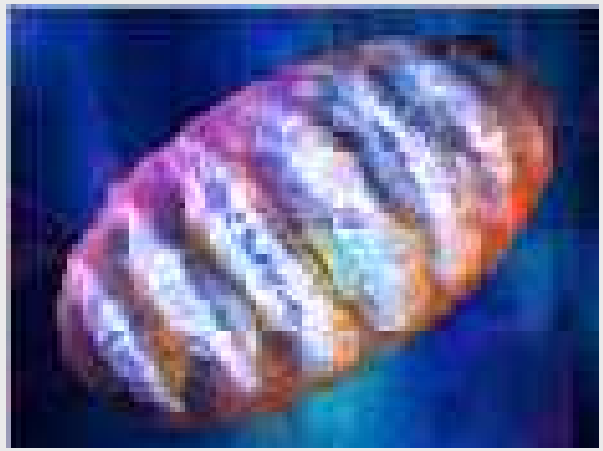
Mitochondrial Disorders



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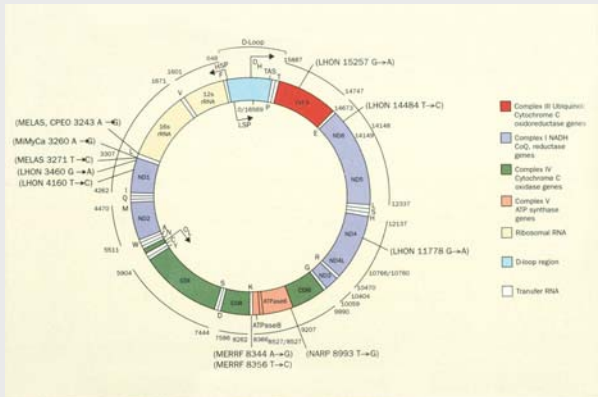
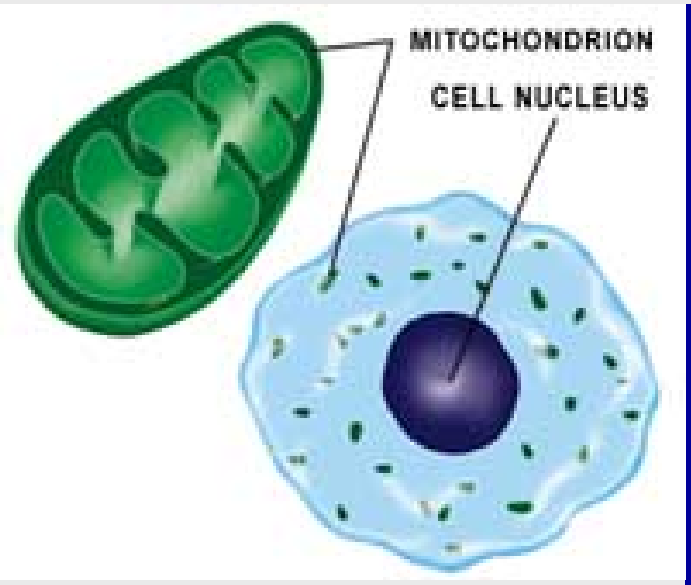


Mitochondrion in Cells



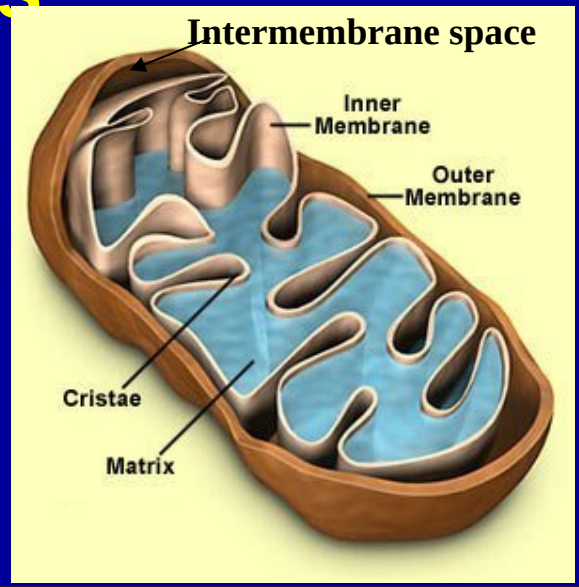
Each cell contains, on average, 500 to 2,000 mitochondria which are responsible for supplying energy needs

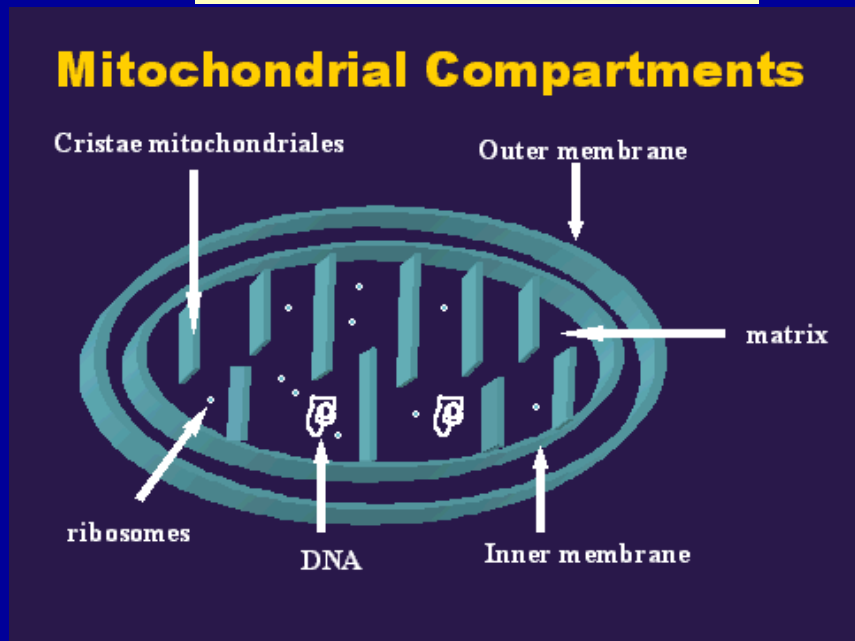
↓

Power House of cells

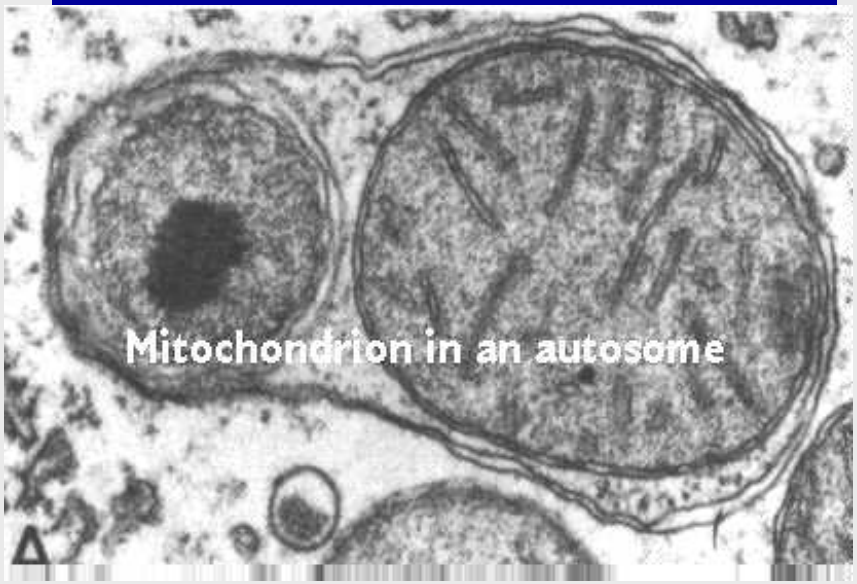
Structural features of mitochondria: 4 compartments

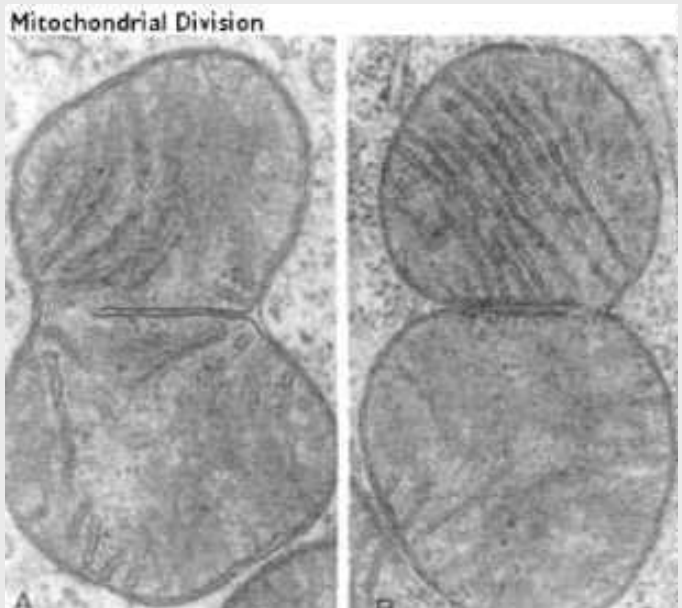
- Outer membrane
- Inner membrane: Composed predominantly of cardiolipin
- Intermembrane space: Between outer & inner membranes
- Matrix: Region inside inner membrane

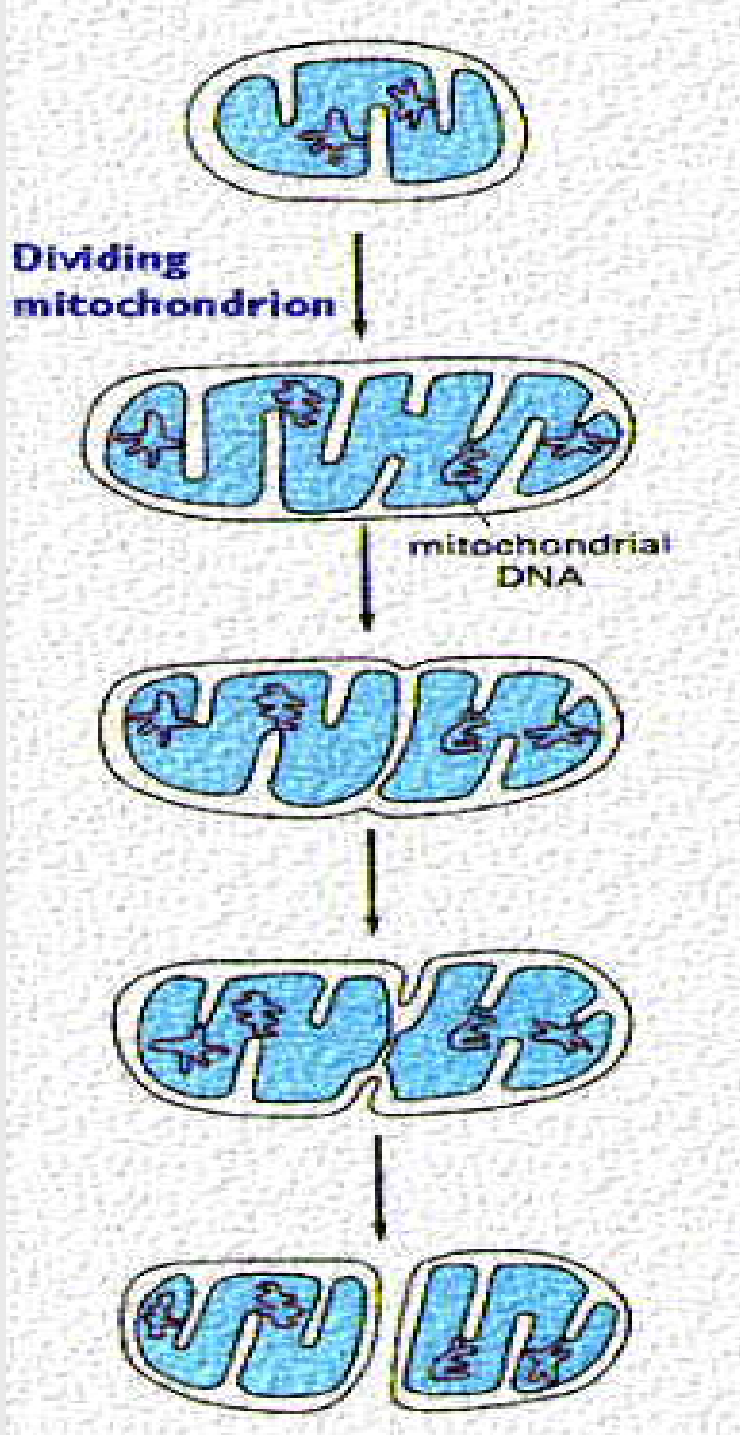




Multiplication of Mitochondria

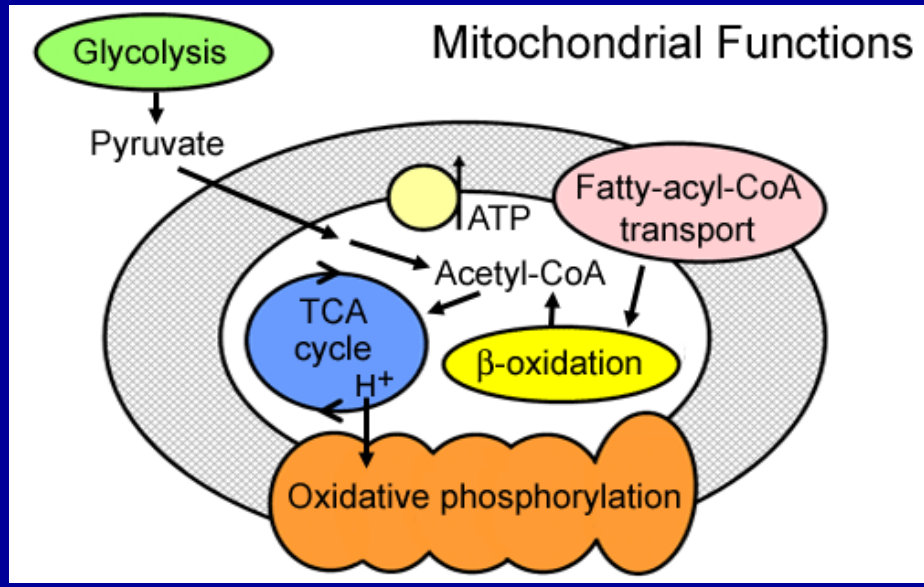


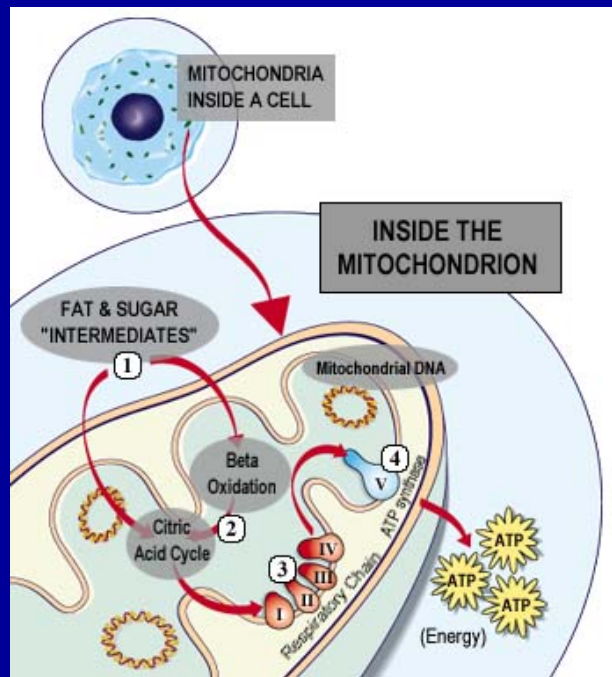




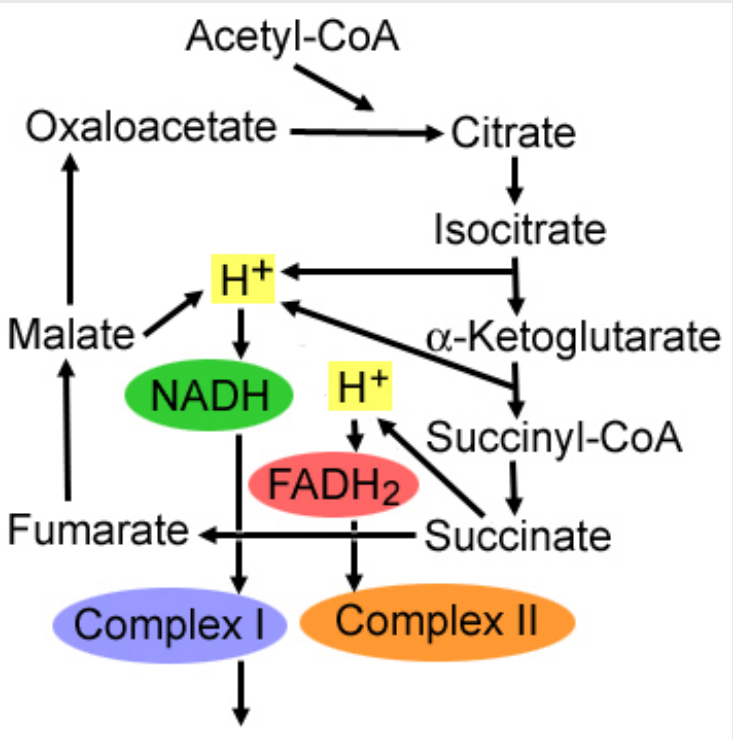
Functions of mitochondria

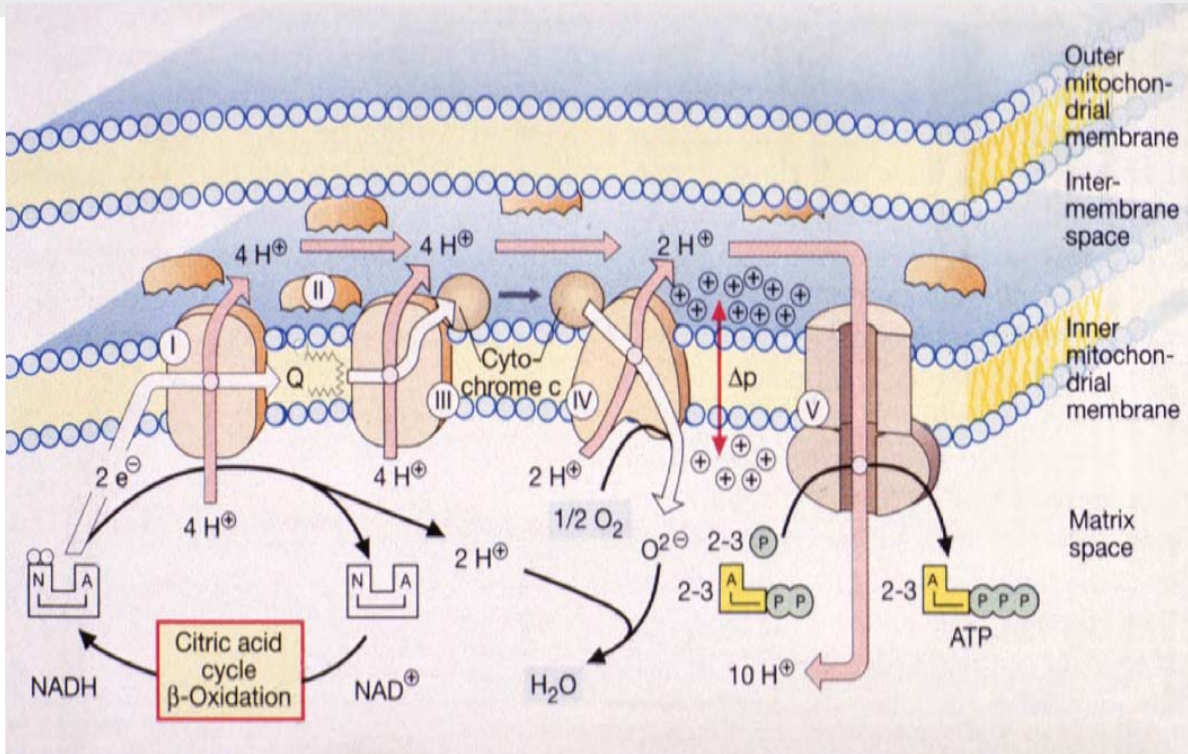
- Pyruvate oxidation
- Krebs cycle
- Metaboloism: Amino acids;Fatty Acids; Steroids
- Generation of Energy as (ATP): Via
  - Electron-transport chain
  - Oxidative Phosphorylation (Respiratory chain)
  - Location: Inner mitochondrial membrane





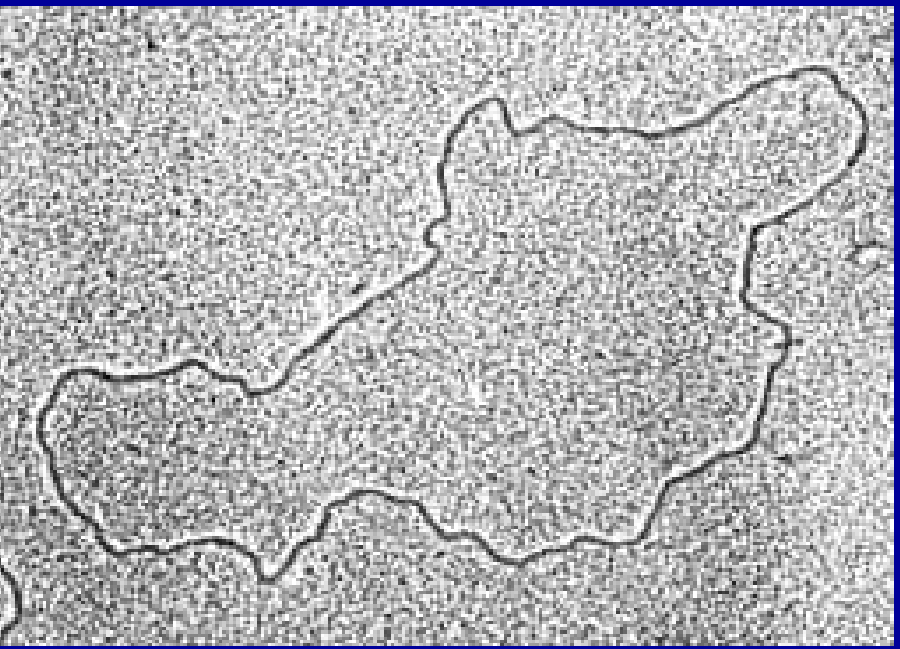
TCA cycle and Electron Transport chain in Mitochondrial Matrix



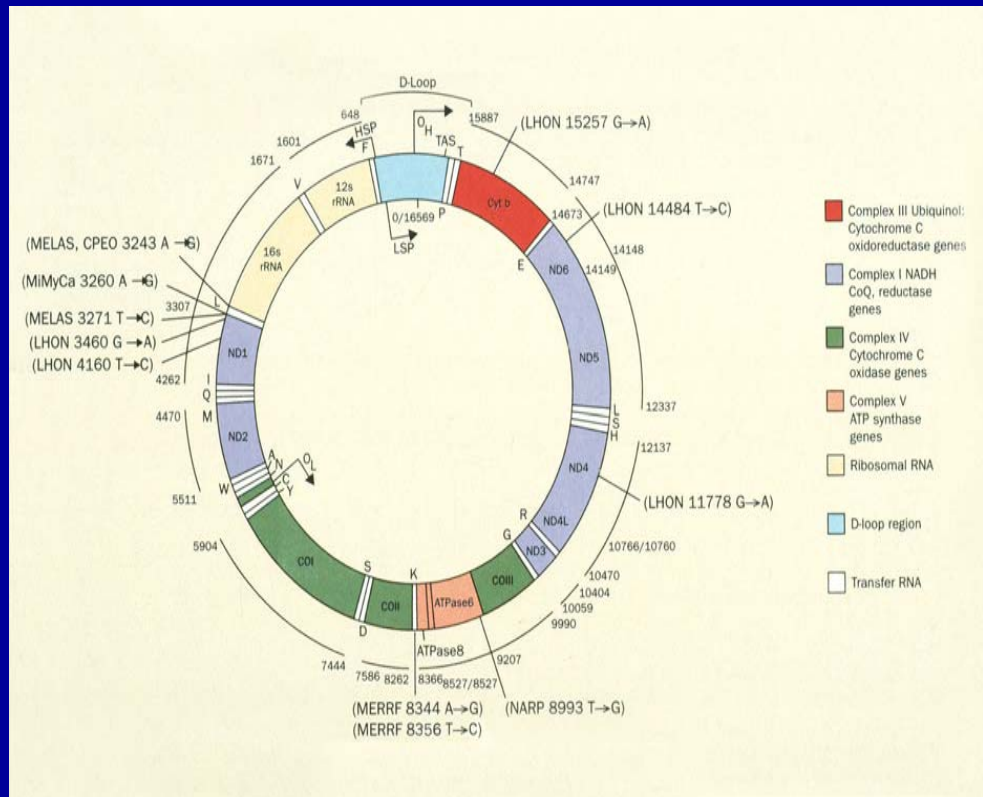


Mitochondrial DNA (Mt DNA)

- Only organelle other than nucleus with own DNA, in humans
- Different structure than nuclear DNA

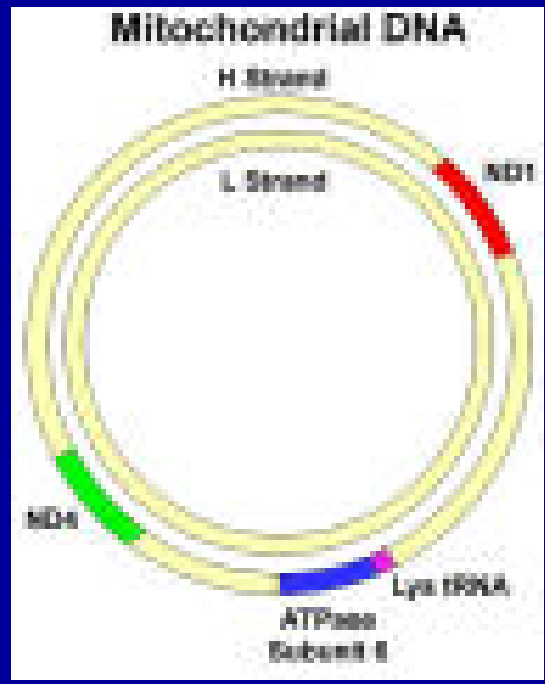


Circular Mt DNA

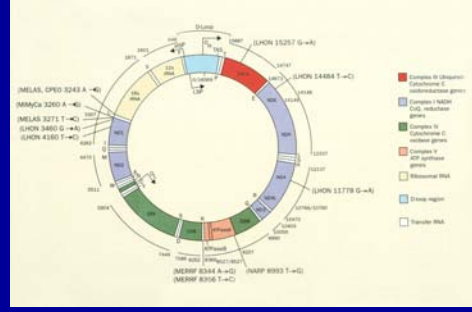


Structure of MtDNA

- Double-stranded, circular molecule: Except for D-loop which is triple stranded (Contains extra 7S DNA)
- 16,569 nucleotide pairs
- Copies: 2 to 10 in each mitochondrion
- Polyplasmy: > 1,000 in each cell
- Strands
  - Heavy (H) strand
    - Rich in guanines
    - 28 genes
  - Light (L) strand
    - Rich in cytosines
    - 9 genes: ND6; 8 tRNAs

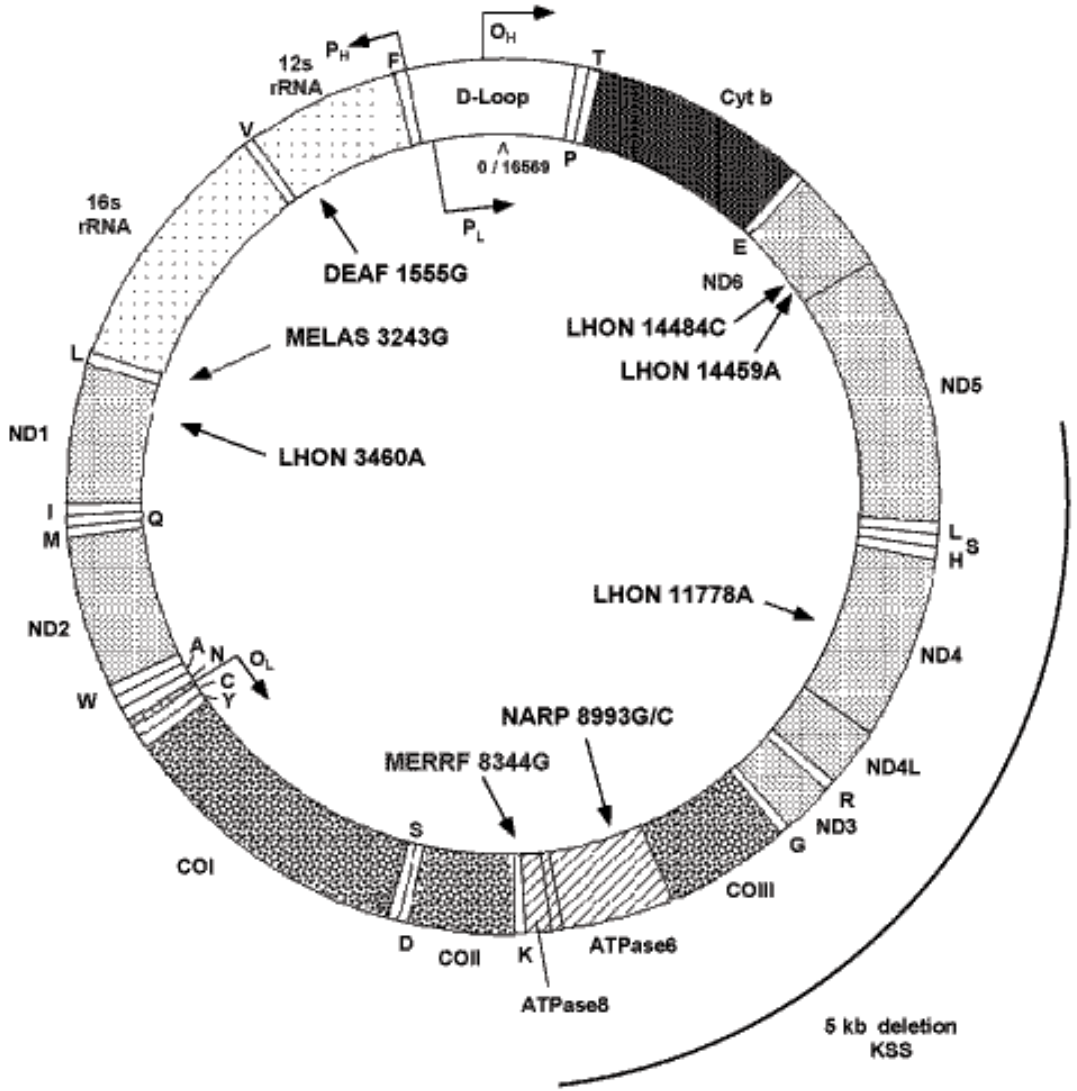


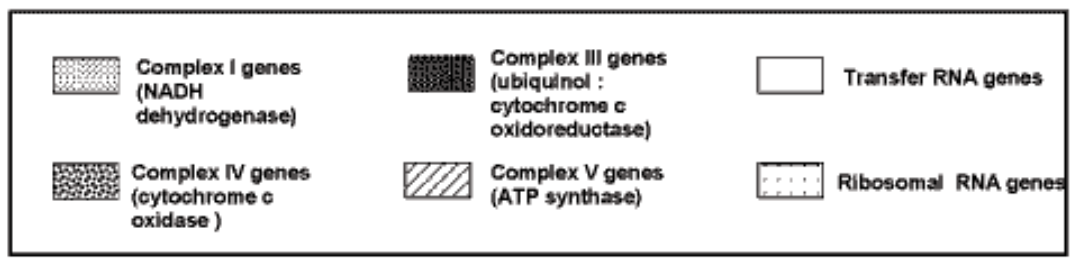
Genes and control regions on Mitochondrial DNA



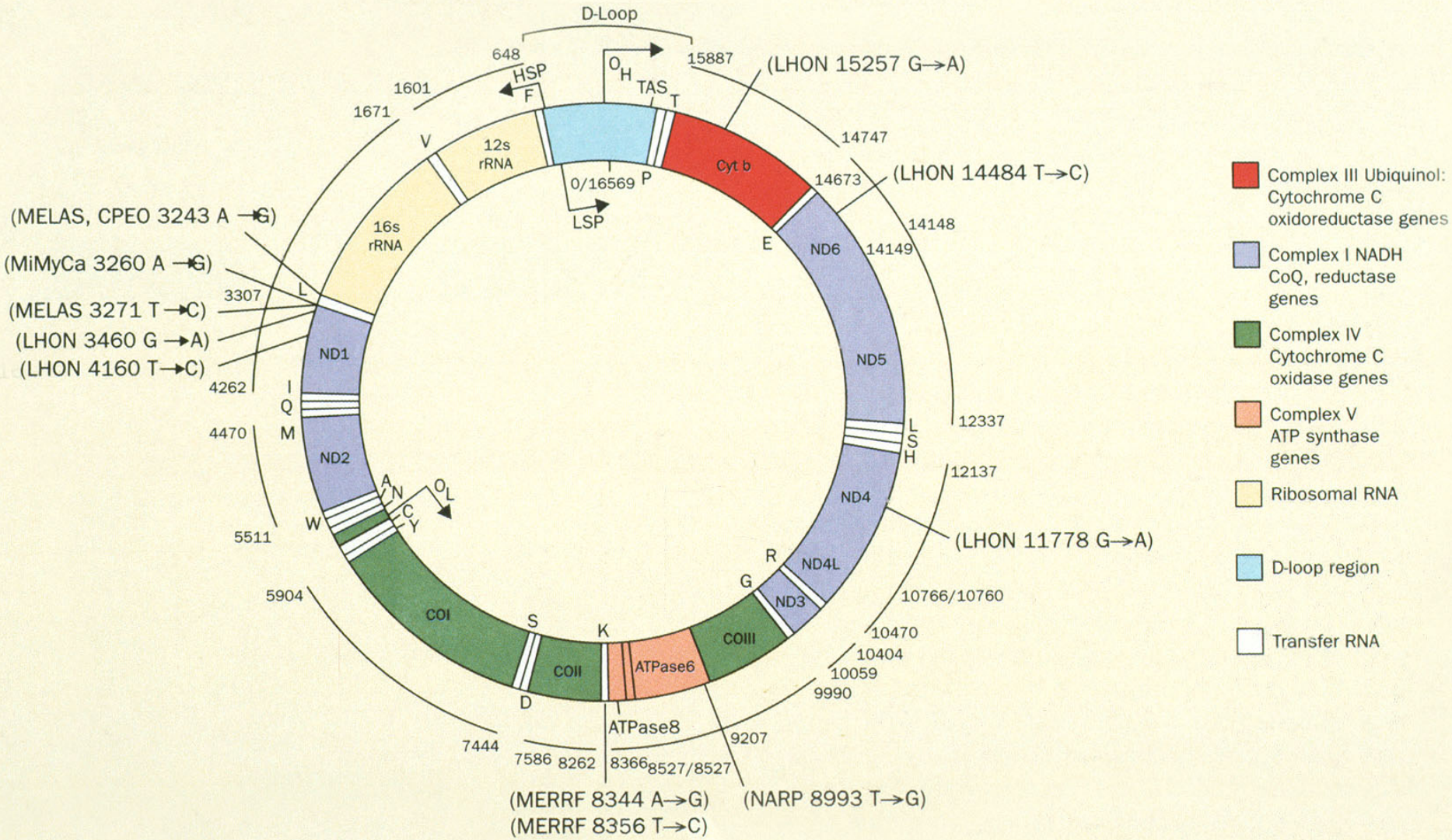
- Peptides
  - Encodes 13 of mitochondrial peptide subunits
  - All 13 peptides are in mitochondrial respiratory chain (OXPHOS)
  - Remaining > 67 OXPHOS subunits are nuclear encoded
- rRNAs: 2
- tRNAs: 22; Located between every 2 rRNA or Protein coding genes
- Non coding region: Triple stranded (D) displacement-loop
  - Produced from additional synthesis of a piece of mitochondrial DNA, 7s DNA
- Contains promoter region
- Origins of replication for H and L strand replication
- Contains elements for initiation of leading strand replication
- Other mitochondrial proteins encoded by nuclear DNA

Human Mt DNA map showing some diseases produced due to mtDNA mutations





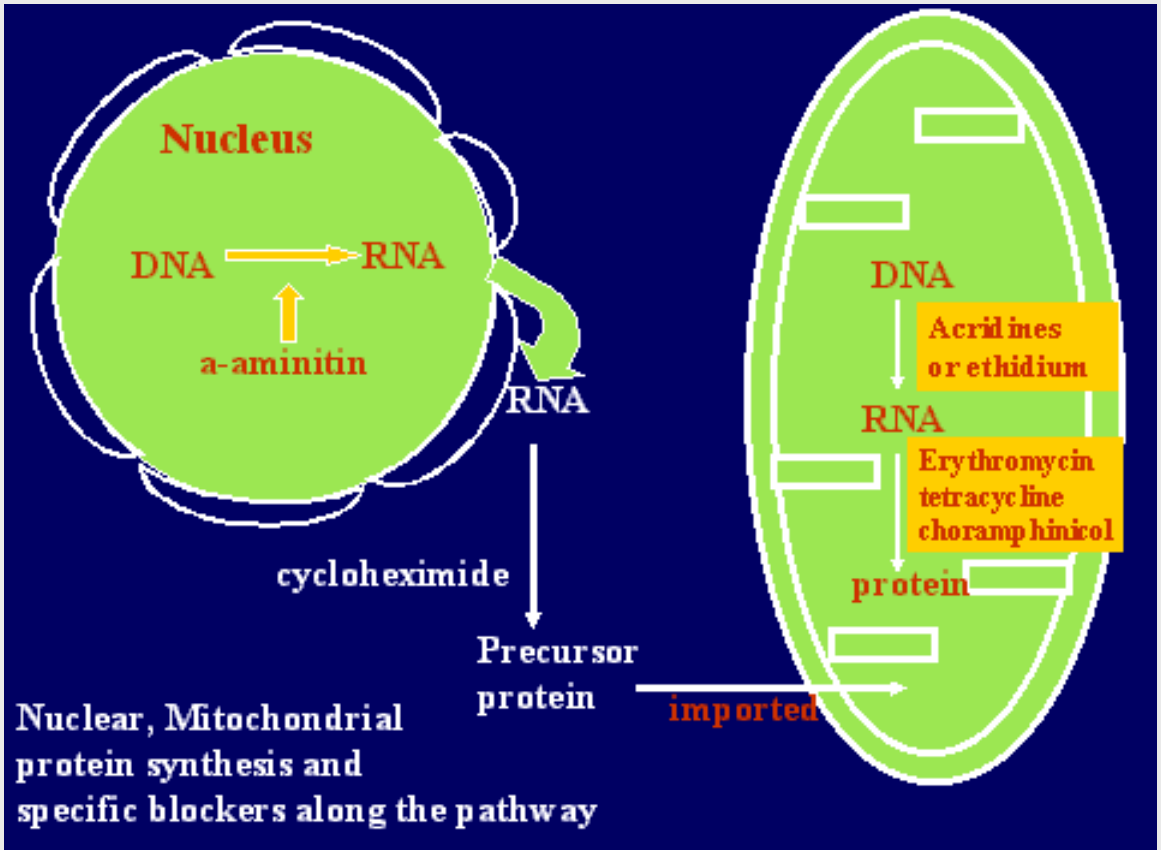
Mitochondrial DNA



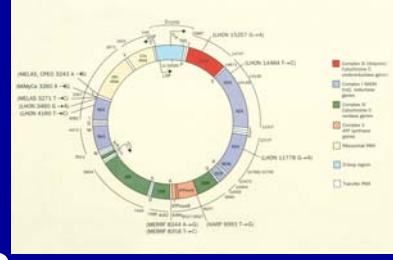
Transcription & translation of mtDNA

- Promoters: Controlled by nuclear DNA
  - 3 Promoters
- H1: H-strand; Produces complete symmetric transcription of heavy strand of mtDNA
- L: L-strand; Produces complete symmetric transcription of light strand of mtDNA
- H2: Synthesis of 2 rRNAs; Acts with factor, mTERF
- Promoters started by: Mitochondrial RNA polymerase + Specificity factor (mtTFA)
- Promoter location: D-loop; Only non-coding region of mtDNA
  - 7% of mtDNA length
  - Contains *cis* elements involved in mtDNA replication & transcription
- Transcripts: Large
- Produced by H1- & L-strand
- Subsequently cleaved into individual genes

All factors involved in maintenance, replication & expression of mtDNA: Nuclear encoded



Differences from nuclear DNA



- No introns
  - Small intergenic spacing: Coding sequences of most genes are contiguous or separated by 1 or 2 bases
- Codon sequences
  - Different start, stop & arginine, tryptophan, isoleucine
- Some mitochondrial genes lack termination codon: Insertion of UAA at the transcriptional level instead
- Replication of mtDNA
  - Rapid rate
  - Lacks proofreading
- Mutation rate 10 to 100x > than nuclear DNA
  - Mitochondria lack an adequate DNA-repair mechanism
- Mode of inheritance: mtDNA inherited maternally

Mitochondrial DNA variation

- Normal: **Homoplasmy**; All copies of mtDNA are identical within coding region
- **Heteroplasmy**: Single cells contain different mtDNA populations
  - Occurs with some mtDNA mutations
  - Due to presence of multiple mitochondria in one cell, each containing several mtDNA copies
  - Produces tissue variation
- **Post-mitotic tissues**
  - Usually contain highest levels of mutated mtDNA
- Mutations in mtDNA % vs normal in mtDNA can **vary widely among tissues** in an individual
- Tissues rich in mitochondrions: **Neurons; Skeletal & Cardiac muscle; Endocrine tissue**
- **Mutational loads may change over time**
- Tissues are **differentially sensitive** to levels of mtDNA mutations: ? Related to oxidative energy requirements
- Pathogenic heteroplasmic mutations: Severity of related symptoms increase with higher proportion of mutated mtDNA
  - Relation to severity not necessarily linear
- Frequency of mtDNA-related disorders: 6 to 17 per 100,000 population

Nuclear DNA and Mitochondrial Diseases

- Mutations in nuclear DNA coding for mitochondrial components
  - Some identified mutations cause defects in oxidative phosphorylation
  - Defects of intergenomic communication
  - Probably nuclear DNA mutations
- Alter control of replication & expression of mitochondrial genome leading to
  - Multiple mtDNA deletions
  - Reduced amounts of mtDNA
- Mutation distribution
  - **Homoplasmic:** Similar distribution of mtDNA mutation in all tissues
  - **Heteroplasmic:** Variable distribution of mtDNA mutation in different cells or tissues

Specific disorders with mtDNA mutations

mtDNA Point mutations
Cardiomyopathy
Leber's optic neuropathy
Leigh's syndrome
MELAS
MERRF
NARP/MILS

Single deletion or duplication
Ataxia, Leukodystrophy
Diabetes: Maternal inheritance
Kearns-Sayre
Pearson's
PEO: Sporadic

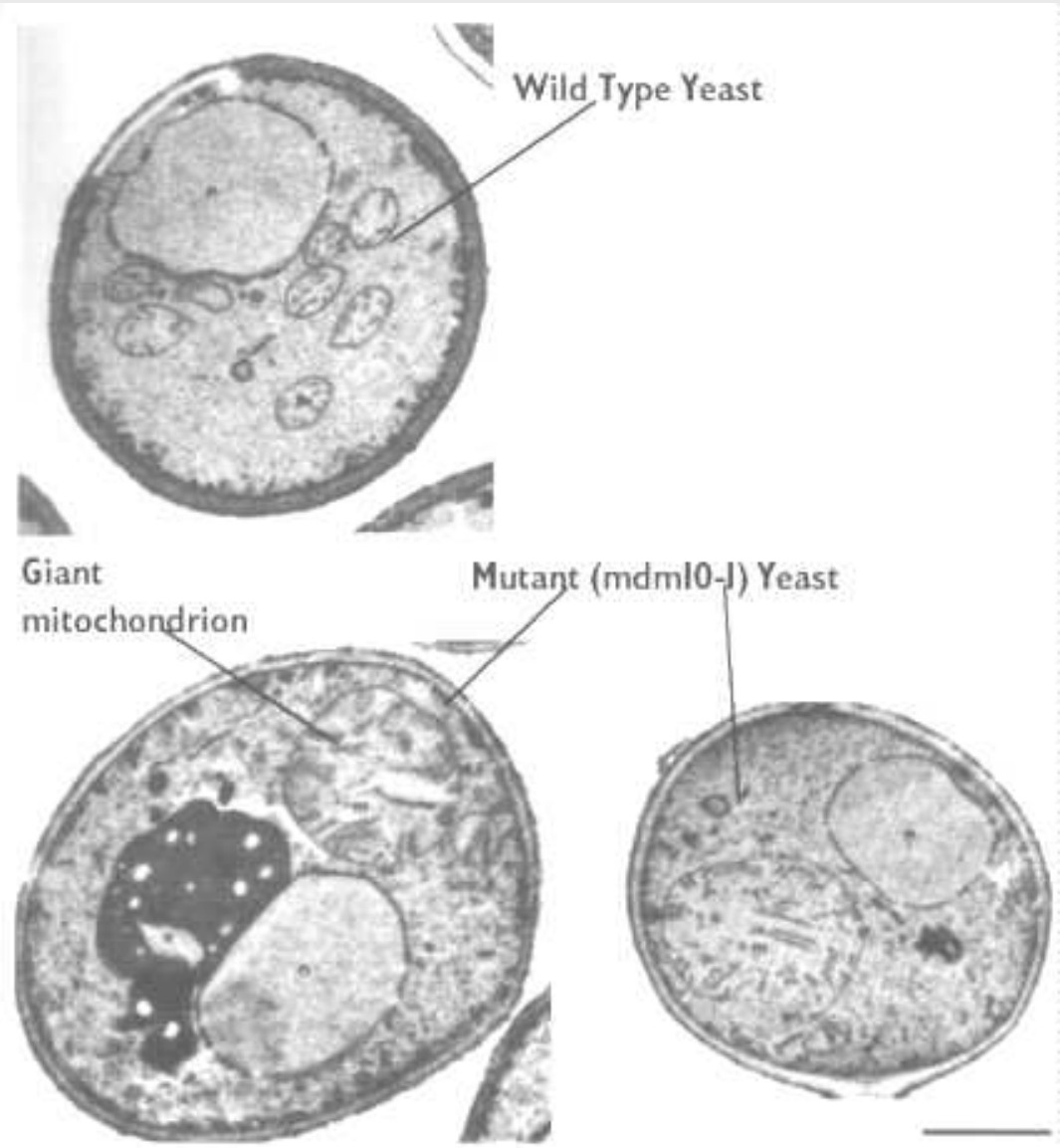
Multiple deletions
Aging
Myositis
- Inclusion body
- COX- muscle fibers

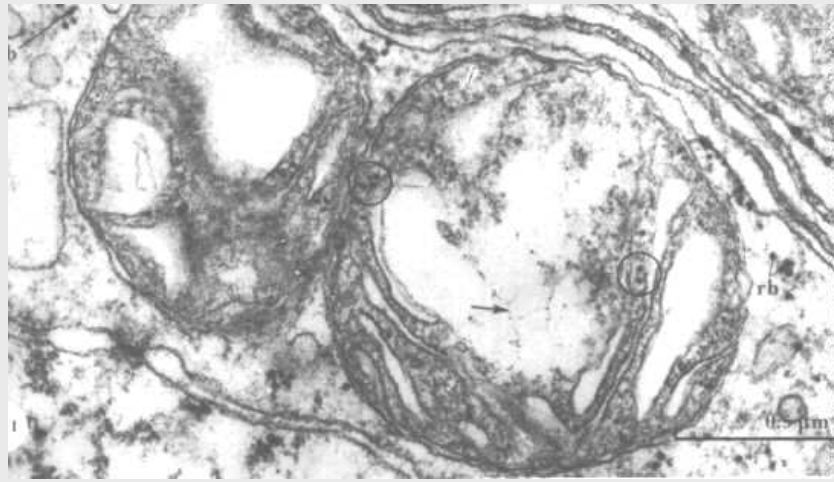
Depletion of mtDNA
Infantile myopathy
- Fatal
- "Later-onset"

Several types of mtDNA defect
Deafness
Diabetes
External ophthalmoplegia (PEO)
- Sporadic
- Maternal
- Dominant
- Recessive

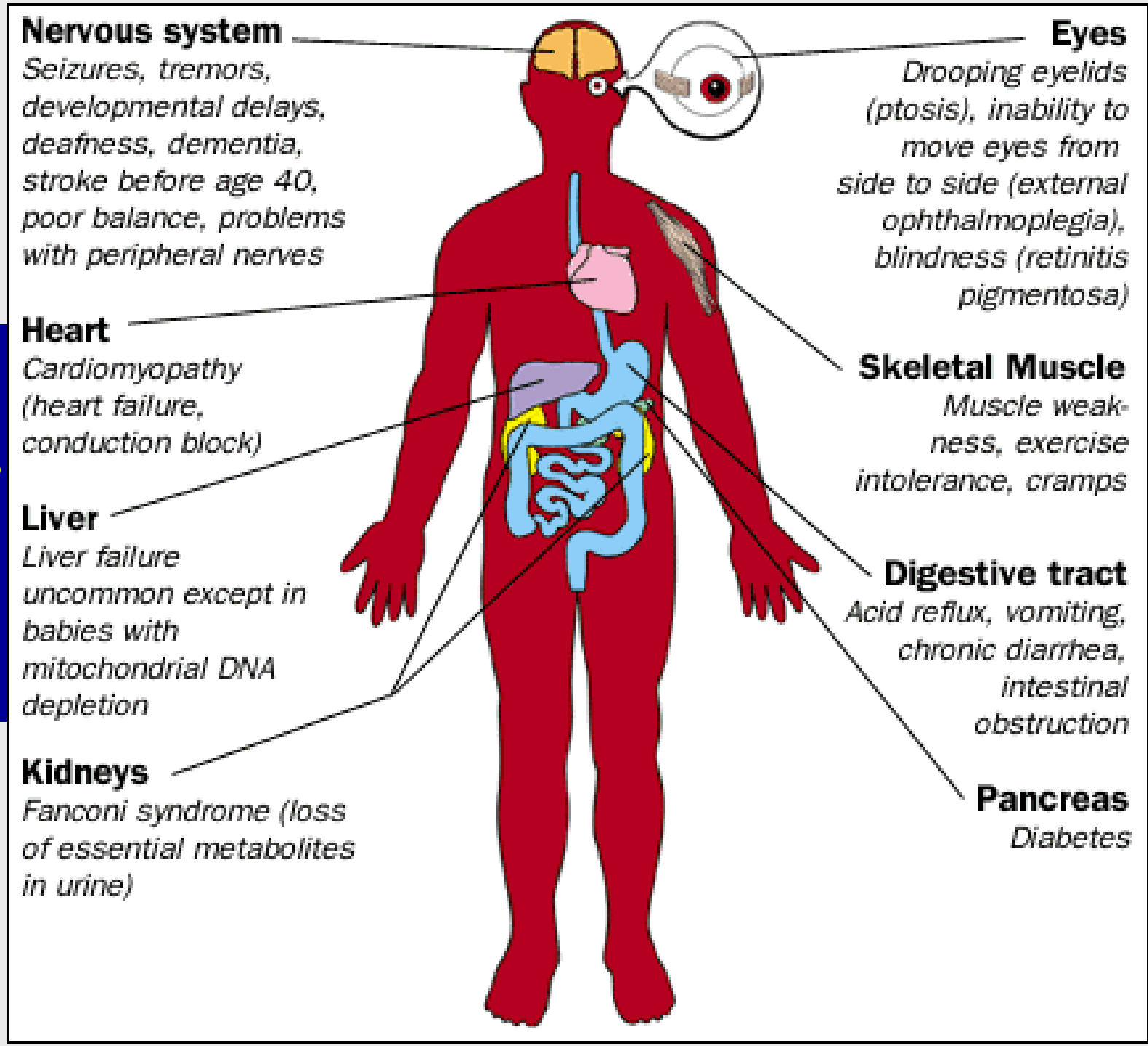
Leigh's Myopathy
Rhabdomyolysis
Sensory neuropathy
Systemic disorders

Abnormalities in mitochondria resulting from mutations

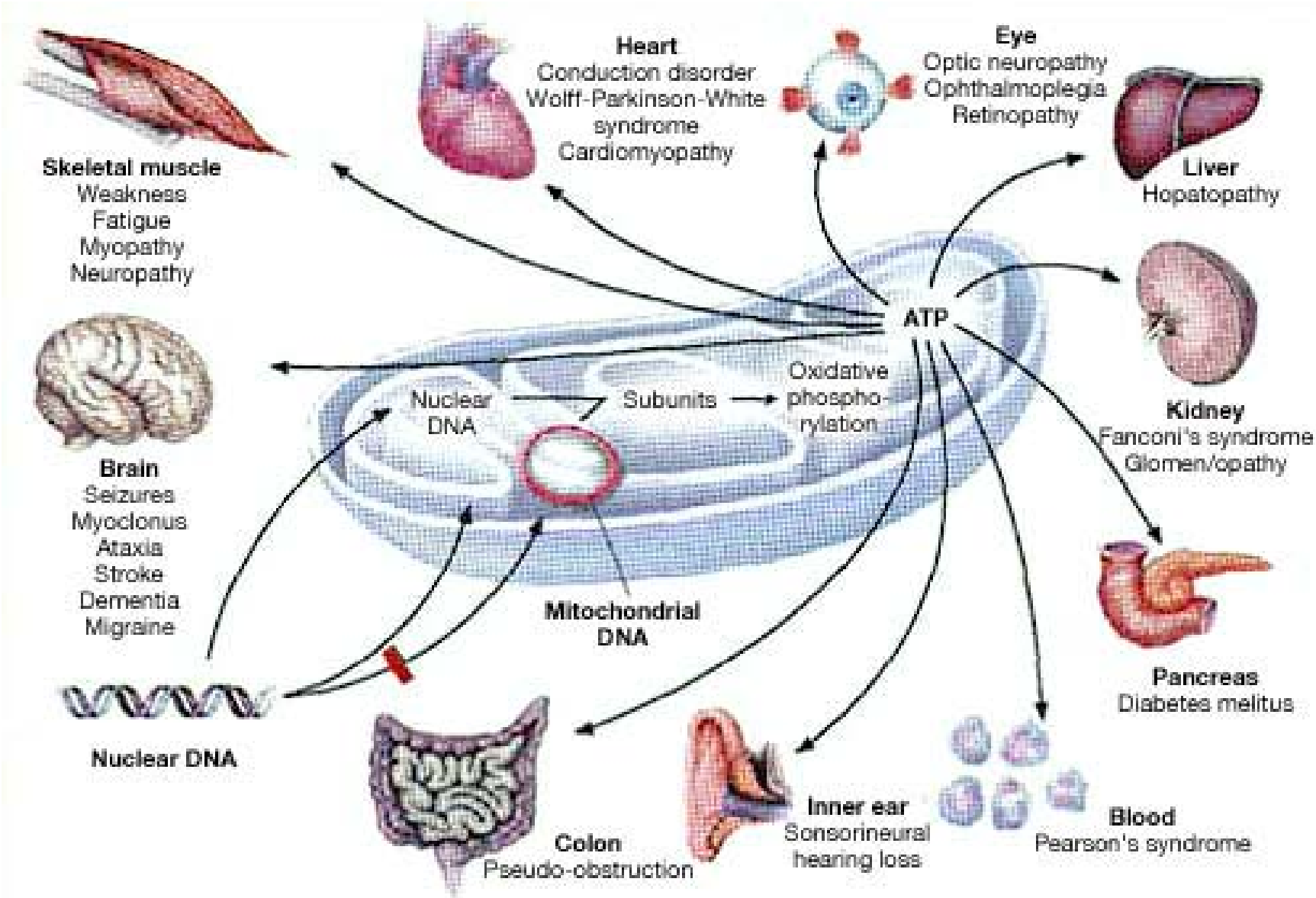




Mt mutations affect different tissues



Tissues affected and examples of mitochondrial diseases



Consequence of Mt DNA Mutations

Tissues that need large amounts of energy, such as the brain, heart, skeletal muscles, eye muscles and the renal tubules of the kidney, probably malfunction, because they run out of fuel. The result is muscle weakness, cardiomyopathy, renal problems, droopy eyelids, cognitive problems and general fatigue.

Mitochondrial disorders: General pathogenic mechanisms

- Often cause deficient function in respiratory-chain
  - Abnormal Oxidative Phosphorylation enzymes
- Threshold effect
  - % of mutant mtDNAs must be above a threshold to produce clinical manifestations
  - % of mutant mtDNAs needed to cause cell dysfunction varies according to tissue oxidative requirements
- Disease signs especially manifest in Tissues with a high energy expenditure: Dependent on oxidative metabolism
- Specific tissues: Brain, Heart & Muscle
- Mitotic segregation
  - % of mutant mtDNAs in daughter cells can shift at cell division
- Produces rapid changes of genotype that may lead to crossing of threshold
- Skewed heteroplasmy
- mtDNA mutation surpasses pathogenic threshold in 1 tissue
  - Examples: A3243G may produce only cardiomyopathy; Myopathy with early respiratory involvement

Mitochondrial disorders

- **Differ from other genetic disorders affecting the muscles:**
  - **Most significantly, although mitochondrial disease can present as a "pure myopathy, i.e: only the skeletal or heart muscles are affected, it more often causes problems in many different organ systems, including the nervous, visual, renal (kidneys), digestive and circulatory systems.**
- **Mitochondrial encephalomyopathies (those disorders affecting brain and muscle) are most often caused by defects in the proteins that make up the respiratory chain.**

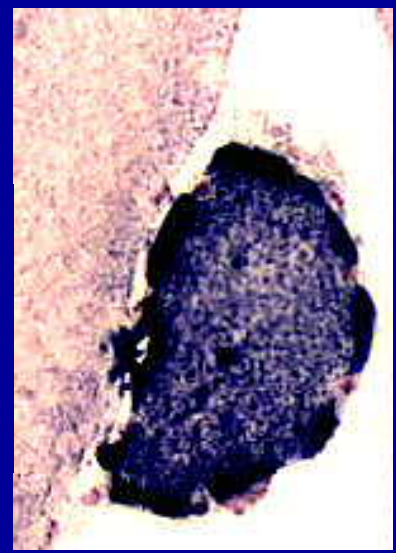
Prime suspects as causes of the symptoms of mitochondrial disease:

- Energy deficit,
- Free radical generation and
- The buildup of toxic metabolites.

mtDNA: General disease features

- Lactic acidosis
- Mitochondrial proliferation in muscle
  - Massive
- Produces ragged-red fibers
- Mutant mtDNAs accumulate preferentially in ragged-red fibers
- Ragged-red fibers are typically negative for cytochrome c oxidase activity

Intense staining
of a muscle fiber with
mitochondrial proliferation



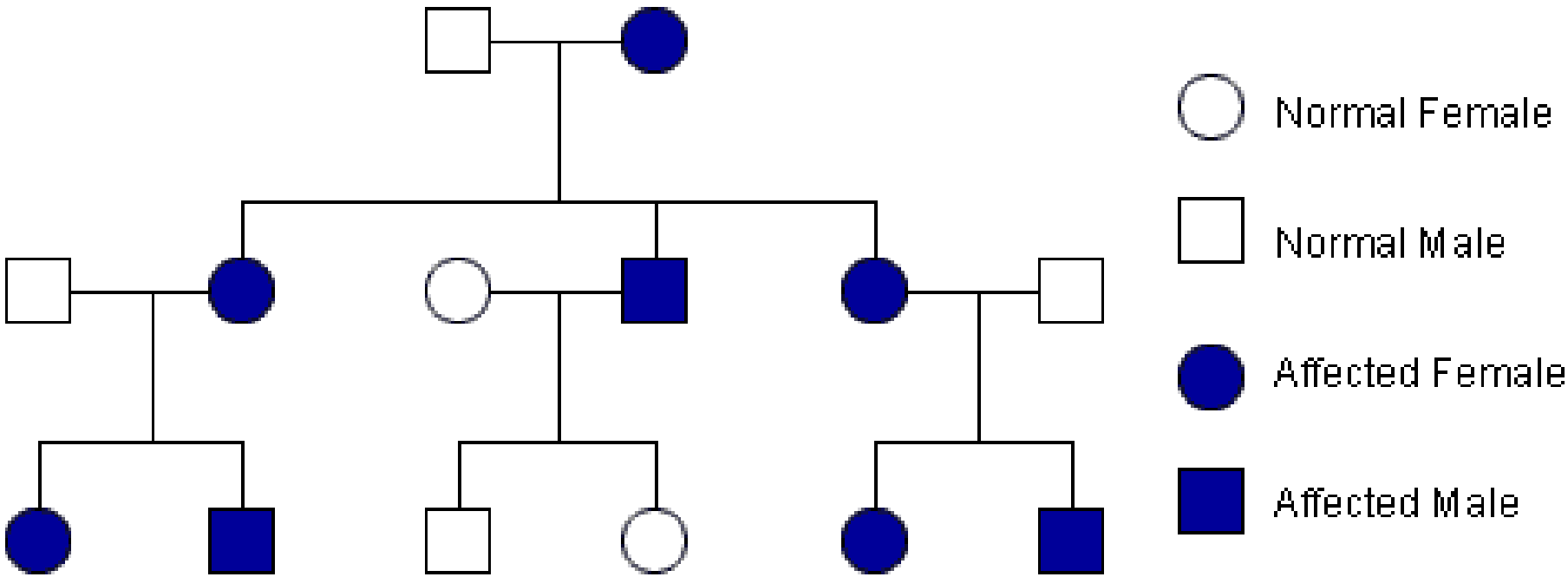
Symptoms common to most Mitochondrial Diseases

- Muscle weakness,
- Muscle cramps,
- Extreme fatigue,

- Gastrointestinal problems (constipation, acid reflux), droopy eyelids (ptosis),
- Eye muscle paralysis (external ophthalmoplegia),
- Retinal degeneration (retinitis pigmentosa) with visual loss,
- Seizures,
- Ataxia (loss of balance and coordination) and learning disorder
- “Soft signs" deafness, mild exercise intolerance, diabetes, short stature and migrainous headaches.

Inheritance of mtDNA

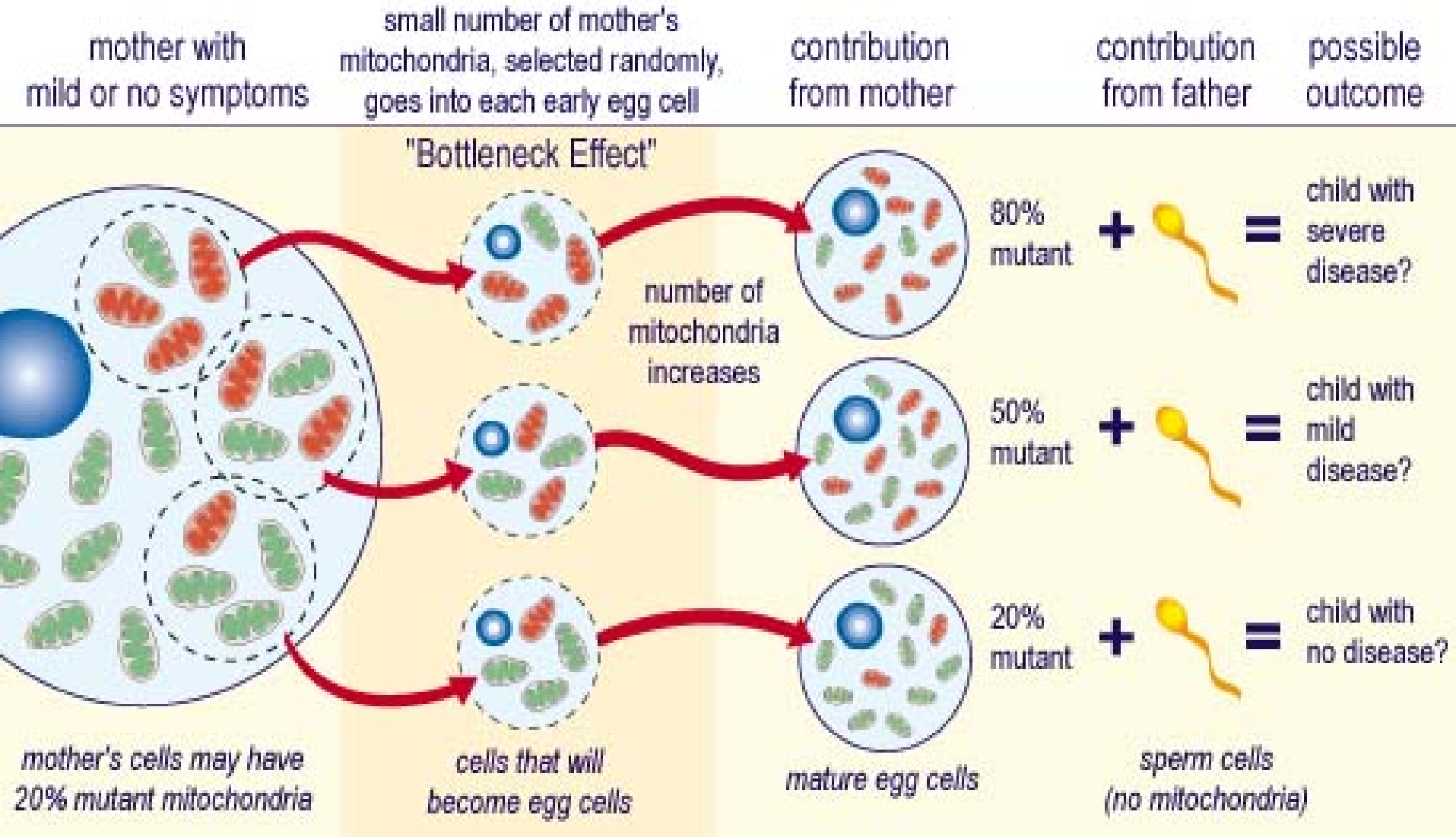
- **Maternal**
  - **Usual pattern**
- **Sperm mtDNA is actively degraded**



Inheritance

- During fertilization mtDNA is derived only from the oocyte
- Maternal inheritance: mtDNA mutations transmitted only from mother
- Mutations transmitted to all offspring, Male & Female
- Increased Mutant mtDNA in the mothers' blood ® Increased Frequency of affected offspring
- Risks of having affected offspring differ between different mtDNA mutations

Variations in Inheritance of Mt mutations




Thank you for Listening