Mitochondrial DNA

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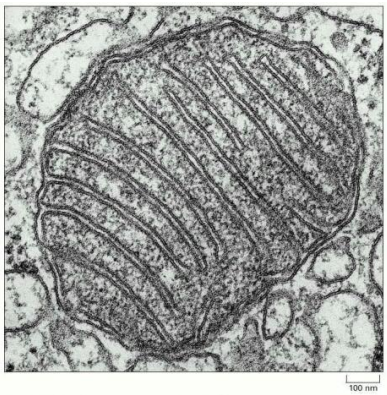
The Mitochondrion

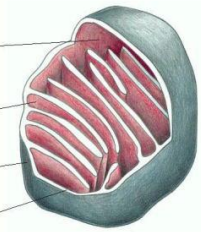


Matrix. This large internal space contains a highly concentrated mixture of hundreds of enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle. The matrix also contains several identical copies of the mitochondrial DNA genome, special mitochondrial ribosomes, tRNAs, and various enzymes required for expression of the mitochondrial genes.

Inner membrane. The inner membrane *(red)* is folded into numerous cristae, greatly increasing its total surface area. It contains proteins with three types of functions: (1) those that carry out the oxidation reactions of the electron-transport chain, (2) the ATP synthase that makes ATP in the matrix, and (3) transport proteins that allow the passage of metabolites into and out of the matrix. An electrochemical gradient of H^+, which drives the ATP synthase, is established across this membrane, so the membrane must be impermeable to ions and most small charged molecules.

Outer membrane. Because it contains a large channel-forming protein (called porin), the outer membrane is permeable to all molecules of 5000 daltons or less. Other proteins in this membrane include enzymes involved in mitochondrial lipid synthesis and enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix.

Intermembrane space. This space *(white)* contains several enzymes that use the ATP passing out of the matrix to phosphorylate other nucleotides.



The Mitochondrion

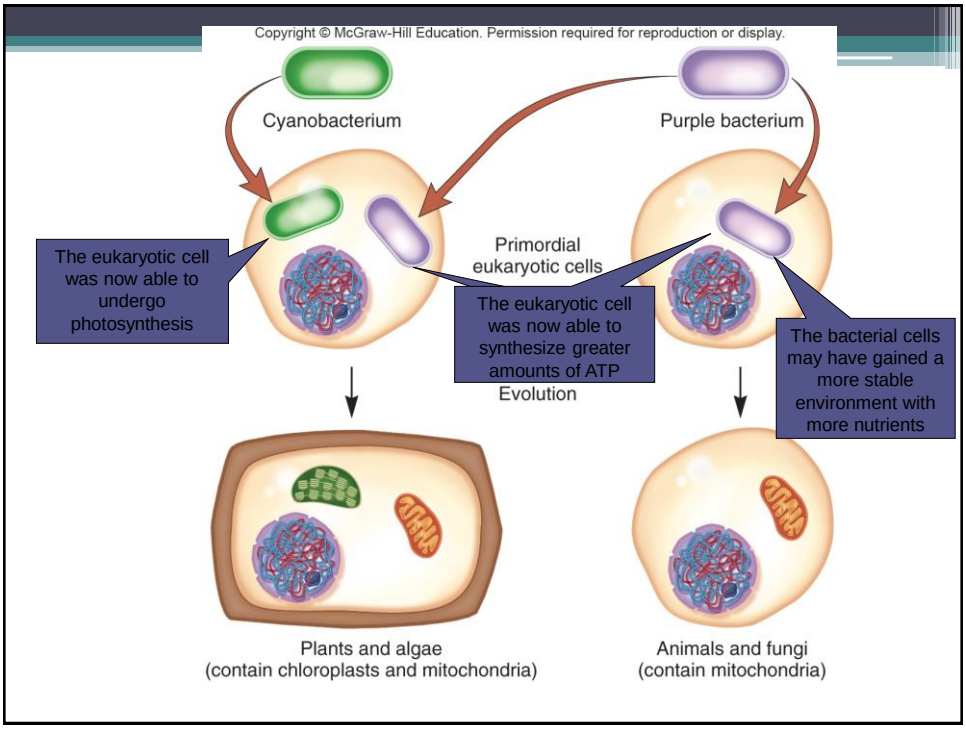
- They are the sites of cellular respiration, which is the process of generating ATP by extracting energy from sugars, fats and other fuels with the help of oxygen
- It is enclosed by two membranes (inner and outer) but is not part of the endomembrane system.
- One of the membranes is highly folded (these infoldings are called the cristae) inside the other.
- The semi-fluid that fills the inside of the mitochondrion is called the matrix
- It is comparable in size to a whole bacterial cell
- It contains its own DNA and ribosomes and therefore can code for and synthesize some of their own proteins
- Some of the reactions that take place inside the mitochondrion are tricarboxylic acid (TCA) cycle, fat oxidation, ATP generation

The Mitochondrion continued

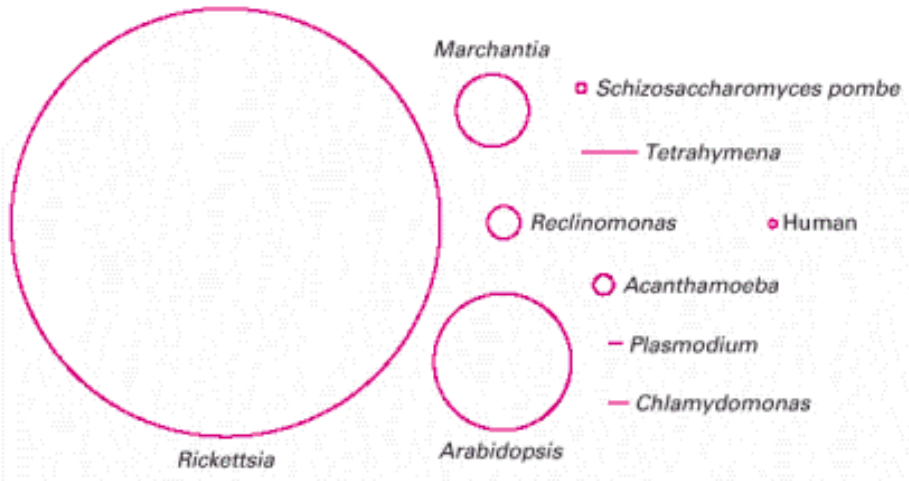
- They are about 1- 10 μm long
- The number and location of the mitochondria can be related directly to their role in the cell and also by species. The yeast might have only one. The liver cells of humans contain about 500 – 1000 mitochondria.
- They move around, change their shape, fuse and divide.
- Plants also have mitochondria. Can you guess why?
- Why is the inner membrane folded? What advantage would this give to the mitochondrion?

Localization of functions within the mitochondrion

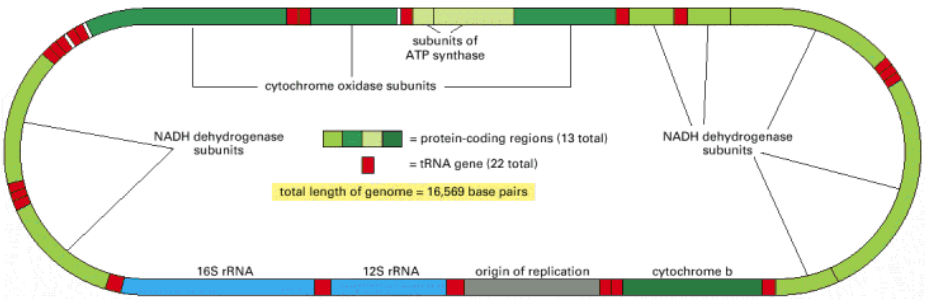
Membrane or Compartment	Functions
Outer membrane	Phospholipid synthesis Fatty acid desaturation Fatty acid elongation
Inner membrane	Electron transport Oxidative phosphorylation Transport of metabolites
Intermembrane space	Phosphorylation of nucleotides
Matrix	Pyruvate oxidation TCA cycle β – oxidation of fats DNA replication RNA synthesis Protein synthesis



Human Mitochondrial genome compared

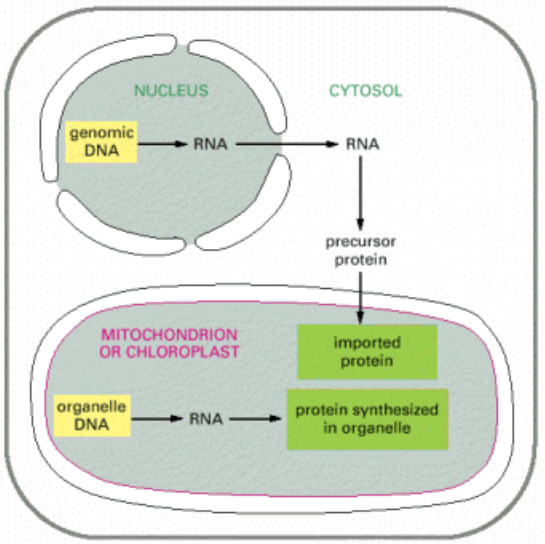


Human mitochondrial genome

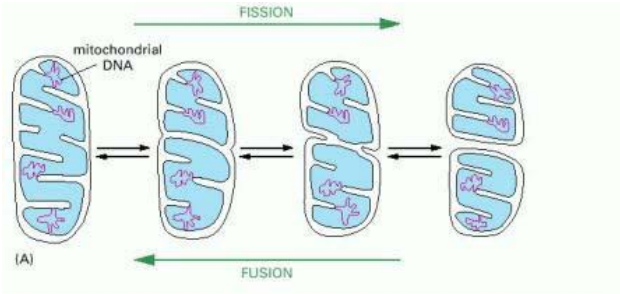


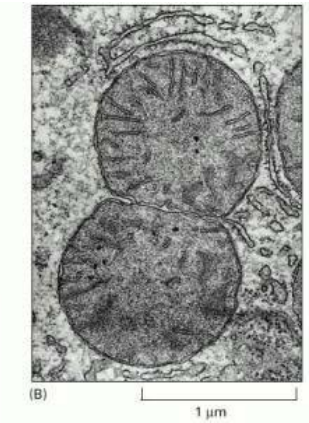
37 genes. The proteins and RNA produced are only 5% of the total needed by the mitocondrion. 13 polypeptides needed for the electron transport system.

Mitochondria proteins

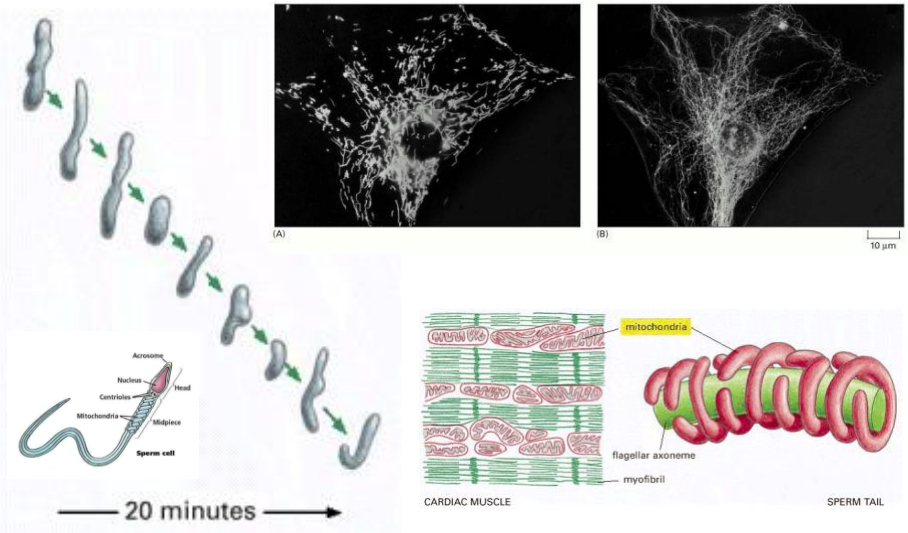


Fusion and fission of Mitochondria

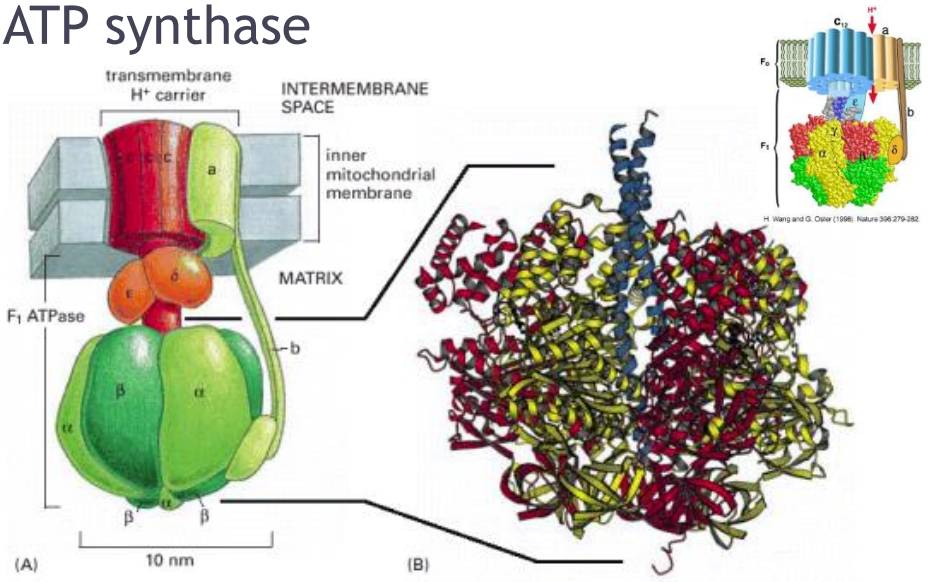




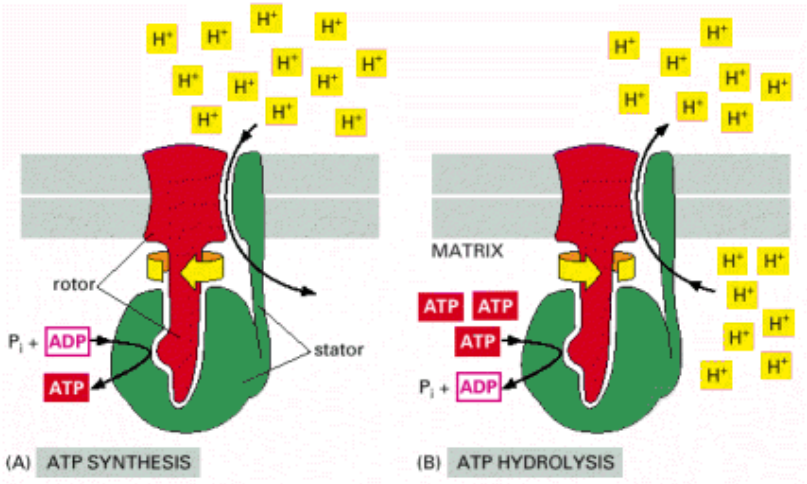
Mitochondria locations and different shapes



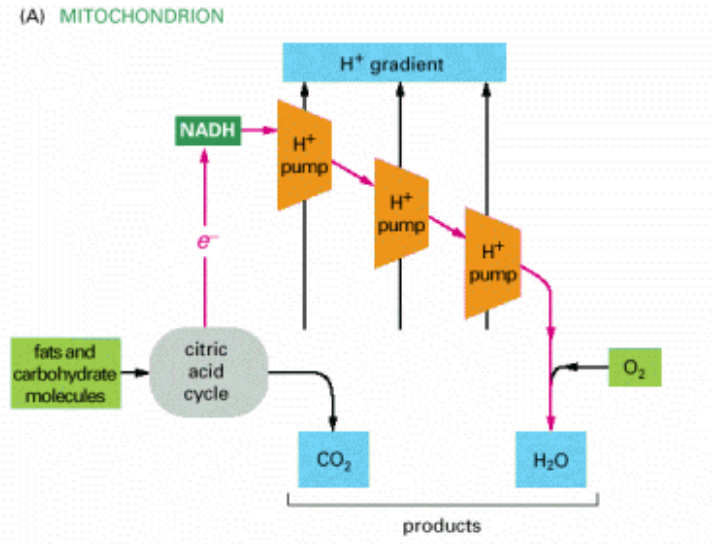
ATP synthase



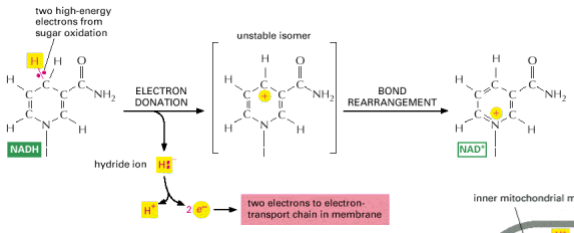
ATP Synthase can be reversed

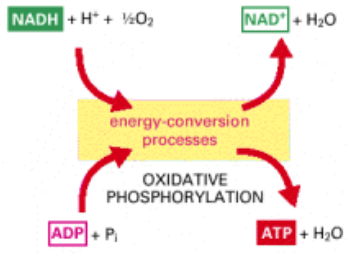


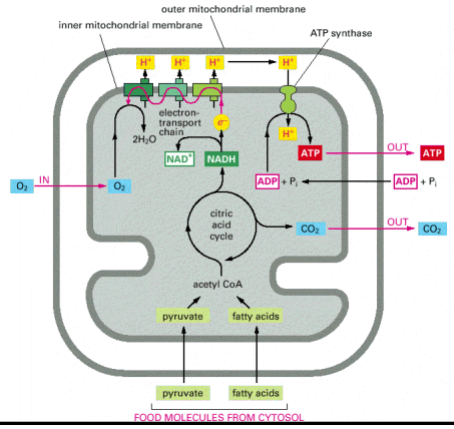
Electron transfer



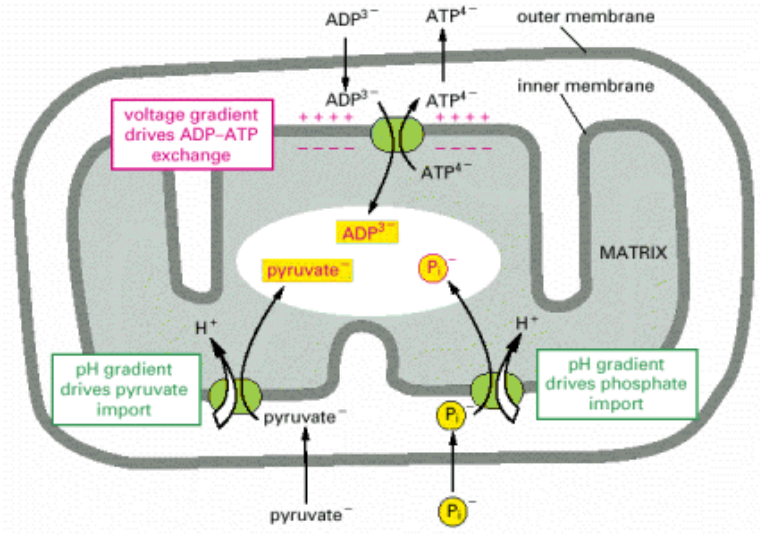
How is the electron used ?

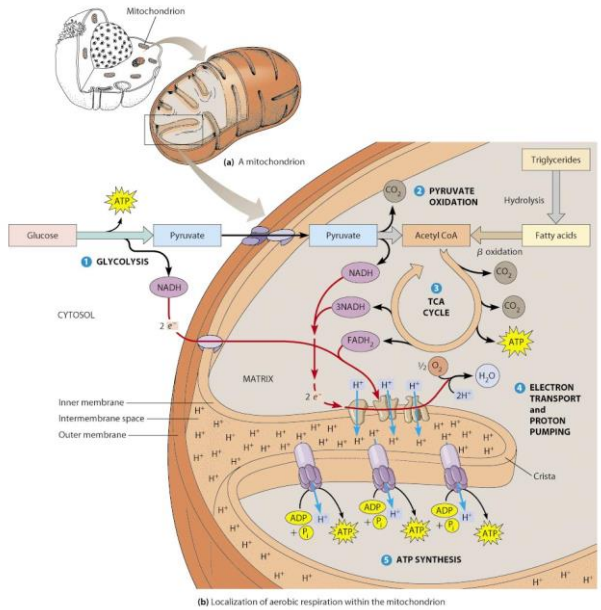






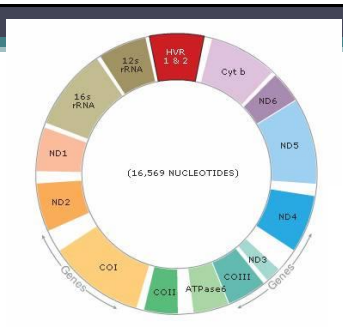
Everything comes together





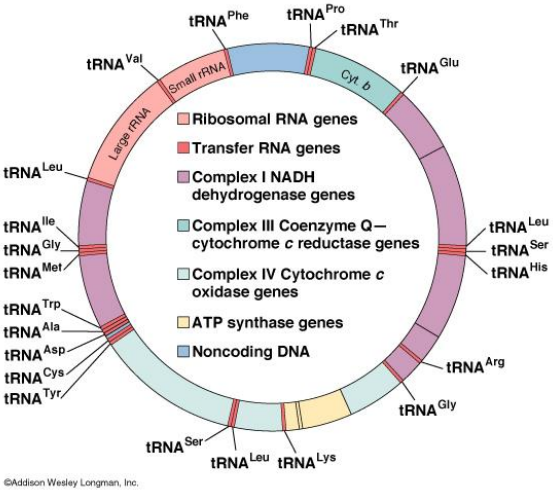
Mitochondrial DNA

- There are ten to hundreds of mitochondria per cell.
- The human mitochondrial genome (mtDNA) is contained on a single circular chromosome with 16,569 basepairs.
- Each mitochondrion contains 5 to 10 copies of the mitochondrial chromosome.
- Circular, double stranded genome with no recombination.
- Many mitochondria are homoplasmic.
- Very little non-coding regions are present. About 90% of the genome is made up of genes. The remaining 10% are the control regions for those genes.
- The genetic code used in the mitochondria is slightly different than the nuclear genetic code.
- Mitochondrial DNA has a higher mutation rate. Why would this be?

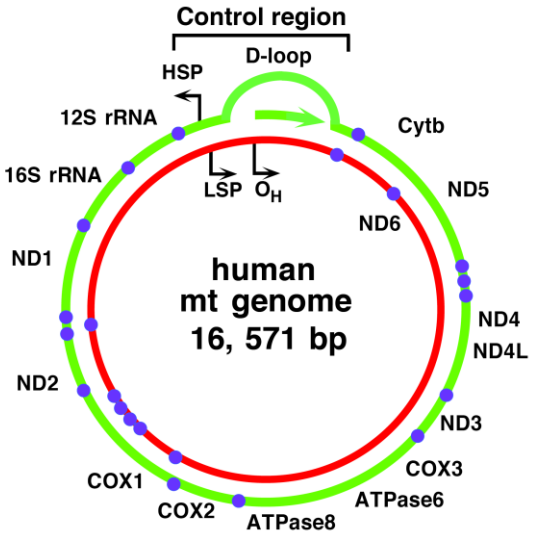


- 2 ribosomal RNAs
- 22 transfer RNAs
- 13 polypeptides involved in ATP synthesis
- 80-90 polypeptides of the oxidative phosphorylation complex are produced by the nuclear genome

Most of the 1500 mitochondrial proteins are prodcued by the nuclear genome.



Organization of the mitochondrial genome



- tRNAs

D-loop: displacement loop, central control region
HSP and LSP: heavy- and light- strand promoters for transcription

Mitochondrial D-loop (Control Region)

- Contains single site for origin of replication
- Contains promoters for transcription
  - L strand: 9 genes
  - H strand: 28 genes
- Control region highly polymorphic compared with remainder of mtDNA

mtDNA has its own tRNA and rRNA

- 37 genes, these genes have no introns. (Does this make sense?)
  - 13 protein-coding
  - 2 rRNA
  - 22 tRNA
- More than 80 proteins needed for oxidative phosphorylation come from the nuclear genome.

mtDNA versus nuclear DNA

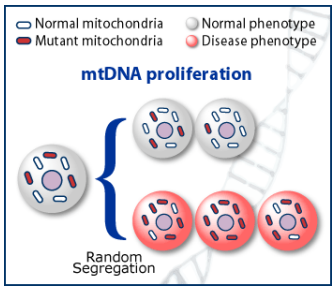
mtDNA	Nuclear DNA
▫ 1 circular chromosome ▫ 16.6 kb ▫ 37 genes	▫ 22 autosomes ▫ X & Y sex chromosomes ▫ 3.1 X 10^9 bp = 3,100 Mbp = 3,100,000 kb ▫ approximately 30,000 genes

Inheritance of mtDNA

- Inherited from the mother.
- Each ovum contains approximately 100,000 mitochondria (in contrast the sperm contains less than 100. What happens to these mitochondrial sperm?)
- When a cell is dividing, the mitochondria of the mother cell are divided randomly between the daughter cells. (stochastic segregation).

Distinctive pattern of inheritance for mutations in mtDNA

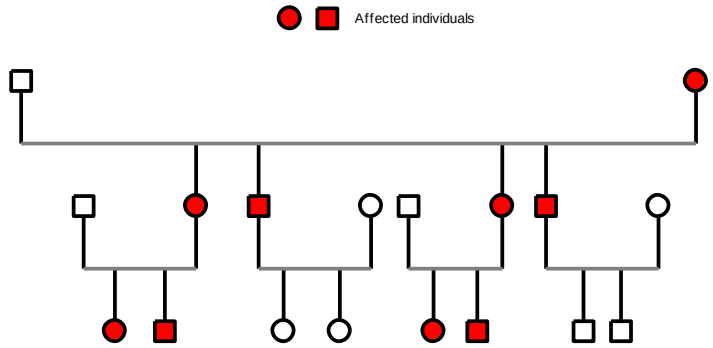
- These distinctive patterns are due to 3 features of the mitochondrial chromosomes:
  - Replicative segregation
  - Homoplasmy and heteroplasmy
  - Maternal inheritance

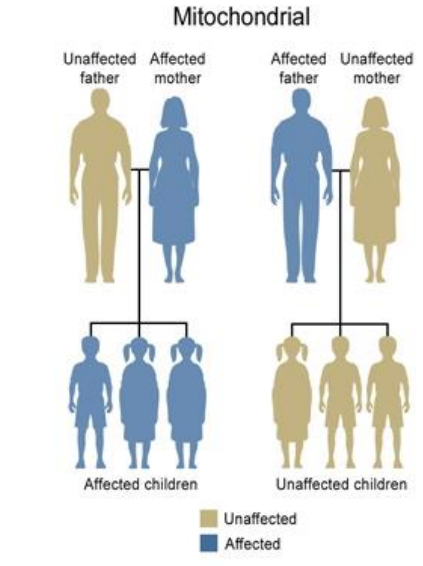


Heteroplasmy

- **Heteroplasmy**: more than one kind of mitochondrial DNA present in cell. Leads to variable disease symptoms due to
- Differentiation of good to bad mitochondria in different cells.
- Affected cells may contain a mixed mitochondrial population.
- Threshold effect

Typical pedigree of mitochondrial inheritance





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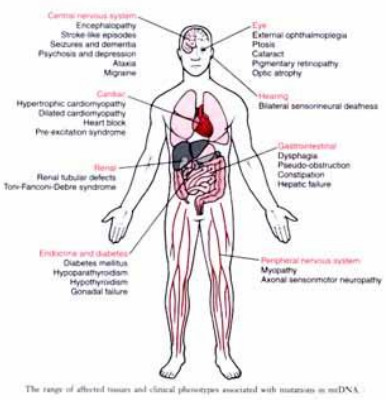
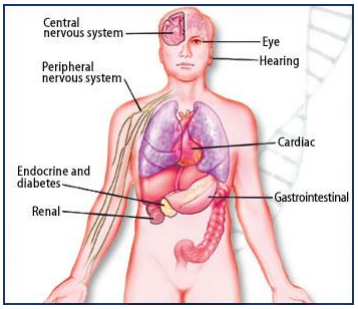


Mitochondrial Disease

- Relatively unprotected and unrepaired, mtDNA suffers more than ten times the damage that nuclear DNA does.
- About 150 different types of hereditary mitochondrial defects are known.
- Which cell-types should be most affected by mtDNA mutations?
  - Non-dividing cells
  - Constitutively oxidative tissues
    - heart, brain, kidney
  - Episodically oxidative tissues
    - skeletal muscle



Types of mutations in mtDNA

- Missense mutations in the coding regions of proteins that alter the activity of an oxidative phosphorylation protein.
- Point mutations in tRNA and rRNA genes that impair mitochondrial protein synthesis.
- Rearrangements that generate deletions or duplications of the mtDNA molecule. (generally somatic)

Heteroplasmy and characteristics of mtDNA genetic disorders

- The risk of transmission of deleted mtDNA molecules is low but carriers of heteroplastic mtDNA point mutations and or of mtDNA duplications do transmit some mutant mtDNA molecules
- The number of molecules within an oocyte is reduced before being subsequenty amplified to the large number mentioned before (mitochondrial genetic bottleneck).
- The mother with a high proportion of mutant mtDNA are more likely to have affected offspring compared to mother with a lower proportion.

Mutations in tRNA and rRNA in mtDNA

- Especially importnant as they demonstrate that not all disease causing mutations occure in genes that encode proteins.
- 90 pathogenic mutations in 20 of the 22 tRNA genes.
- Most common cause of the oxidateive phosphorylation abnormalities.
- Wobble is effected.
- May cause MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes) and sensorineural prelingual deafness.

Mitochondrial disease caused by deletions

- Deletions 1-9 kb with at least 1 tRNA gene
- Cells homoplasmic for deletions do not survive
- Progressive external ophthalmoplegia
  - gradual loss of eye movement control
- Kearns Sayre Syndrome
  - progressive external ophthalmoplegia, eye pigment disorders, heart disease, cerebellar dysfunction, diabetes, hearing loss, muscle weakness
- Pearson Syndrome
  - Childhood onset of anemia, dysfunction of pancreas, liver, kidneys (usually fatal)
- Both of the above disease are mostly from sporadic somatic mutations. Around 5% of cases result from the transmission of maternal deletions. Why the low transmission frequency?

Mitochondrial disease caused by point mutations

- Leber hereditary optic neuropathy (LHON)
  - degeneration of optic nerve, rapid onset of blindness
  - mostly affects men in their 20s.
  - There is a strong gender bias (50% of male carriers have visual loss vs 10 % of females). WHY?
  - individuals are usually homoplasmic for mutation
  - mutation in one of subunits of complex I (NADH-ubiquinone) of electron transport chain
  - partial penetrance

Autosomally transmitted deletions in mtDNA

- Phenotype resembles chronic progressive external opthalmoplegia.
- One protein encoded by one such protein is called Twinkle (mitochondrial-specific DNA polymerase γ. Loss of function of this is associated with both dominant and recessive multiple deletion syndromes.
- mtDNA depletion syndrome. This is due to mutations in any of the six nuclear genes that are required to maintain nucelotide pools in the mitochondrion.

Mitochondrial conditions

- They can be due to nuclear or mtDNA problems.
- They can be very variable even within the same family especially with mtDNA problems.
- May present at any age (In general, nuclear problems present in early childhood and mtDNA problems present in late childhood and adulthood.)
- Mitochondrial conditions can often represent as neuropathies, myopathies, diabetes, endocrionpaties and sometimes as other systemic manifestations.
- Some affect single organs like **Leber hereditary optic neuropathy** others present in multiple systems.
- **Kearns-Sayre syndrome** is an example of a mitochondrial genetic condition. Thought to be due to the rearrangement of mtDNA
- Some forms of diabetes and deafness thought to be due to point mutations in the mtDNA
- **Leigh syndrome** is due to a problem in the respiratory chain.

Human Mitochondrial Diseases

TABLE 5.5

Examples of Human Mitochondrial Diseases

Disease	Mitochondrial Gene Mutated
Leber hereditary optic neuropathy	A mutation in one of several mitochondrial genes that encode respiratory chain proteins: *ND1, ND2, CO1, ND4, ND5, ND6,* and *cytb*
Neurogenic muscle weakness	A mutation in the *ATPase6* gene that encodes a subunit of the mitochondrial ATP-synthetase, which is required for ATP synthesis
Mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes	A mutation in genes that encode tRNAs for leucine and lysine
Mitochondrial myopathy	A mutation in a gene that encodes a tRNA for leucine
Maternal myopathy and cardiomyopathy	A mutation in a gene that encodes a tRNA for leucine
Myoclonic epilepsy with ragged-red muscle fibers	A mutation in a gene that encodes a tRNA for lysine

mtDNA and aging

- mtDNA somatic deletions accumulate with age
- Especially prevalent in non-dividing cells
- Are mtDNA somatic deletions associated with neurological and muscular function loss?
  - Noted accumulation of partially deleted mtDNAs in heart and brain
  - may be partially causative of degenerative disorders: Parkinson's, Alzheimer's, Huntington's