Molecular Biology of the Cell
Fifth Edition

Chapter 14

1. The Genetic System of Mitochondria and Plastids
2. The Evolution of Electron-Transport Chains
Mitos, gr. Faden; Chondros, gr. Korn
Apart from the oxidation of pyruvate and fatty acids

**STARVATION CONDITIONS**
- use amino acids to fuel ATP production

**CELLS RELYING ON GLYCOLYSIS FOR RAPID ATP PRODUCTION**
- remove excess NADH from cytosol to further speed glycolysis

**MITOCHONDRIUM**

**CYTOSOL**

**CONDITIONS OF EXCESS**
- supply cytosol with excess citrate for synthesis of fatty acids and sterols
- supply cytosol with reducing power (as NADPH) for biosynthesis using excess mitochondrial reducing power

Figure 14-32  Molecular Biology of the Cell (© Garland Science 2008)
1. The Genetic Systems of Mitochondria and Plastids

- These two organelles are never made *de novo*, but are inherited by growth and division.

- Even in non-dividing cells, these organelles need to be replenished.

- Plastids and mitochondria contain genetic information:
  
  **Organelle Genome:**
  
  - rRNAs \(\rightarrow\) ribosomes
  - tRNAs
  - mRNA \(\rightarrow\) proteins (always...cytochrome oxidase)

  Genome needs to be replicated, inherited.
Euglenia gracilis stained with a mitotracker dye (green) and a DNA stain red

Note the reticular mitochondrial network with its nucleoids
1.1. Mitochondria and Chloroplasts contain complete genetic systems

- Biogenesis of these two organelles requires contribution of nuclear genes and organelle genome
- Unidirectional import of nuclear encoded proteins (99%!)
- Organelle protein synthesis resembles that of bacteria i.e., chloroplast ribosomes are very similar to that of *E. coli* sensitive to chloramphenicol etc.
  - protein synthesis starts with *N*-formyl methionine
Figure 14-53 Molecular Biology of the Cell (© Garland Science 2008)
1.2. Organelle growth and division determine the number of mitochondria and plastids in a cell

- In mammalian cells mitochondrial DNA makes up 1% of the total cellular DNA, but proportion higher in some plants or amphibian eggs (99%)

- Live cell images of mitochondria (mitotracker, membrane potential sensitive dyes) => dynamic organelles: fuse and divide (fission) constantly

- Thus, number and shape of mitochondria vary dramatically
  - In different cell types
  - Under different physiological conditions
  - Controlled by rates of fusion and fission
  - Large mass increase (5-10fold) upon exercise in skeletal muscle
<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>TISSUE OR CELL TYPE</th>
<th>DNA MOLECULES PER ORGANELLE</th>
<th>ORGANELLES PER CELL</th>
<th>ORGANELLE DNA AS PERCENTAGE OF TOTAL CELLULAR DNA</th>
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<tbody>
<tr>
<td>MITOCHONDRIAL DNA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>liver</td>
<td>5–10</td>
<td>1000</td>
<td>1</td>
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<tr>
<td>Yeast*</td>
<td>vegetative</td>
<td>2–50</td>
<td>1–50</td>
<td>15</td>
</tr>
<tr>
<td>Frog</td>
<td>egg</td>
<td>5–10</td>
<td>10⁷</td>
<td>99</td>
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<tr>
<td>CHLOROPLAST DNA</td>
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<td></td>
<td></td>
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<tr>
<td>Chlamydomonas</td>
<td>vegetative</td>
<td>80</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Maize</td>
<td>leaves</td>
<td>0–300**</td>
<td>20–40</td>
<td>0–15**</td>
</tr>
</tbody>
</table>

*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.
1.2. Organelle growth and division determine the number of mitochondria and plastids in a cell

- => Number of genome per organelle varies
- DNA organized in clusters, nucleosids
- Replication is random, generally not coordinated with the cell cycle
- *Genome can be circular or linear*
Topological complex fusion and fission involves a double membrane
Figure 14-54  Molecular Biology of the Cell (© Garland Science 2008)
1.3. Mitochondria and chloroplasts have diverse genomes

- Mitochondrial genome size similar to that of viruses:
  Range 6kb - 300kb

- Chloroplasts: 70-200kb

- Size of genome does not correlate with number of encoded proteins:
  - Human, 16kb, 13 proteins
  - Arabidopsis 22x larger, 32 proteins (2.5-fold)
  - Reclinomonas americana, 98 proteins (max.)

- Rickettsia prowazekii, small pathogenic bacterium, genome most closely resembles that of present-day mitochondria
Various sizes of mitochondrial genomes

- **Marchantia**
- **Schizosaccharomyces pombe**
- **Tetrahymena**
- **Reclinomonas**
- **Human**
- **Acanthamoeba**
- **Plasmodium**
- **Chlamydomonas**
1.4. Mitochondria and chloroplasts probably both evolved from endosymbiotic bacteria

- Prokaryotic character of organellar genetic systems suggests origin from bacteria

- **Endosymbiotic hypothesis:**
  - 1 Mia years ago
  - First eukaryotic cells were anaerobic
  - Established stable endosymbiotic relation with bacteria to employ their oxidative phosphorylation
  - Occurred while oxygen entered the atmosphere (due to photosynthesis by cyanobacteria)
  - Gene-transfer from organelle to nuclear DNA
    - Complex, different structures
    - May still continue today
Figure 14-58 Molecular Biology of the Cell (© Garland Science 2008)
1.4. Mitochondria and chloroplasts probably both evolved from endosymbiotic bacteria (2)

- Gene transfer was a gradual process:
  Reduction of mitochondrial genome over time
  Superoxide dismutase of chicken mitochondria resembles more that of bacteria than the cytosolic iso-enzyme does

- Core set of genes encoded by all mitochondrial genomes:
  Cytochrome oxidase subunit, cox1
  Cytochrome b, cob
  rRNAs, rns, rnl
1.5. Mitochondria have a relaxed codon usage and can have a variant genetic code

- Small genome size -> attractive target of early DNA sequencing projects

- Human mitochondrial genome: 16’560bp, 1981 sequenced

- Surprising features:
  1. *Dense gene packing*, fully coding for protein or r/tRNA
     - Little room for regulatory elements
  2. *Relaxed codon usage*, only 22 tRNAs
     - “2 out of 3” pairing, wobble position not discriminatory
  3. *Variant genetic code*, 4 of the 64 codons (34) have different meaning compared to nuclear codons
     - Random drift in small genomes but not large ones
     [explain?]
### Table 14-3 Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

<table>
<thead>
<tr>
<th>CODON</th>
<th>&quot;UNIVERSAL&quot; CODE</th>
<th>MAMMALS</th>
<th>INVERTEBRATES</th>
<th>YEASTS</th>
<th>PLANTS</th>
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<tr>
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<td>Leu</td>
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<td>Leu</td>
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<td>Leu</td>
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<tr>
<td>AGA</td>
<td>Arg</td>
<td>STOP</td>
<td>Ser</td>
<td>Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>AGG</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Red italics indicate that the code differs from the "Universal" code.*
1.6. Animal mitochondrial genomes contain the simplest genetic systems known

- Sequence comparison: Rate of nucleotide substitutions in mitochondrial genes 10 times higher than that of nuclear genes

- Why?
  - Fidelity of replication / repair low
  - Low selective pressure
  - Multiple copies
  - Others [?]
1.7. Some organelle genes contain introns

- Processing of mitochondrial pre-mRNAs

- Human: both strands are transcribed symmetrically at the same rate => 2 giant RNAs
  Extensive processing of both transcripts:
  nuclease cleavage etc., but 90% of the antisense transcript is being degraded again

- Mitochondrial mRNAs lack CAP structure but contain polyA tail

- Yeast and some plant mitochondrial mRNAs contain introns
  Also true for some plant chloroplast genes
  Some are self-splicing (≠ bacterial origin [?])
  Optional introns in yeast, i.e., strain-specific
1.8. The chloroplast genomes of higher plants contains about 120 genes

• More than 20 chloroplast genomes sequenced

• Are highly similar: functions for transcription, translation, photosynthesis, biogenesis of small molecules (amino acids, fatty acids, and pigments)

40 proteins of unknown function

• All protein are part of protein complex that also contains nuclear encoded subunits.....[paradoxically, why ?]

• Chloroplast genomes show striking similarities with bacterial genomes (transcription promoters, terminators
1.8. The chloroplast genomes of higher plants contains about 120 genes (2)

• Conclusions:

1. Chloroplast in higher plants arose from photosynthetic bacteria

2. Many of the original bacterial genes are now in the nucleus, for example ca 40 of the 60 ribosomal proteins
The organization of a chloroplast genome

2 copies of 16S and 23S rRNA

Figure 14-61 Molecular Biology of the Cell (© Garland Science 2008)
1.9. Mitochondrial genes are inherited by a Non-Mendelian mechanism

- Yeast as experimental model to probe mitochondrial function - why?
  - Because yeast can grow fermentative on glucose → ethanol without respiration (lethal in most other organisms...)
  - Or, it can grow on “non-fermentative” carbon sources; i.e. Ethanol, lactate, glycerol :: but then needs to respire

- Thus, by simply testing whether a strain or a mutant grows on glucose media compared to lactose media one can determine whether mitochondrial function/genome is intact

- mito− mutants are also called “petite”
Figure 14-62 Molecular Biology of the Cell (© Garland Science 2008)
1.9. Mitochondrial genes are inherited by a Non-Mendelian mechanism (2)

- Non-Mendelian = cytoplasmic inheritance of mitochondrial traits through stochastic mitotic segregation
- 4:0; 0:4 segregation instead of 2:2 segregation
- Recombination between nucleoids is rare, they are anchored to the inner mitochondrial membrane
1.11. Petite mutants in yeast demonstrate the overwhelming importance of the cell nucleus to mitochondrial biogenesis

- Yeasts mutants with large deletions in the mitochondrial genome, or those that completely lack the mitochondrial genome:
  - form small colonies, "cytoplasmic petite mutants" [?]
  - cannot grow on non-fermentable carbon sources

- BUT THEY CONTAIN MITOCHONDRIA!

- Contain virtually all mitochondrial proteins

- Similar for chloroplast in *Euglena* mutants (algae)
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1.10. Organelle genes are maternally inherited in many organisms

- Consequences of cytoplasmic inheritance:
  - Inheritance in yeast is biparental (both parents contribute)
  - In higher animals, egg contains much more mitochondria than the sperm - uniparental inheritance, maternal also true for humans
  - In 2/3 of higher plants, the chloroplast from the male pollen does not enter the zygote => chloroplast and mitochondria are maternally inherited
  - In those that enter: variegation can be observed, mixture between normal an non-functional chloroplasts
1.10. Organelle genes are maternally inherited in many organisms (2)

- **Variegation** of mitochondrial defects can also be observed in human:
  - i.e., oocyte contains mixture of normal and defective mitochondria -> offspring with defects in muscle and nervous tissue functions

- **Inheritance**: daughters produce mutant offspring but not the sons
1.12. Mitochondria and plastids contain tissue-specific proteins that are encoded in the cell nucleus

- Mitochondria can have specialized functions in particular cell types:
  
  - Urea cycle in liver, nuclear encoded enzymes synthesized and imported into liver mitochondria
  
  - Tissue specific component of electron transport chain skeletal muscle specific subunit of cytochrome oxidase
1.13. Mitochondria import most of their lipids; chloroplast make most of theirs

- Biosynthesis of mitochondria and chloroplasts requires lipids in addition to proteins and nucleic acids
- Chloroplast make their lipids and have a bacterial type of fatty acid synthetase
- In contrast, mitochondria need to import most of their lipids, particularly phosphatidylcholine and phosphatidylserine, PS decarboxylase is located in mitochondria
- Cardiolipin (“double” phospholipid), makes up 20% of the inner mitoch. membrane, synthesized locally
1.14. Mitochondria may contribute to aging of cells and organisms

- At the end of the electron transport chain, **cytochrome oxidase** reduces $O_2$ to $H_2O$

- Superoxide, $O_2^-$, is produced as an intermediate and escapes at a slow rate (1 in every 2000 $e^-$ transfers)

- Superoxide damage is prevented by:
  - *superoxide dismutase* (SOD): $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
  - *catalase* $2H_2O_2 \rightarrow 2H_2O + O_2$
  - or *glutathione peroxidase* $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$

- 90% of $O_2^-$ is formed inside mitochondria, have their own SOD and glutathione peroxidase

- MnSOD mutant mice die early
1.14. Mitochondria may contribute to aging of cells and organisms (2)

- Mn DNA is 10-fold more oxidized than nuclear DNA

- “Vicious cycle” hypothesis to explain why cells and organisms age time-dependent accumulation of oxidative damage

- But mice with only half the level of MnSOD live as long as normal animals, even though they clearly accumulate more oxidative damage
1.15. Why do mitochondria and chloroplasts have their own genetic systems?

- Peroxisomes and lysosomes have no genome of their own.

- Maintaining a genetic system is costly; for example, nucleus requires 90 genes only to maintain mitochondrial genome.

- Cox1 and cob are present in all mitochondria genomes; large proteins, hydrophobic, many TMDs, so may need to cotranslationally insert into the inner mitochondrial membrane and cannot be synthesized in the cytosol and then imported.

- Alternative: evolutionary dead-end, because transfer to the nucleus has stopped, due to alternative codon usage...
2. The Evolution of Electron-Transport Chains

- How did the main energy-generating components arise? ATP synthase, redox-driven H$^+$ pumps, photosystems

- Fundamental mechanism to generate energy from: light or oxidation of glucose are the same, through synthesis of ATP
2.1. The earliest cells probably used fermentation to produce ATP

- First living cells arose $3 \times 10^9$ years ago when the earth was about 1 Mia year old.

- Environment lacked $O_2$, **reducing**, rich in geochemically produced organic molecules.

- Energy through fermentation, i.e. oxidation of an organic molecule, e- flow via NADH to an acceptor which thereby becomes reduced (harnessing of redox potential).

- Excretion of metabolic waste (organic acids, i.e. lactic acid).
2.2. Electron transport chains enabled anaerobic bacteria to use nonfermentable molecules as their major source of energy

- Early fermentation would have produced ATP and NADH
- But the metabolic activity of the organism must have changed the environment (depletion of substrate, decrease of pH due to metabolite excretion)
- => evolution of new pathways:
  - *Stage 1*: proton pumping ATPase which, when run in reverse, could synthesize ATP
  - *Stage 2*: energy-independent H\(^+\) pumps as used in the electron transport chain
  - *Stage 3*: H\(^+\) pumping electron transport chains
Generation of a $H^+$-transmembrane potential

-420 mV

+30 mV

Figure 14-67  Molecular Biology of the Cell (© Garland Science 2008)
Figure 14-68  Molecular Biology of the Cell (© Garland Science 2008)
2.3. By providing an inexhaustible source of reducing power, photosynthetic bacteria Overcame a major evolutionary obstacle

- Evolutionary steps (1-3) solved major problems of
  - maintaining neutral cytosolic pH
  - produce energy

but, depletion of substrate would still be a problem

- => find alternative sources for carbohydrates:
  - fixation of atmospheric CO$_2$ to CH$_2$O

- Strong electron donors for reverse electron flow to drive
  NADH dehydrogenase $\rightarrow$ NADPH for carbon fixation

- Major breakthrough with evol. of photochemical reaction centers
Electron transport pathways in present-day bacteria
2.4. The photosynthetic electron-transport chains of cyanobacteria produced atmospheric oxygen and permitted new life-forms

- Development of cyanobacteria, ca 3 Mia years ago - use water as the electron source for $CO_2$ fixation/reduction

- Evolution of water splitting enzyme and second photosystem to bridge gap in redox potential between water and NADPH

- Combination of photosystem I from green bacteria with PS II from purple bacteria

- $\Rightarrow$ oxygen entered the atmosphere, toxic! kills anaerobic bacteria
Electron flow in green sulfur bacteria

Figure 14-70 Molecular Biology of the Cell (© Garland Science 2008)
2.4. The photosynthetic electron-transport chains of cyanobacteria produced atmospheric oxygen and permitted new life-forms (2)

- Increase in atmospheric $O_2$ was slow and was buffered by $Fe^{2+}$ content of early seas
  -> $Fe^{3+}$ precipitation visible in sediments 2.7-2 Mia years ago

- => availability of $O_2$ led to evolution of respiratory chain, cytochrome oxidase

- Many present-day purple bacteria can switch between photosynthesis and respiration
Major events during the evolution of life on Earth

- **Formation of the Earth**
- **Formation of oceans and continents**
- **First living cells**
- **First photosynthetic cells**
- **First water-splitting photosynthesis releases O₂**
- **Start of rapid O₂ accumulation**
- **Origin of eukaryotic photosynthetic cells**
- **Aerobic respiration becomes widespread**
- **First multicellular plants and animals**
- **First vertebrates**
- **Present day**

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