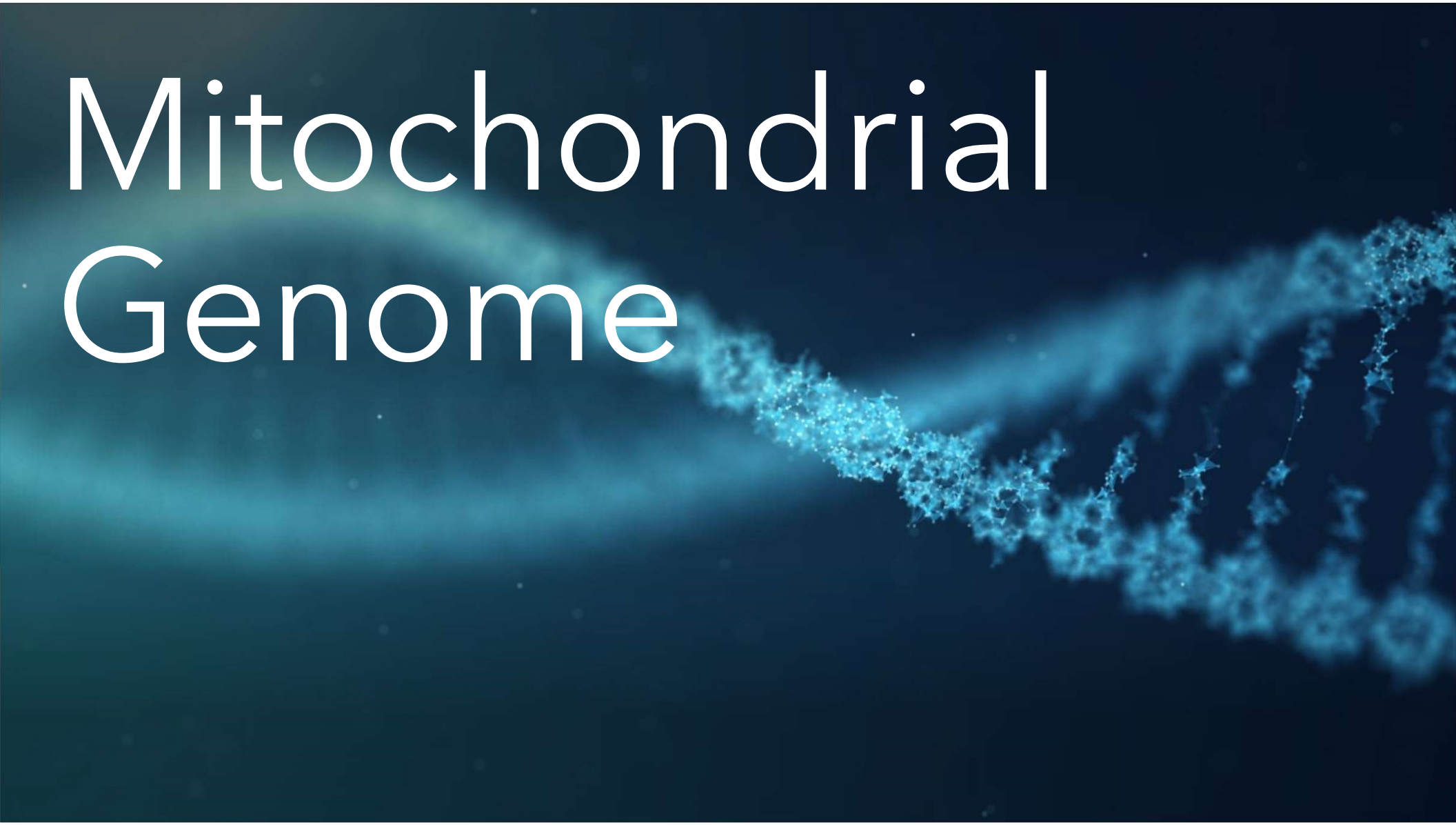
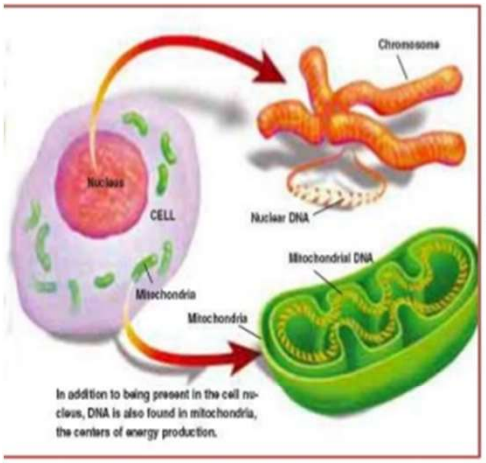
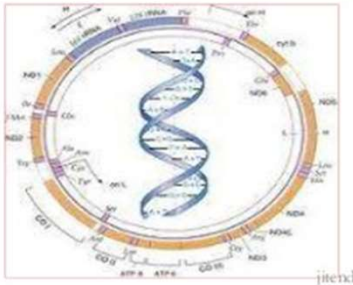
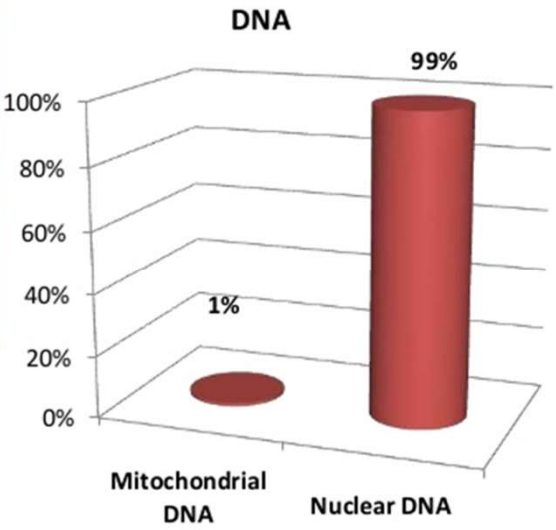

Mitochondrial
Genome

MITOCHONDRIA

- Found in ALL eukaryotic cells (even in plant cells)
- Site of aerobic respiration
  - sugars + O_2 - - > ATP + CO_2 + H_2O
- Contain DNA which codes for mitochondrial proteins, ribosomes, etc.
- Divide by a process similar to binary fission when cell divides

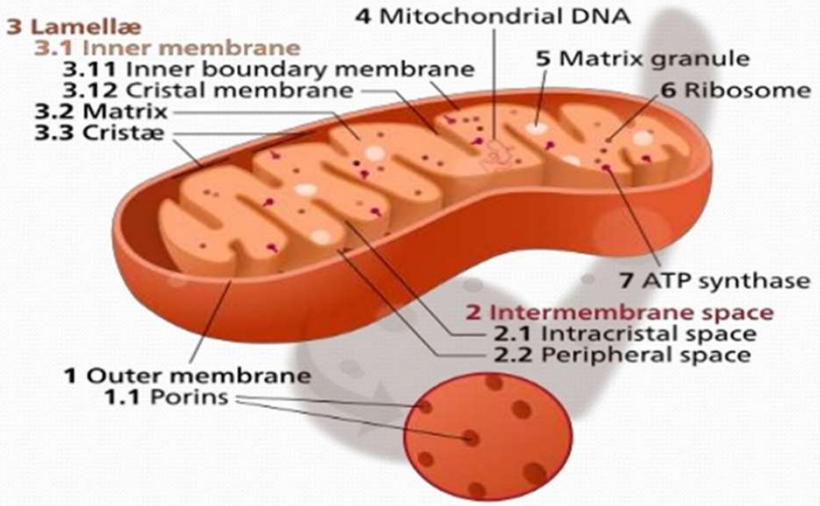




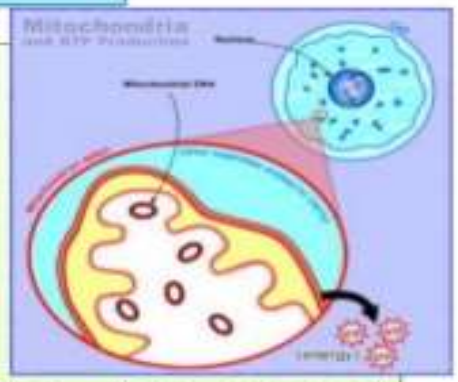


- Enclosed in a double membrane system
- Inner Membrane forms the Cristae (invaginations into interior region)
- Site of energy generation
- Matrix is the soluble portion of the mitochondira
- Site of carbon metabolism
- Location of mDNA
- Site of mitochondrial protein synthesis

The Mitochondria



Mitochondrial DNA



mtDNA is small genome and **double stranded** circular DNA molecule

Haploid in nature

mt DNA contain **37 genes** all of essential for normal mitochondrial function

13 genes making enzymes involved in oxidative phosphorylation (**ATP production**)

process involved use of **oxygen** and simple **sugars** to form **ATP**

Origin

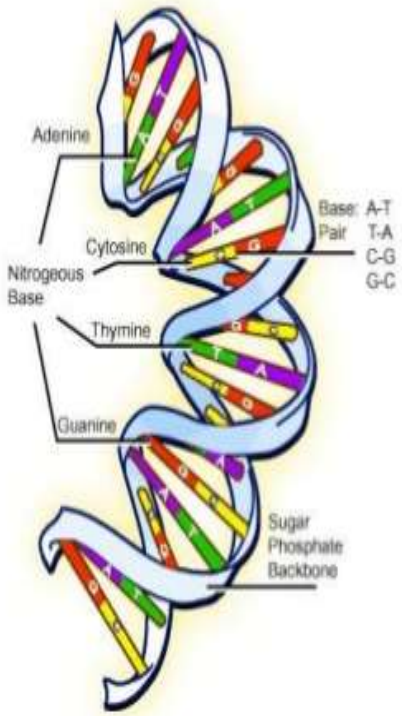
Nuclear and mitochondrial DNA are thought to be of separate evolutionary origin, with the mtDNA being derived from the circular genomes of the bacteria that were engulfed by the early ancestors of today's eukaryotic cells. This theory is called the endosymbiotic theory. Each mitochondrion is estimated to contain 2–10 mtDNA copies.[4] In the cells of extant organisms, the vast majority of the proteins present in the mitochondria (numbering approximately 1500 different types in mammals) are coded for by nuclear DNA, but the genes for some of them, if not most, are thought to have originally been of bacterial origin, having since been transferred to the eukaryoticnucleus during evolution.

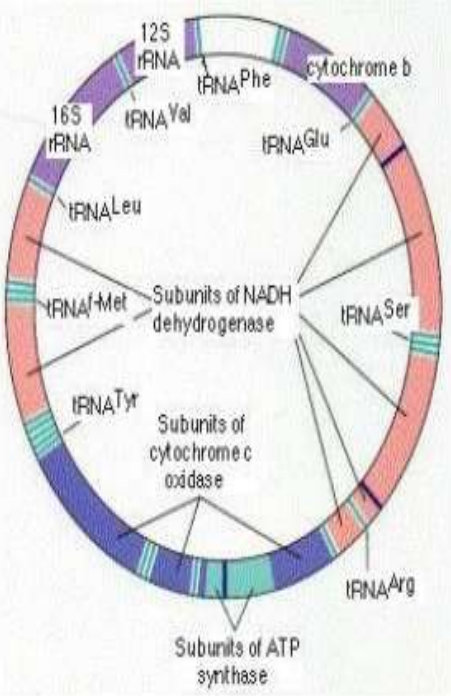
Nuclear DNA vs. Mitochondrial DNA

Nuclear DNA vs. Mitochondrial DNA

- **Nuclear DNA**
  - found in **nucleus** of the cell
  - 2 sets of **23 chromosomes**
  - **maternal and paternal**
  - can "**discriminate** between individuals of the same maternal lineage"
  - **double helix**
  - **bounded** by a nuclear envelope
  - DNA **packed** into chromatin

- **Mitochondrial DNA**
  - found in **mitochondria** of the cell
  - each mitochondria may have **several copies** of the single mtDNA molecule
  - **maternal only**
  - **cannot "discriminate** between individuals of the same maternal lineage"
  - **Circular**
  - **free** of a nuclear envelope
  - DNA is **not** packed into chromatin





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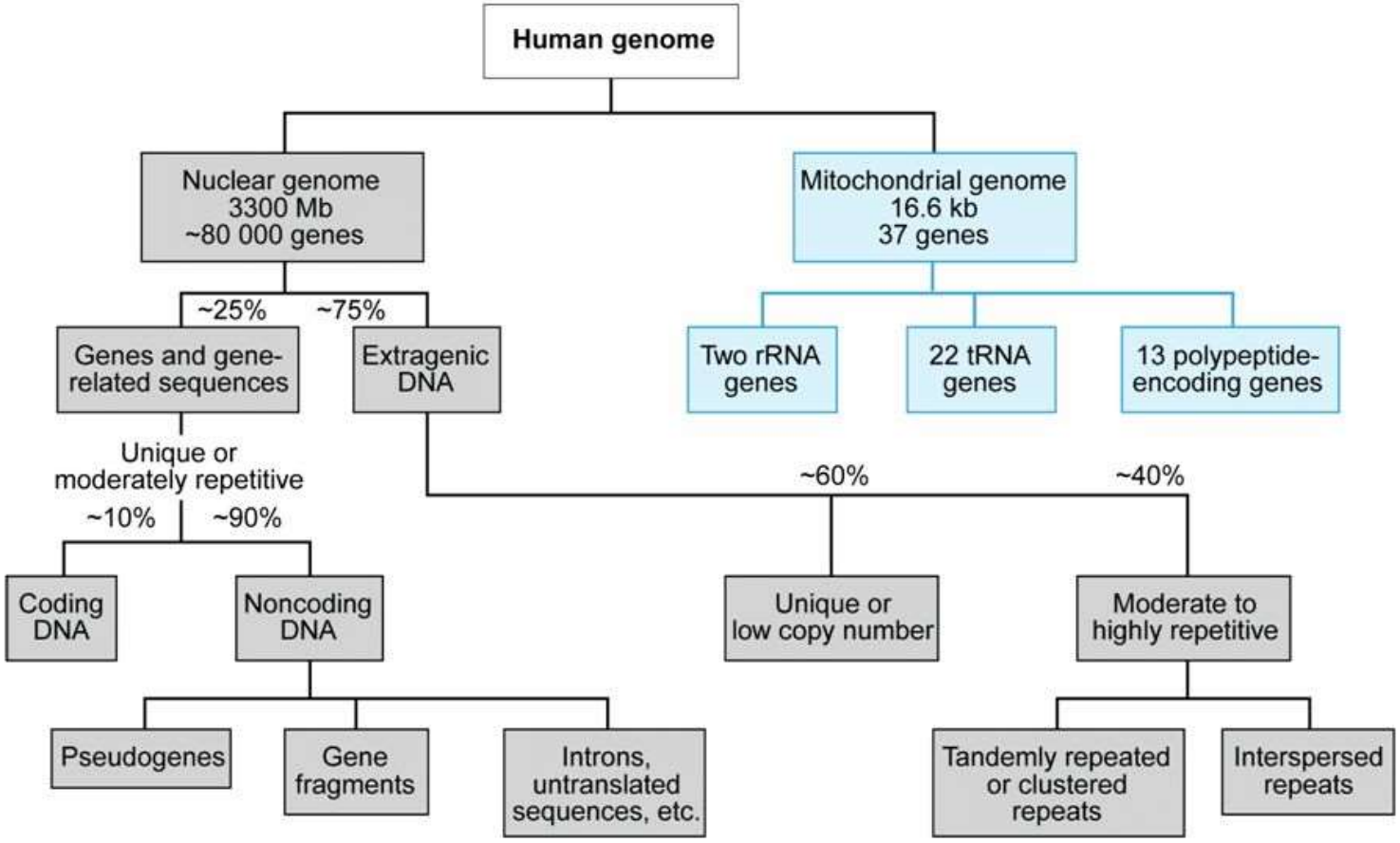
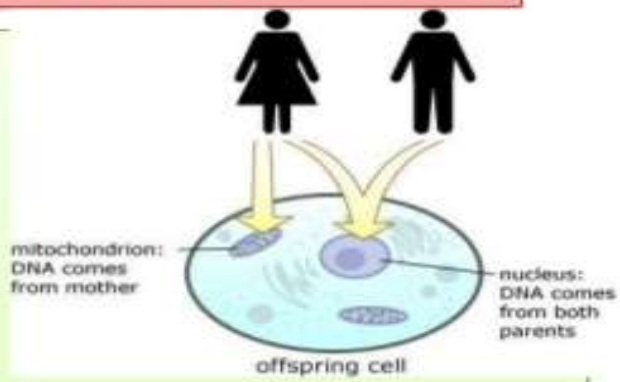

Human genome
Nuclear genome
3300 Mb
~80 000 genes
Mitochondrial genome
16.6 kb
37 genes
~25%
~75%
Genes and gene-related sequences
Extragenic DNA
Two rRNA genes
22 tRNA genes
13 polypeptide-encoding genes
Unique or moderately repetitive
~10%
~90%
~60%
~40%
Coding DNA
Noncoding DNA
Unique or low copy number
Moderate to highly repetitive
Pseudogenes
Gene fragments
Introns, untranslated sequences, etc.
Tandemly repeated or clustered repeats
Interspersed repeats

Maternal Inheritance of mtDNA

During **fertilization**, the sperm only contributes its nucleus (**23 chromosomes**)



Mitochondria of the **sperm cell** are located at the mitochondrial sheath which is **destroyed** upon fertilization

Only available mitochondria (**mtDNA**) is that of the mother's; this is why mtDNA is of maternal origin

jitenderanduat@gmail.com

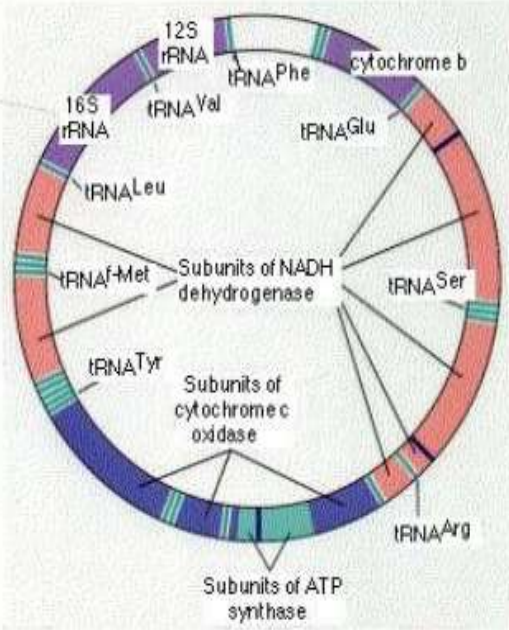
The Mitochondrial Genome

- **16,569** base pairs (**bp**) in length (16-18 kbp)
- encodes **37 genes**, **13 proteins**, **22 tRNAs**, and **2 rRNAs**

two general regions:

- **coding region**: responsible for the production of various biological molecules involved in **"cellular respiration"**
- **control region**: responsible for the **regulation of the mtDNA molecule**

The Mitochondrial Genome

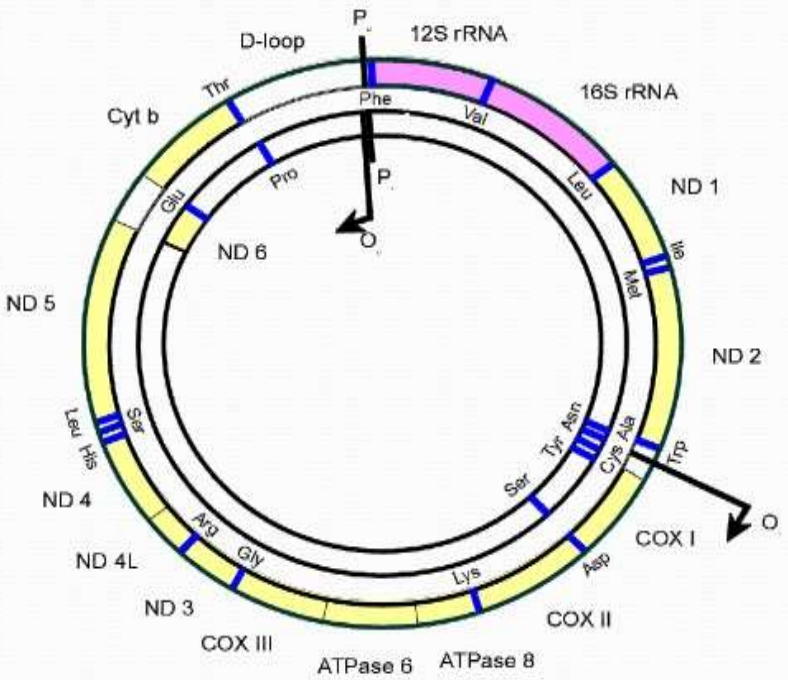


The genome of human mitochondria contains 16,569 base pairs of DNA organized in a closed circle. These encode:

2 ribosomal RNA (**rRNA**) molecules

22 transfer RNA (**tRNA**) molecules

13 polypeptides



- The mitochondrial genome is a circle, 16.6 kb of DNA. A typical bacterial genome is 2-4 Mbp.
- The two strands are notably different in base composition, leading to one strand being "heavy" (the H strand) and the other light (the L strand).
- Both strands encode genes, although more are on the H strand.
- A short region (1121 bp), the D loop (D = "displacement"), is a DNA triple helix: there are 2 overlapping copies of the H strand there.
- The D loop is also the site where most of replication and transcription is controlled.
- Genes are tightly packed, with almost no non-coding DNA outside of the D loop. In one case, two genes overlap: they share 43 bp, using different reading frames. Human mitochondrial genes contain no introns, although introns are found in the mitochondria of other groups (plants, for instance).

Most information is encoded on the heavy (H) strand, with genes for two rRNAs, 14 tRNAs, and 12 polypeptides. The light (L) strand codes for eight tRNAs and a single polypeptide. All 13 protein products are constituents of the enzyme complexes of the oxidative phosphorylation system

The **13 polypeptides** participate in building several protein complexes embedded in the inner mitochondrial membrane.

7 subunits that make up the mitochondrial **NADH dehydrogenase**

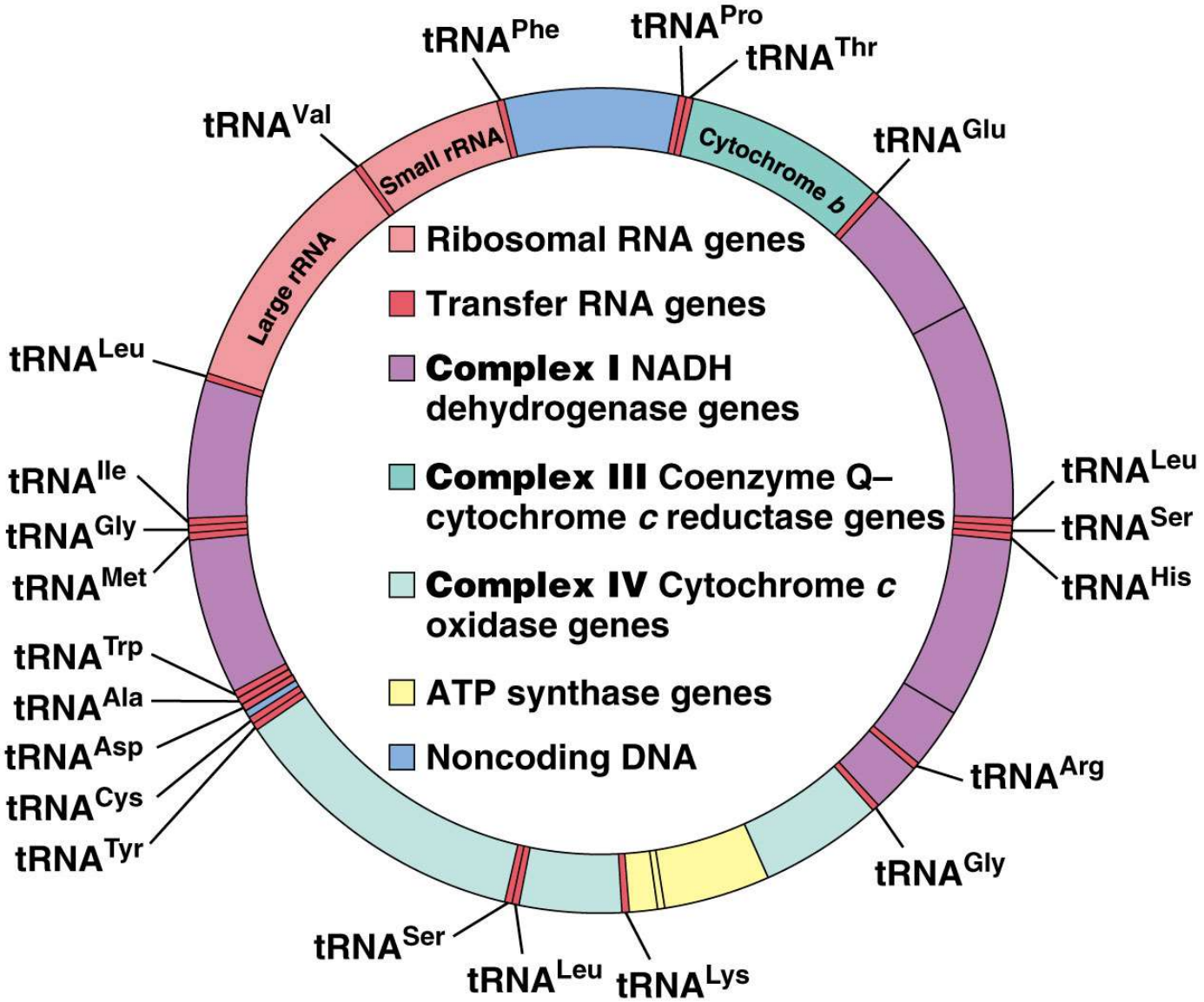
3 subunits of **cytochrome c oxidase**

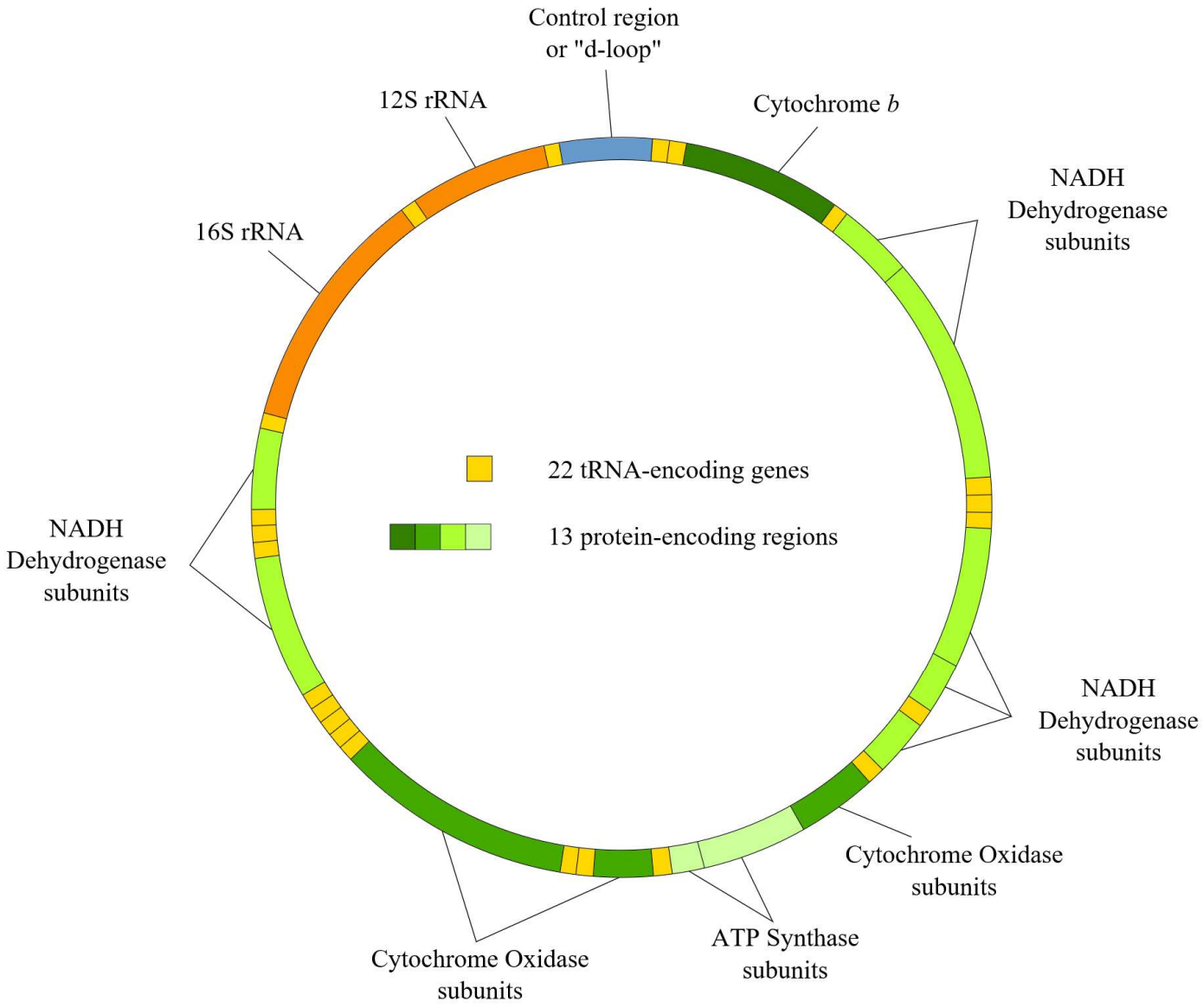
2 subunits of **ATP synthase**

cytochrome b

All these gene products are used within the mitochondrion, but the mitochondrion also needs proteins encoded by nuclear genes. These proteins (e.g., cytochrome c and the DNA polymerases used within the mitochondrion) are synthesized in the cytosol and then imported into the mitochondrion.

- Genes: Total of 37. 22 tRNAs, 2 rRNAs, 13 polypeptides.
- tRNA: only 60 of the 64 codons code for amino acids. 8 tRNAs cover all 4 3rd base positions with the same amino acid, and the remaining 14 tRNAs each cover two 3rd base positions (purines or pyrimidines). Thus, all 60 codons are covered.
- rRNA: 16S and 23S which are standard sizes for bacterial rRNAs. Bacterial ribosomes don't use 5S or 5.8S rRNAs.
- polypeptides: all are components of the electron transport chain. Other components are encoded in the nucleus and transported to the mitochondria after translation.




Control region
or "d-loop"
12S rRNA
Cytochrome *b*
NADH
Dehydrogenase
subunits
16S rRNA
22 tRNA-encoding genes
13 protein-encoding regions
NADH
Dehydrogenase
subunits
NADH
Dehydrogenase
subunits
Cytochrome Oxidase
subunits
ATP Synthase
subunits
Cytochrome Oxidase
subunits

Size of Mitochondrial Genomes

Species	Size (kb)
Human	16
Drosophila	18
Yeast	75
Turnip	218
Corn	570
Muskmelon	2000

- It evolves **faster than nuclear DNA**

  (Brown *et al.* 1982),
- Probably due to inefficient **replication repair**

  (Clayton 1984)
- Mitochondrial DNA is **maternally inherited** in most species. (Gyllesten *et al.* 1991)

- Generally mitochondrial **DNA does not recombine**

  (Hayashi *et al.* 1985)

Though some evidence of **recombination events** has recently been reported

(Eyre-Walker *et al.* 1999, Hagelberg *et al.* 1999).

Uses for mtDNA in Forensics

- mtDNA will be used when "biological evidence may be degraded [i.e. charred remains] or in small quantity“

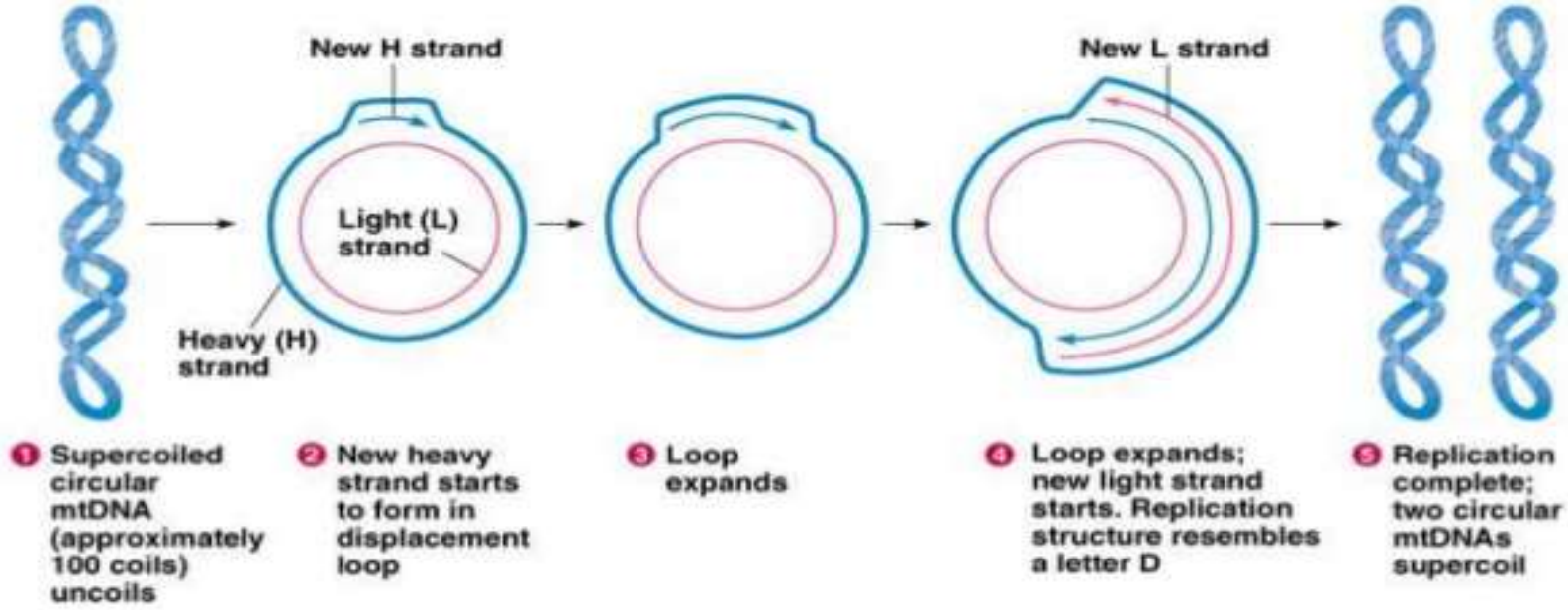
- Cases in which evidence consists only of:
  - –hairs
  - –bones
  - –Teeth

- Missing Persons Cases (use of skeletal remains)

- Establishing Individuals as suspects (hair evidence)

Mitochondrial DNA replication

Replication starts with the H strand.
The origin of replication for the H strand is in the D loop, and it is initiated by an RNA primer generated from the L strand transcript.
After the new H strand is about 2/3 complete, the L strand origin of replication is uncovered. The L strand origin is on the old H strand; it is "uncovered" when the old H strand is displaced by the DNA polymerase synthesizing the new H strand.
The L strand origin folds into a stem-loop structure, which acts as a primer, and replication of the L strand begins.
Replication can be said to be bidirectional by asynchronous, unlike replication of nuclear DNA, which proceeds in both directions simultaneously.


New H strand
Light (L) strand
Heavy (H) strand
New L strand
1 Supercoiled circular mtDNA (approximately 100 coils) uncoils
2 New heavy strand starts to form in displacement loop
3 Loop expands
4 Loop expands; new light strand starts. Replication structure resembles a letter D
5 Replication complete; two circular mtDNAs supercoil

Transcription.

Both strands are transcribed.

The D loop contains one promoter for each strand, and the entire strand is transcribed.

The RNA is then cut into individual RNAs for each gene.

Protein-coding genes are given poly-A tails, and rRNA and tRNA molecules are modified as necessary.

Mitochondrial translation

- mRNAs have no 5'cap
- fMet-tRNA initiates protein synthesis

- Plant mitochondria use universal genetic code
- Mammalian and yeast mitochondria use modified genetic code
- mtDNA tRNAs have more 'wobble'
  - 22 (mammalian) or 25 (yeast) tRNAs needed
  - 32 tRNAs for translation of nuclear mRNA

Why is mitochondrial mutation so high?

- The mitochondrial genome has a very high mutation rate, 10- to 17-fold higher than that observed in nuclear DNA. Although mtDNA repair systems do exist and , they are not sufficient to counteract the oxidative damage sustained by the mitochondrial genome.
- Protective histones are also lacking.
- Oxygenation process is high percentage.
- The mtDNA mutation rate can be increased by environmental agents or by mutation of nuclear genes involved in mtDNA maintenance

Mutations in mtDNA and disease

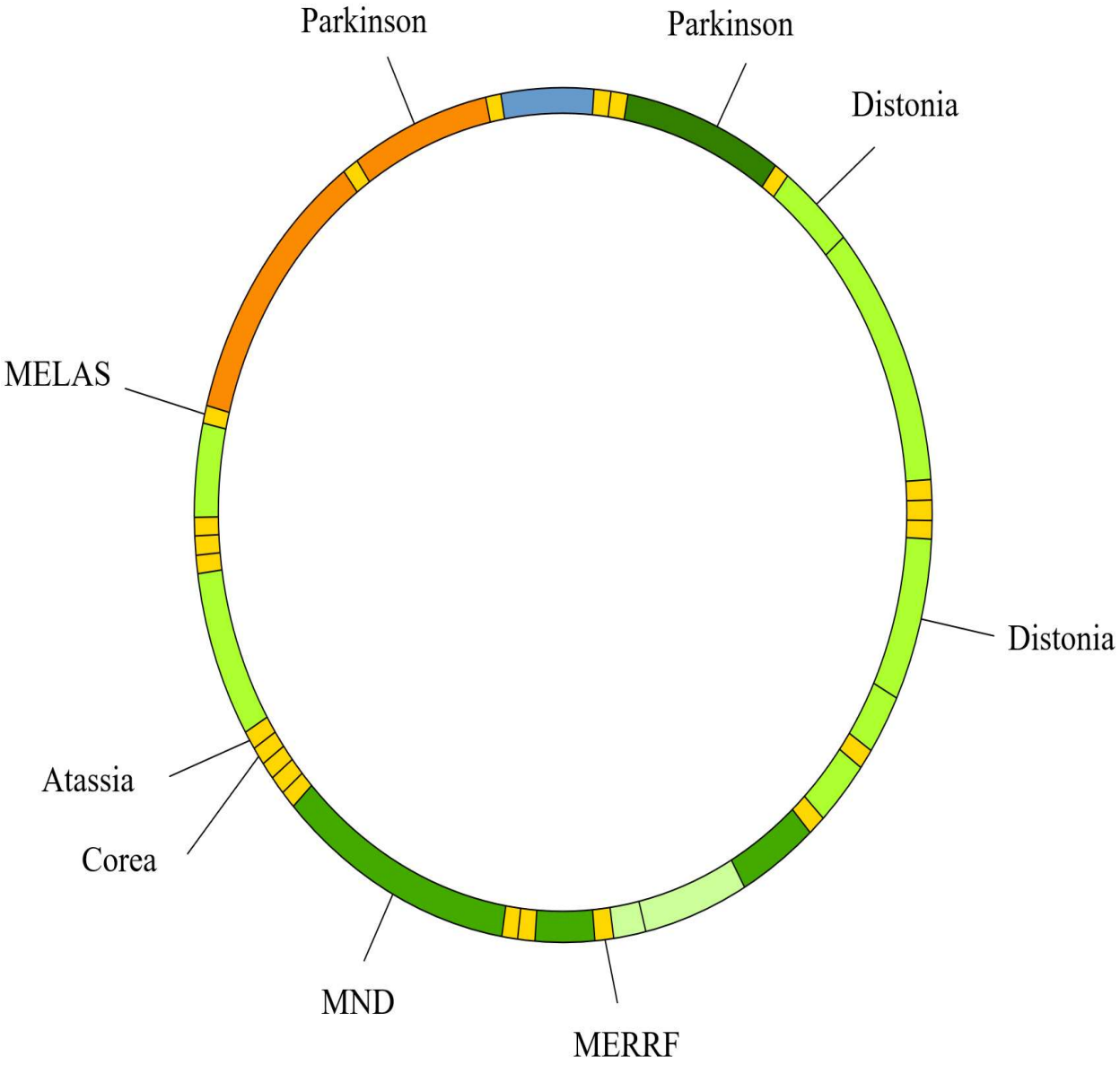
- About 1 in 4,000 children in the US develop mitochondrial disease by the age of 10 years
- mtDNA genome mutates 10 times more frequently than does nuclear DNA
- More than 100 different rearrangements and about 100 different point mutations that are disease-causing have been identified in mtDNA.
- The clinical phenotype resulting from mtDNA mutations is diverse, however the diseases of central-nervous or muscular-skeletal systems are most common.
- Pleiotropy and variable expressivity is common in different affected family members (due to heteroplasmy).
- Pleiotropy - multiple phenotipic effects of a single allele or pair of alleles.

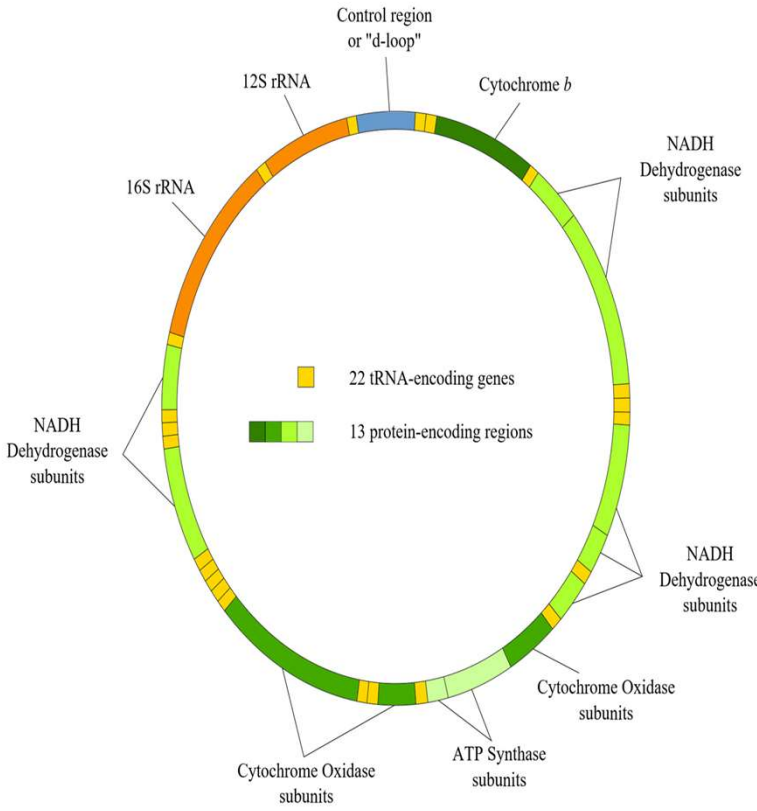
THREE TYPES OF MUTATIONS

- It has been identified in mtDNA:
- (1) missense mutations in the coding regions of genes that alter the activity of an oxidative phosphorylation protein;
- (2) point mutations in tRNA or rRNA genes that impair mitochondrial protein synthesis;
- (3) Deletions or duplications of the mtDNA molecule. They are generally somatic in origin, although a small proportion is inherited, in some diseases.

Some diseases associated with mtDNA

- **MERRF** (Myoclonic Epilepsy with Ragged Red Fibres)
- **MELAS** (Myopathy, Epilepsy, Lactic acidosis, Stroke-like episodes)
- **LHON** (Leber's Hereditary Optic atrophy)
- **Kearn-Sayre** (eye problems, heart block, ataxia and loss of coordination)
- **Leigh syndrome** (rare severe brain disease in infancy, also heart problems)


Parkinson
Parkinson
Distonia
MELAS
Distonia
Atassia
Corea
MND
MERRF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subunits
16S rRNA
22 tRNA-encoding genes
13 protein-encoding regions
NADH
Dehydrogenase
subunits
NADH
Dehydrogenase
subunits
Cytochrome Oxidase
subunits
ATP Synthase
subunits
Cytochrome Oxidase
subunits

Inheritance of organelle genes

- Show non-Mendelian inheritance
- Meiosis-based segregation doesn't occur
- Mendelian ratios aren't observed

- Results of reciprocal crosses are different than those involving nuclear genes
- In humans, mtDNA markers can be tracked using molecular techniques

Plant mitochondrial manipulation:

Genetic manipulations of mitochondria have been attempted in many organisms but it is mostly successful to yeast and C. reinhardtii. Sequencing of mitochondrial genomes in most of the plant species has been done. Plant mitochondria offer great advantages in its manipulation because of following reasons.

1. Maternal inheritance
2. No pleiotropic effect
3. Absence of gene silencing
4. Multigene engineering
5. No position effects
6. No specific degradation of transgene RNA at post transcriptional level.

Despite of these advantages, mitochondrial transformation is less employed due to difficulties in:

1. The introduction of foreign DNA into the mitochondria.
2. Incorporation of transgene into the mitochondrial DNA.
3. Absence of selectable marker for mitochondria.
4. Relatively large number of mitochondria per cell and mitochondrial genome per mitochondria.
5. RNA editing may render foreign genes ineffective that are introduced into the mitochondrial genome.

Methods of mitochondrial transformation:

- Protoplast fusion
- Particle bombardment of cell culture
- Agrobacterium mediated gene transfer
- Microinjection method

Application of plant mitochondrial genetic manipulation in crops:

Transfer of transgene through pollens to related plant/ crop species is a big environmental concern. Mitochondrion is a unique candidate for transgene carrier as it has its own transcription and translation machinery, and maternal inheritance gives assurance of transgene containment with high expression level. These features make mitochondria amenable to engineer and therefore reduce the risk of transgene escape in nature. Genetic manipulation or engineering will add to the efficiency of crop improvement.

In plants, the mitochondrial genome is the source of cytoplasmic male sterility (CMS), a trait of major interest to crop breeders for hybrid generation. Many of the economically important traits including yield, male sterility, heterosis, disease resistance, temperature or drought tolerance etc are affected by genetic interactions between the organellar and the nuclear genes (Frie *et al* 2004, Mackenzie SA 2005, Atienza *et al* 2007)). The mechanisms explaining effects of cytoplasmic inheritance on these traits are still largely unexplored and roles of mitochondrial and chloroplastic genetics remain to be established (Romain Val *et al.* 2011). Due to lack of molecular approaches to engineer and control the genetic system in this organelle, progress in the understanding of mitochondrial genetic processes and regulation pathways, are hindered.

Conclusion:

- Many economically important crop species are devoid of CMS system due to unavailability of cytoplasmic genetic male sterility. In such species mitochondrial manipulation could provide a novel means to develop CMS lines as non-GMO/ transgenic materials
- Once the mitochondrion is transformed with "gene of interest", their maternal inheritances will confind the gene through successive generations thus reducing the risk of transgene escape.
- Use of mitochondrial plasmid as a vector for transgene would be more compatible than the bacterial plasmid being its origin from the plant genome itself.