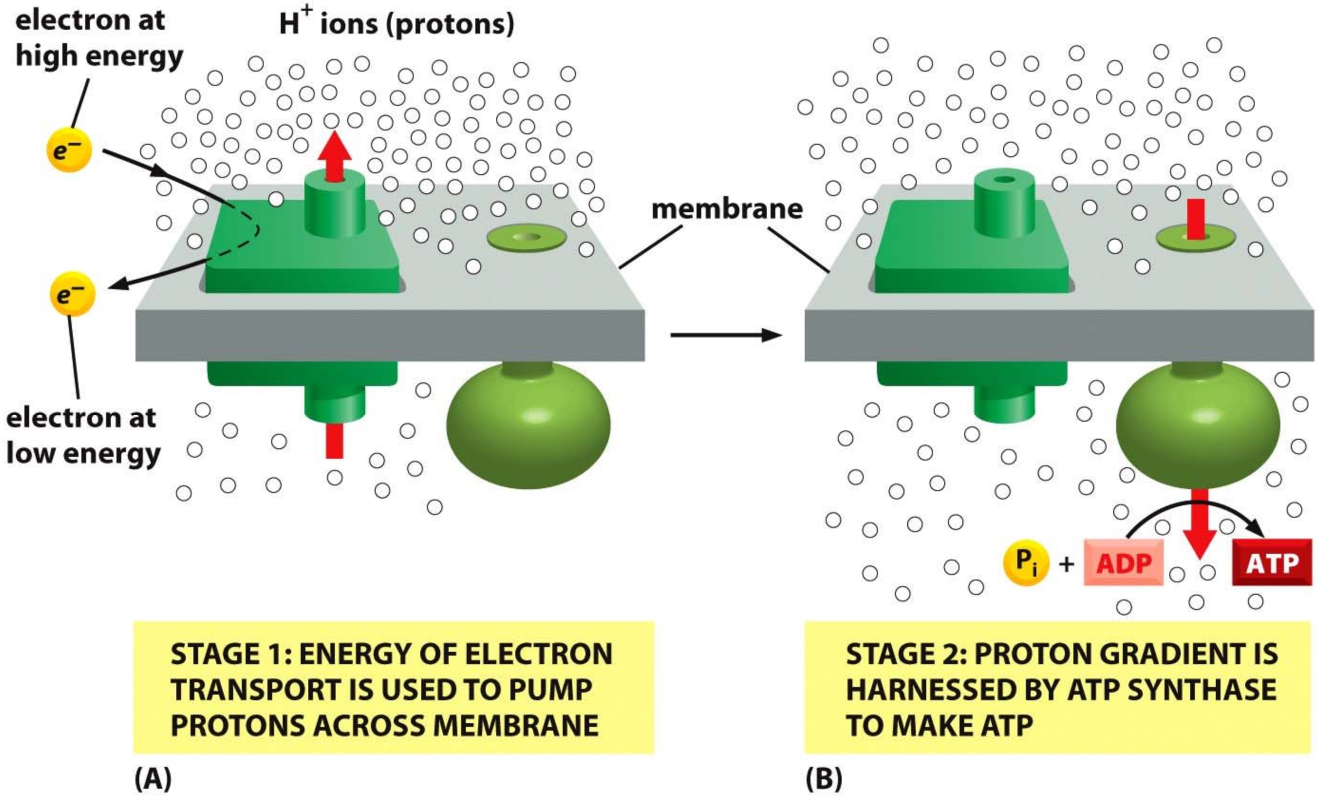


Figure 14-1 Essential Cell Biology 3/e (© Garland Science 2010)

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

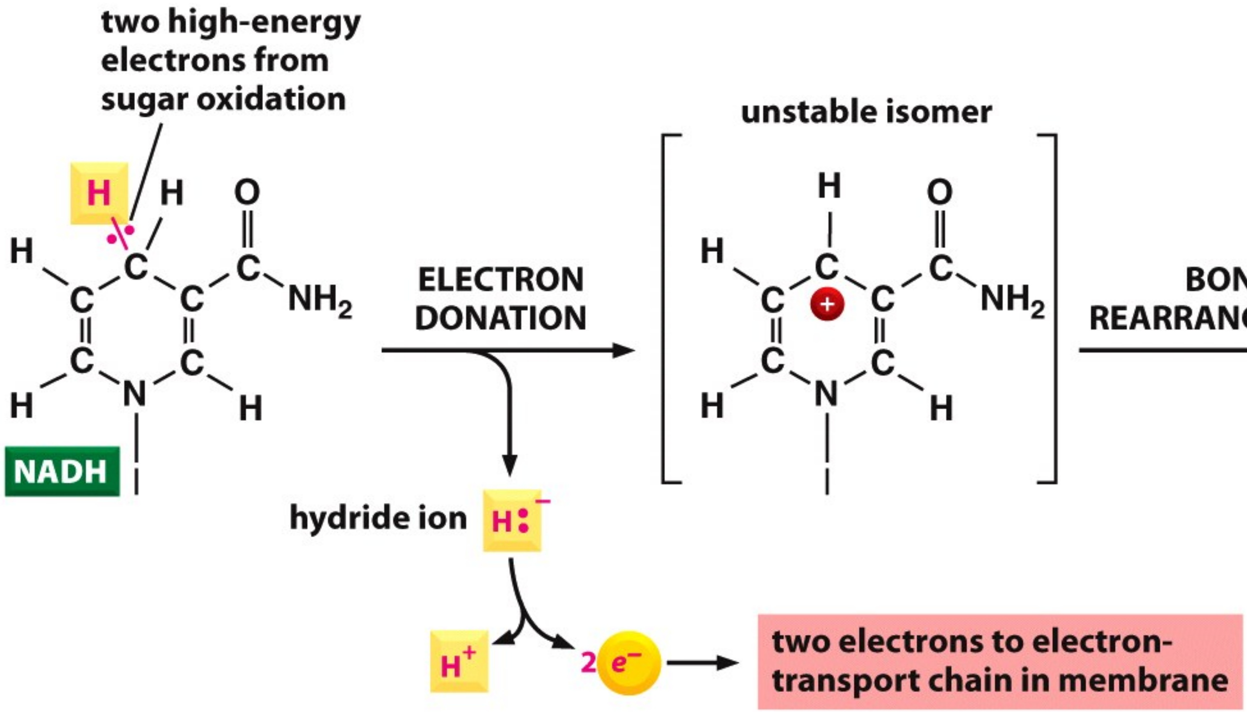


Figure 14-5 Essential Cell Biology 3/e (© Garland Science 2010)

NADH and FADH2 carry high energy electrons

Electrons are transferred by the electron transfer chain (ETC)

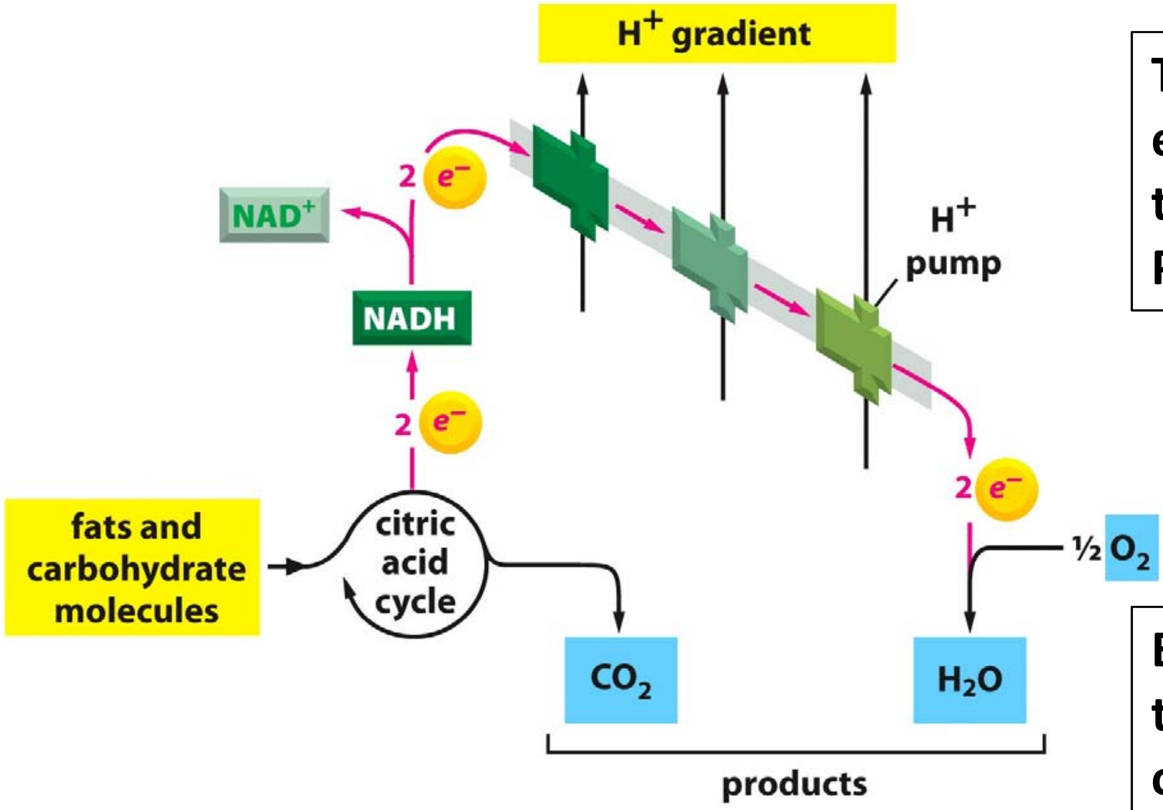


Figure 14-6 Essential Cell Biology 3/e (© Garland Science 2010)

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

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

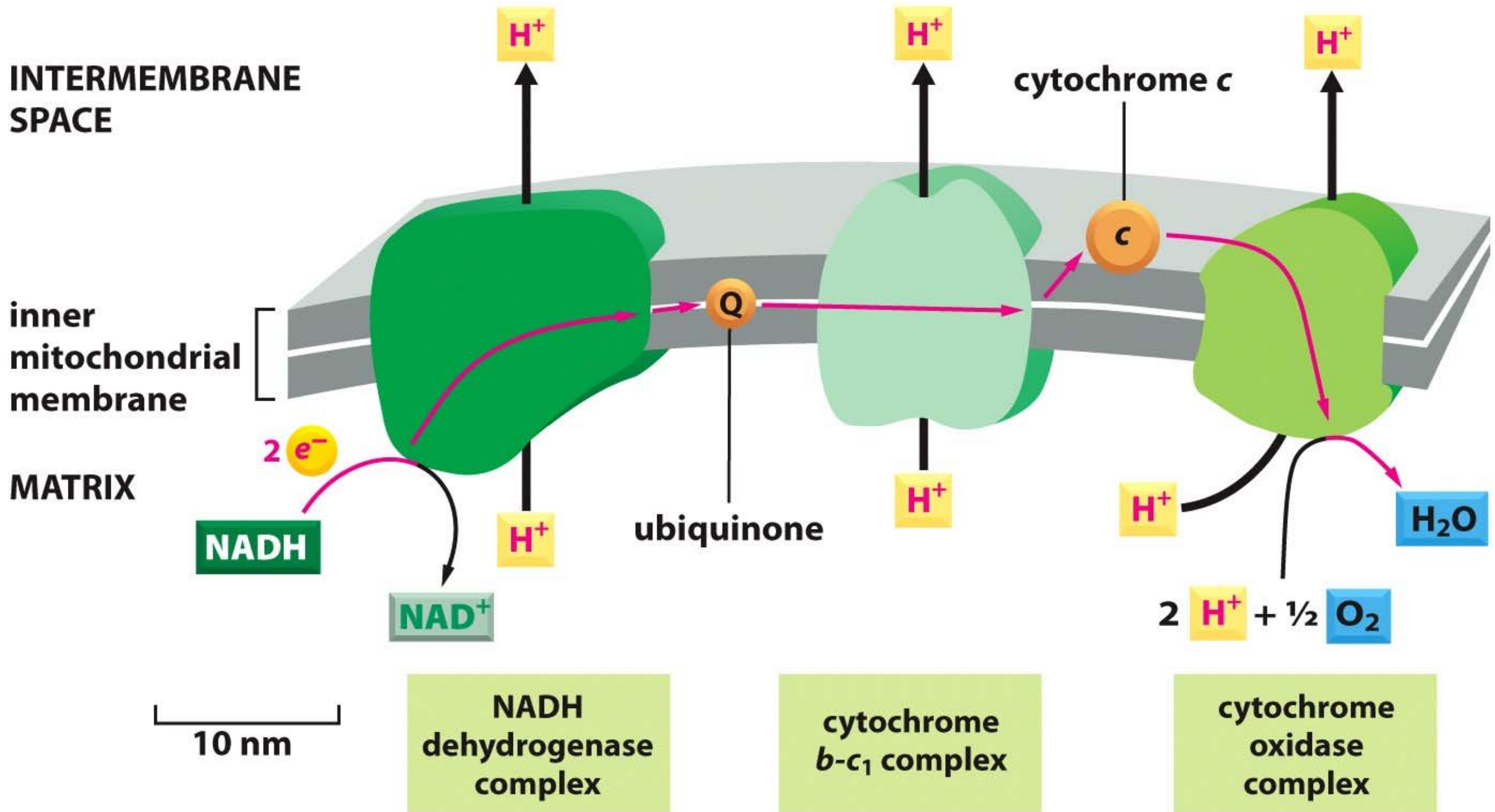


Figure 14-9 Essential Cell Biology 3/e (© Garland Science 2010)

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

The Q cycle

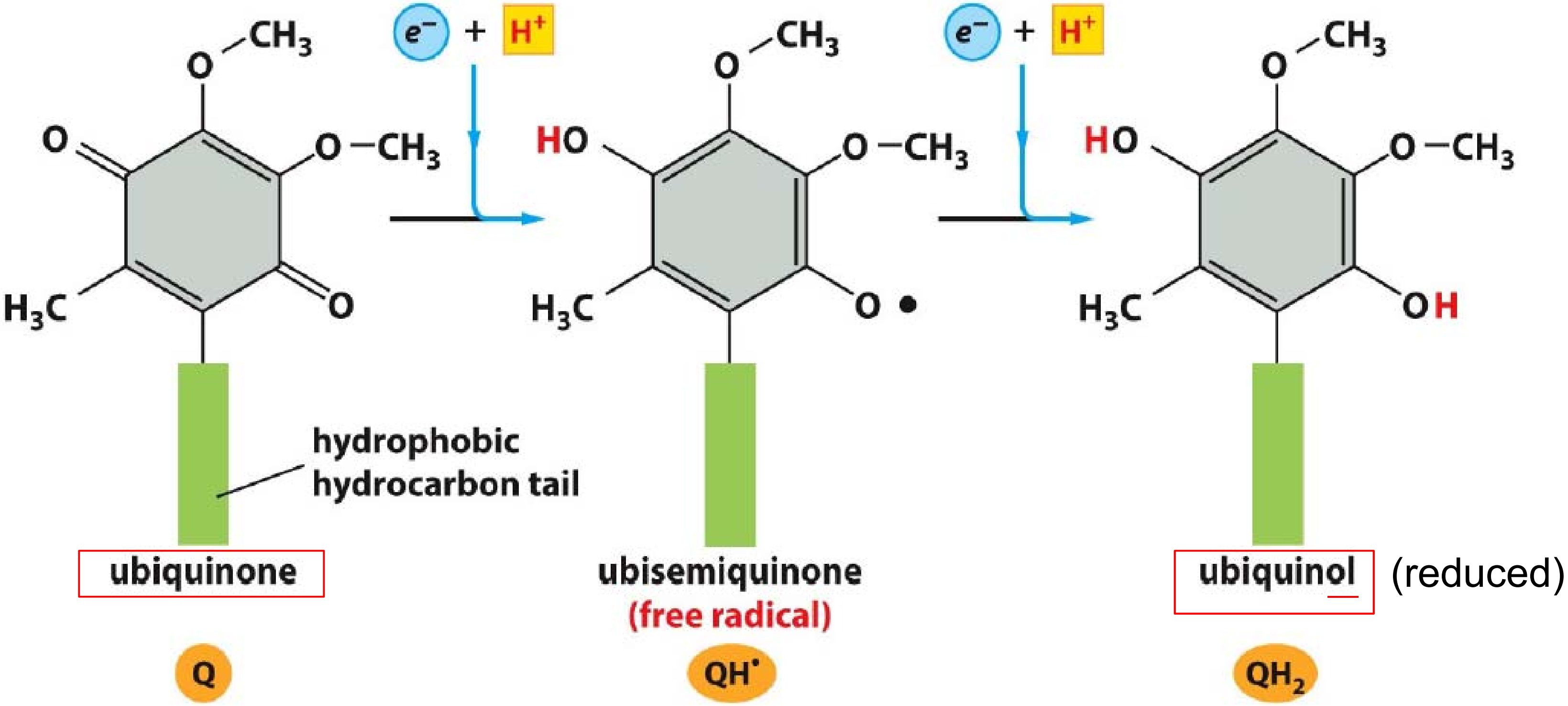


Figure 14-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

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

Proton is pumped back coupled with ATP synthesis

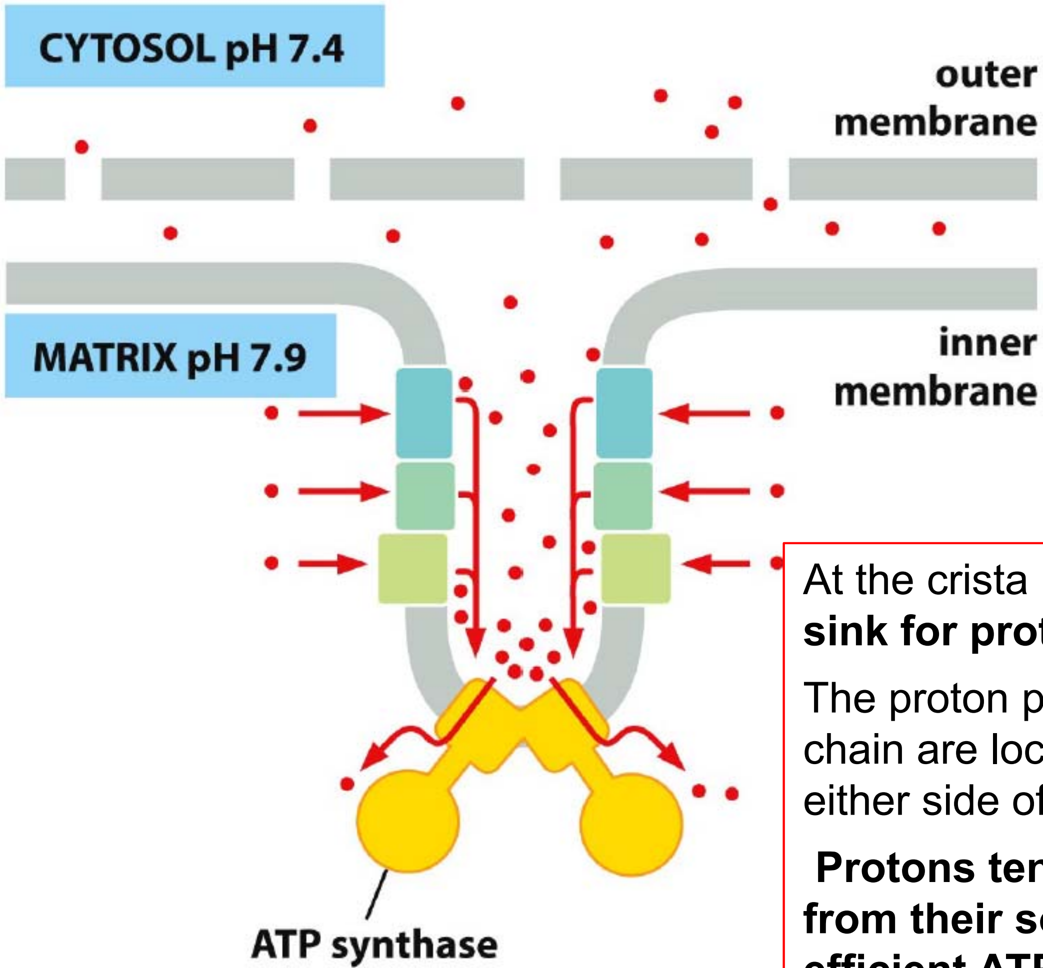


Figure 14-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

At the crista **ridges**, the ATP synthases **form a sink for protons.**

The proton pumps of the electron-transport chain are located in the membrane regions on either side of the crista.

Protons tend to diffuse along the membrane from their source to the proton sink, allowing efficient ATP production despite the small H+ gradient between the cytosol and matrix

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

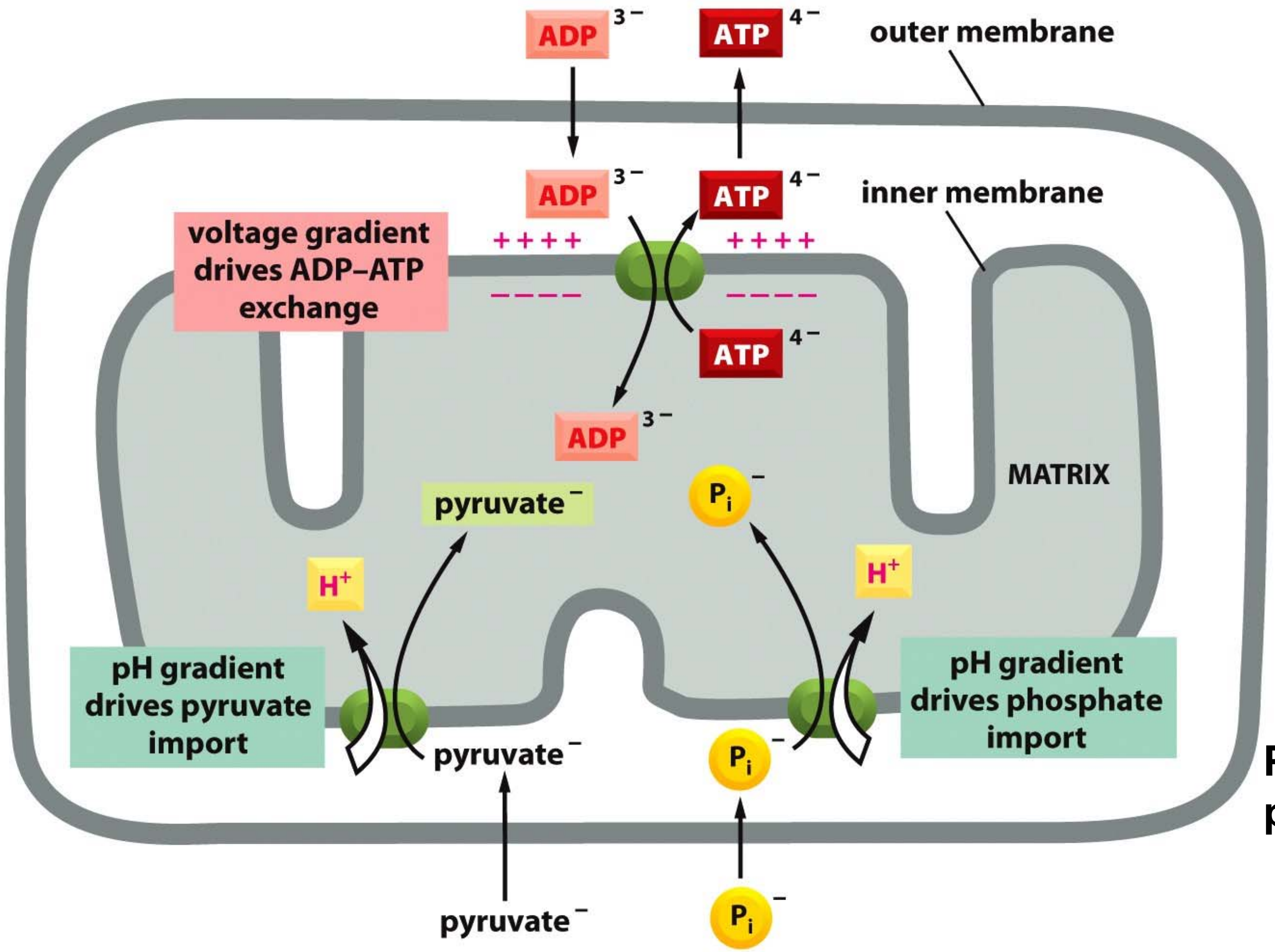
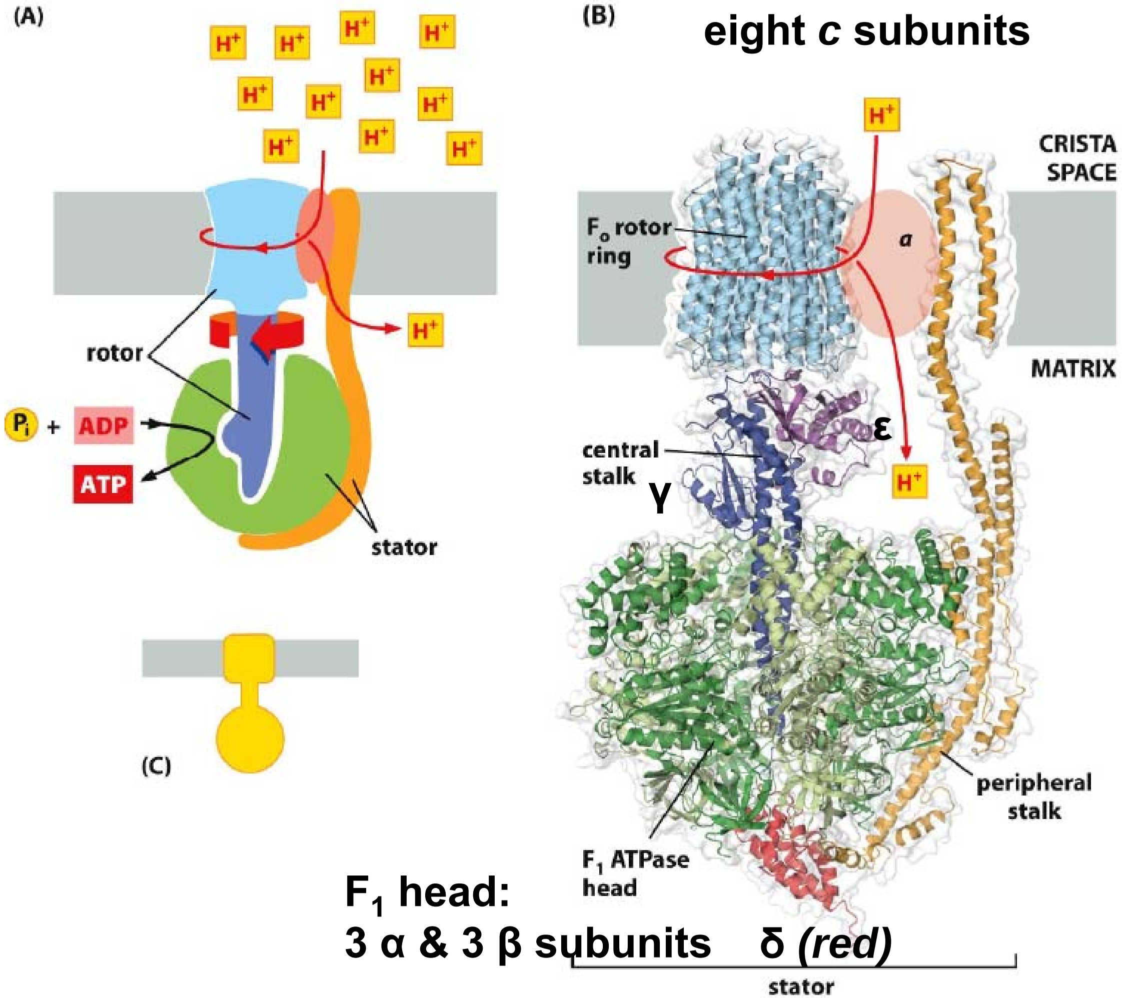


Figure 14-14 Essential Cell Biology 3/e (© Garland Science 2010)

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**

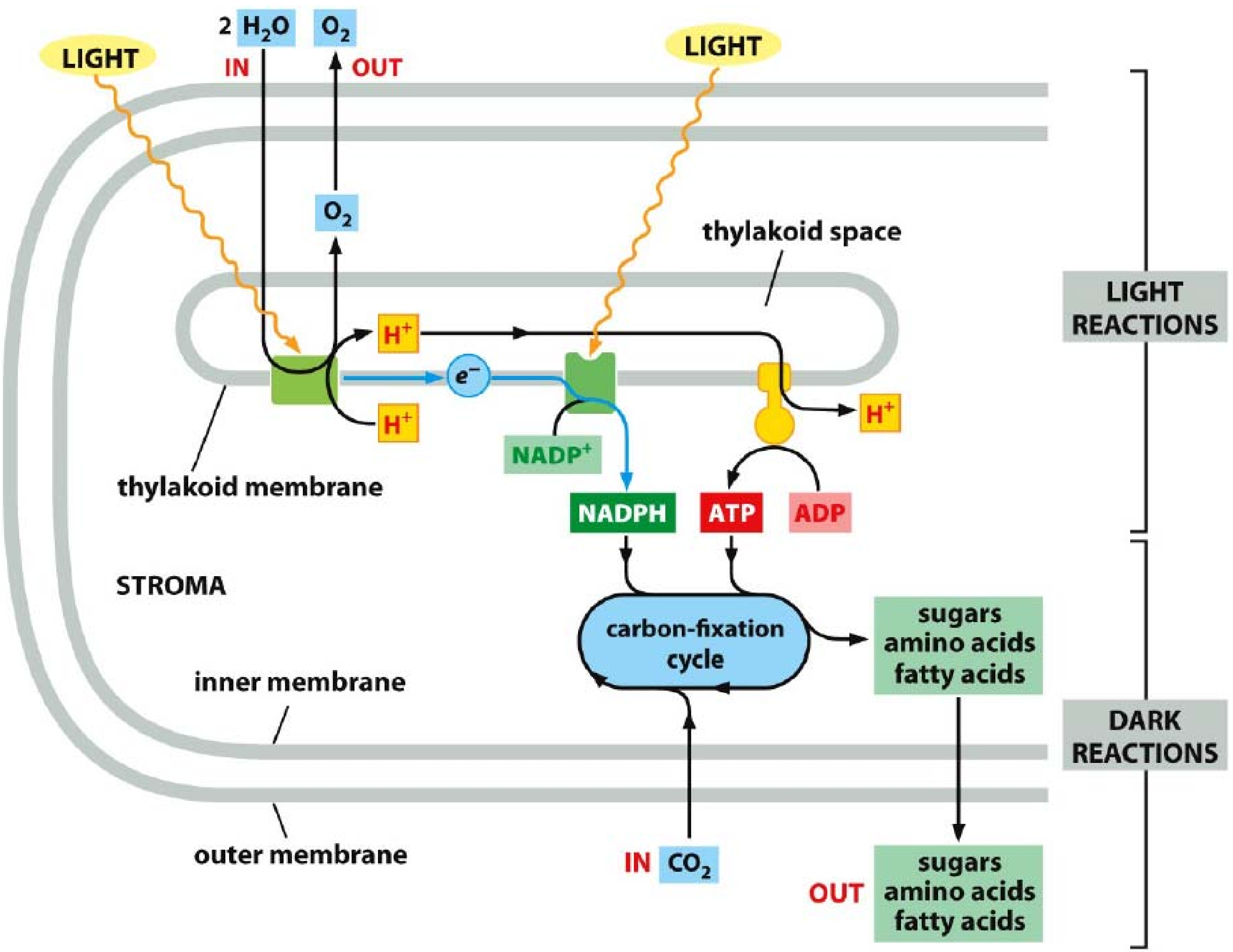


Figure 14-39 Molecular Biology of the Cell 6e (© Garland Science 2015)

Thylakoid membrane contains two different photosystems

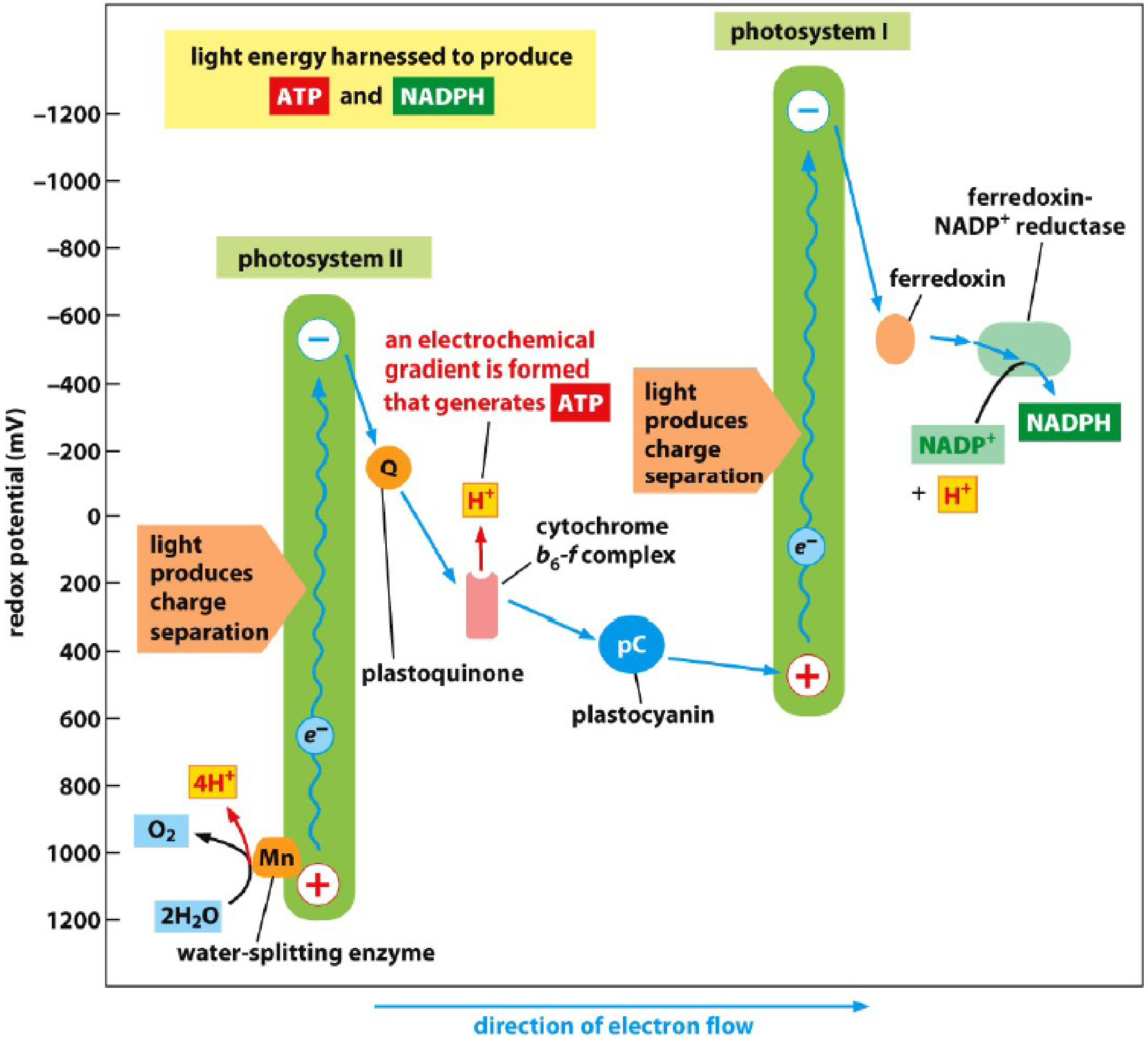


Figure 14-47 Molecular Biology of the Cell 6e (© Garland Science 2015)

PS II uses Mangnese cluster to withdraw electrons from water

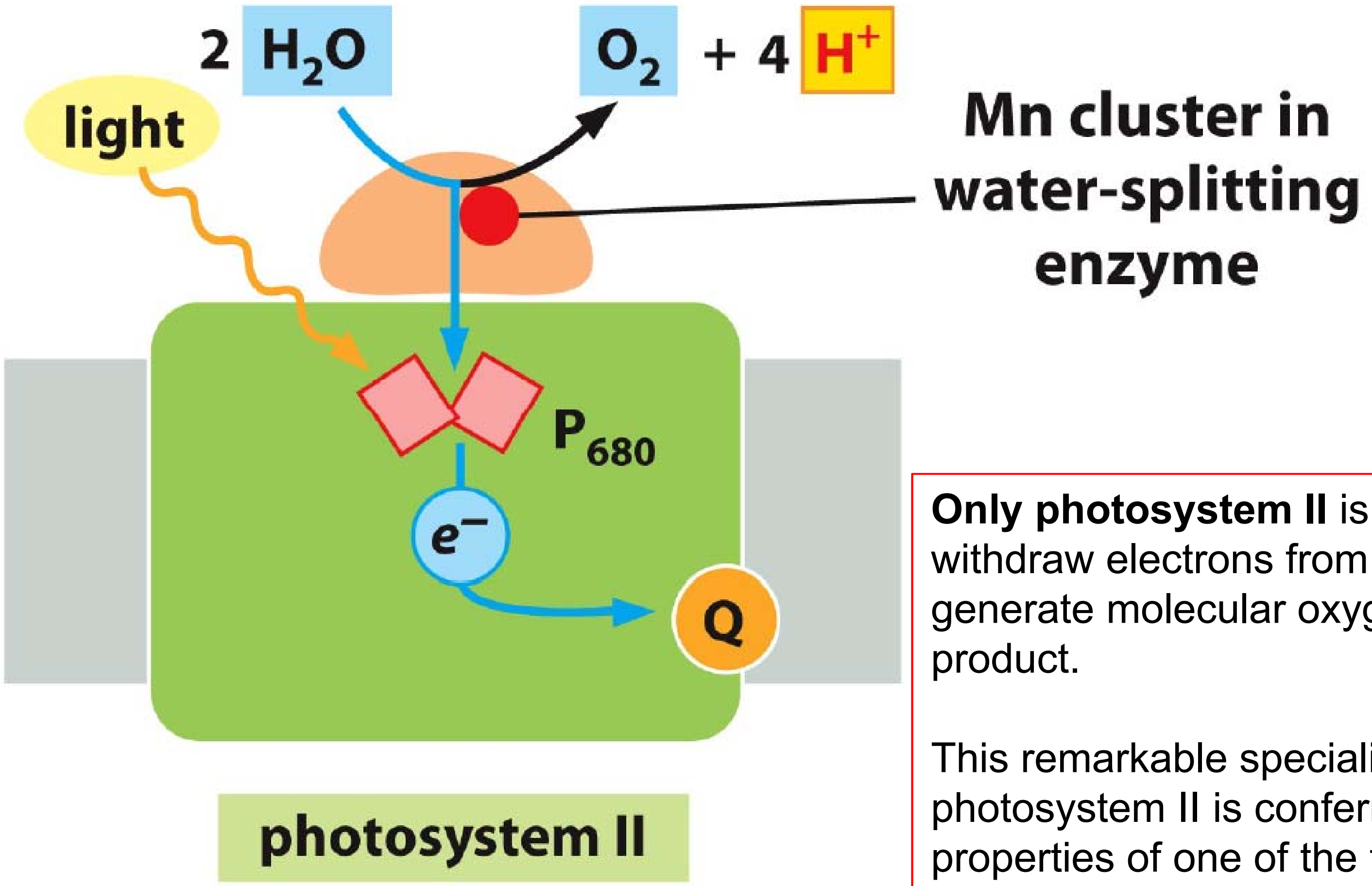


Figure 14-48a Molecular Biology of the Cell 6e (© Garland Science 2015)

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

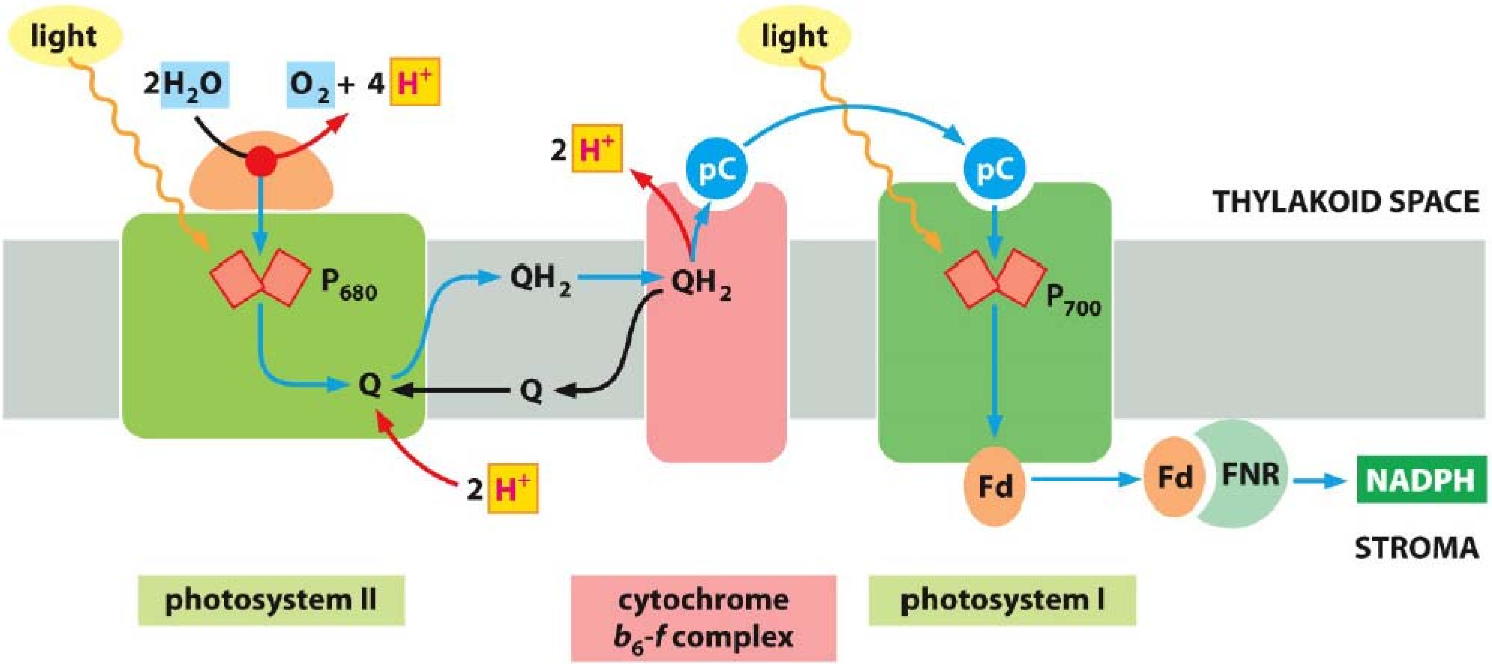


Figure 14-50 Molecular Biology of the Cell 6e (© Garland Science 2015)

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 CO_2 into sugar

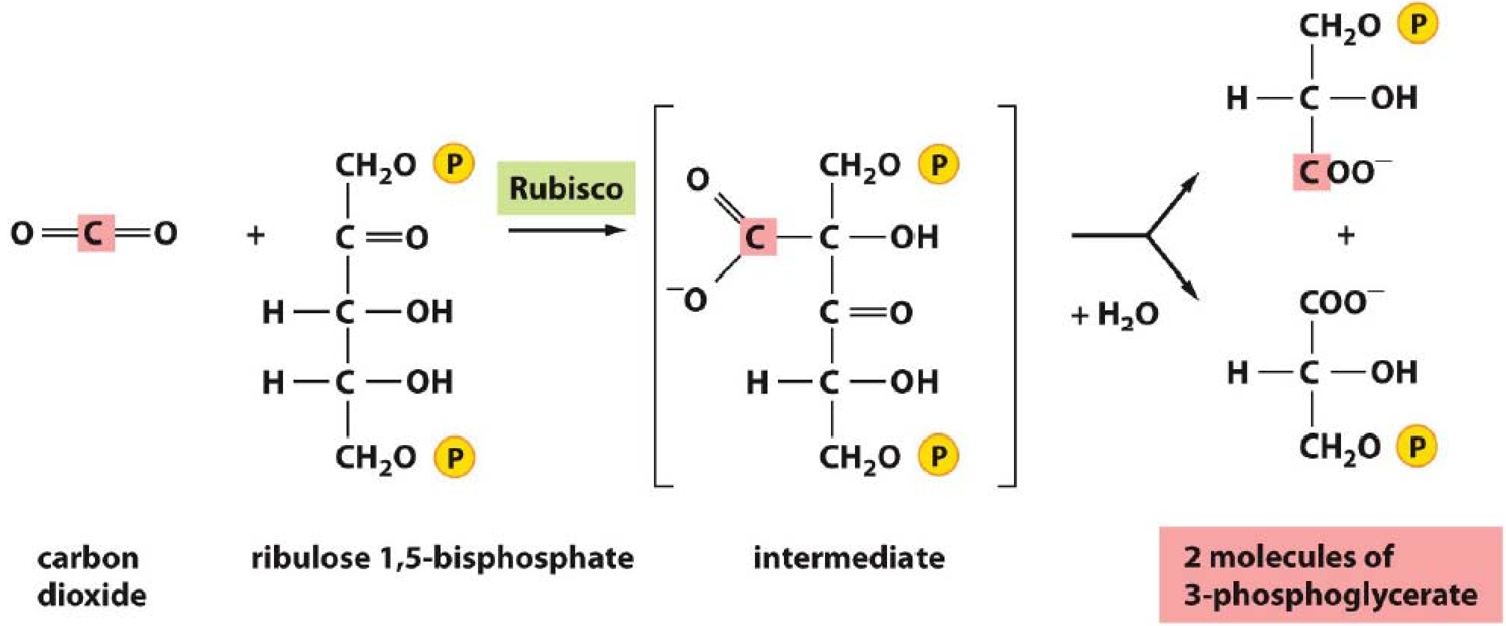
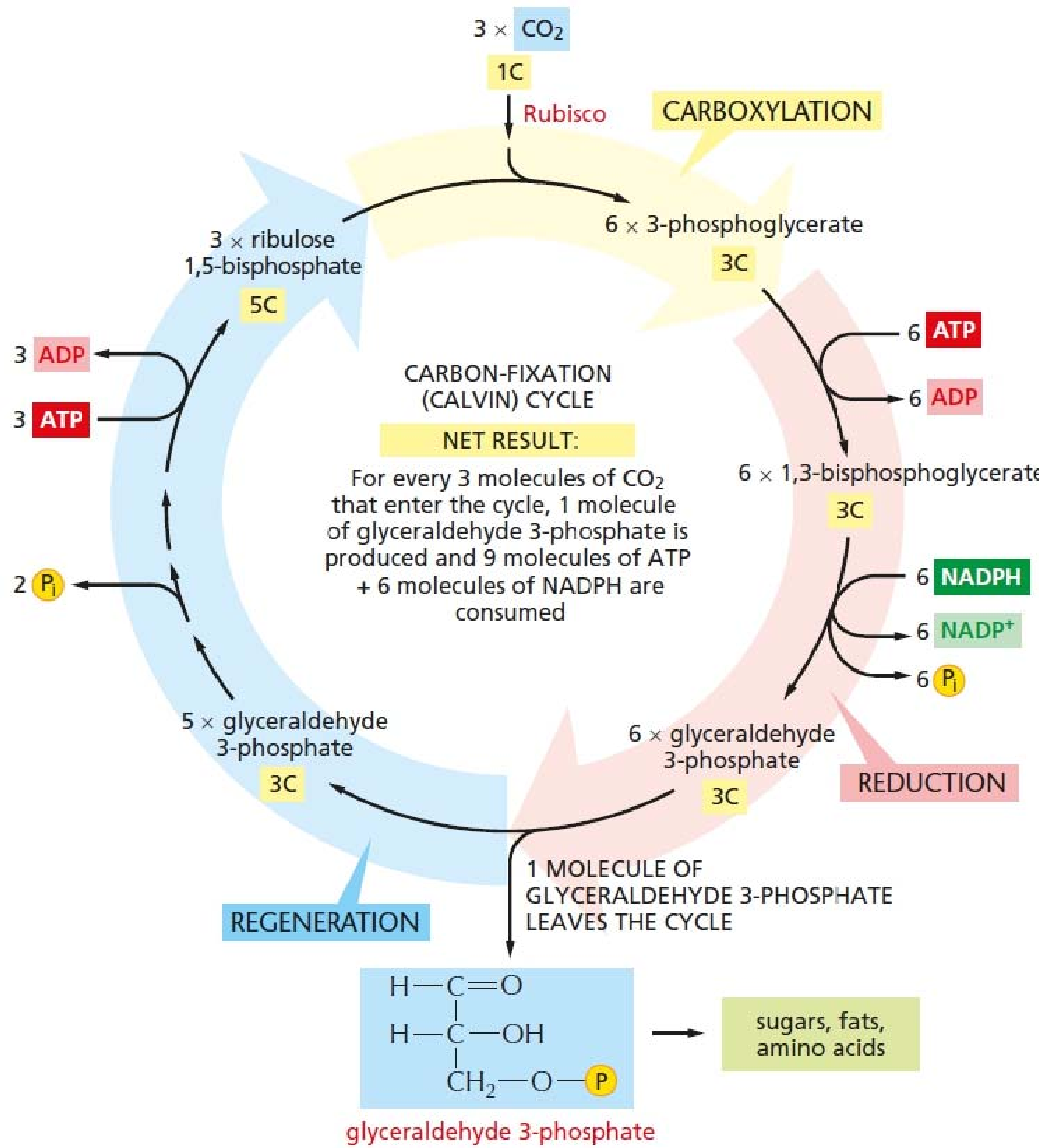


Figure 14-40 Molecular Biology of the Cell 6e (© Garland Science 2015)

ATP and NADPH are used to convert CO_2 into sugar



Summary mitochondria