

Using yeast to study mitochondrial genetics

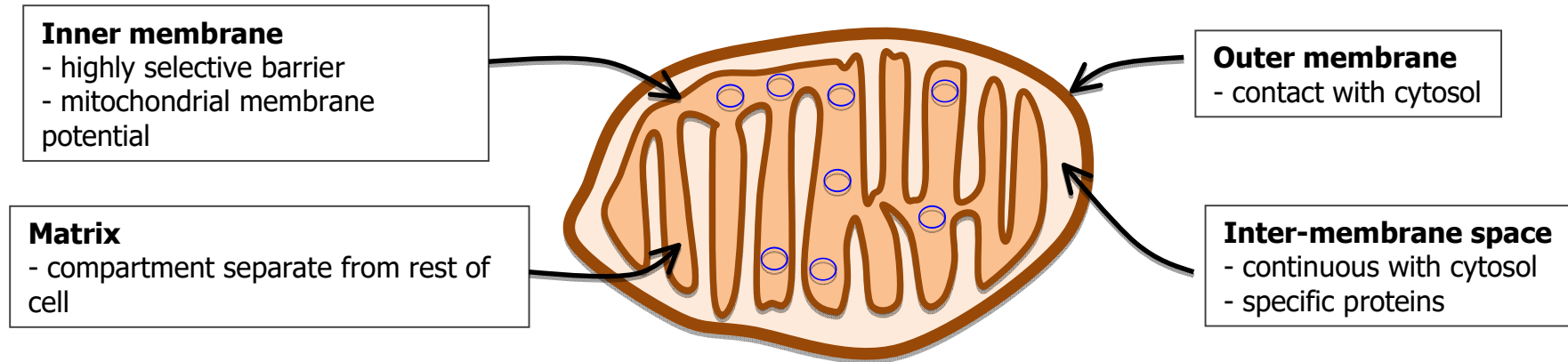
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University of Sao Paulo
13th September 2019

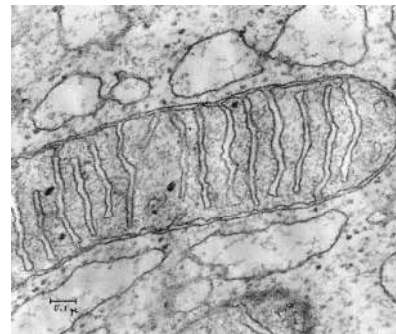
Today's topics

- Mitochondria and mitochondrial DNA
- Origins of mitochondria
- Biology of mitochondria
- How yeast have helped to understand mitochondrial biology
- Applications of mitochondrial research
 - Medicine
 - Human migration
 - Forensics

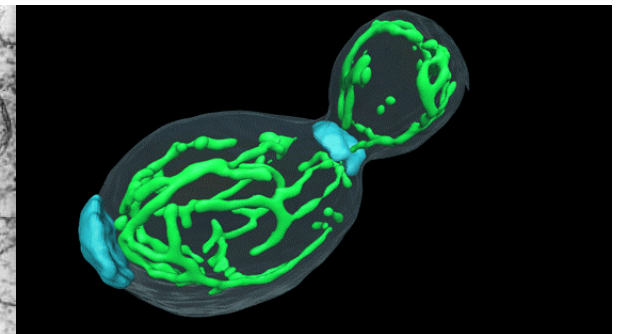
Mitochondria – a mass producer of cellular energy and a metabolic hub



- Double membrane organelle
- Network throughout cytosol
- Energy production
- Metabolic pathways
- Cell death
- Mitochondrial DNA

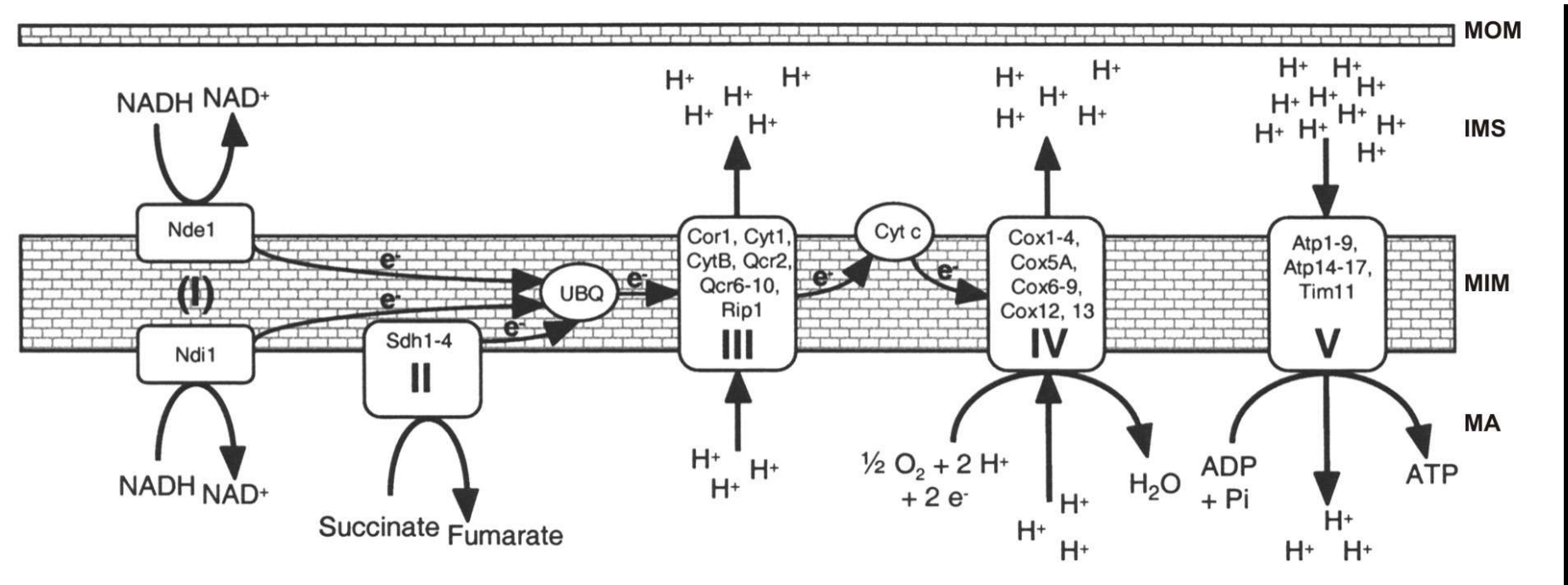


Courtesy of George Palade



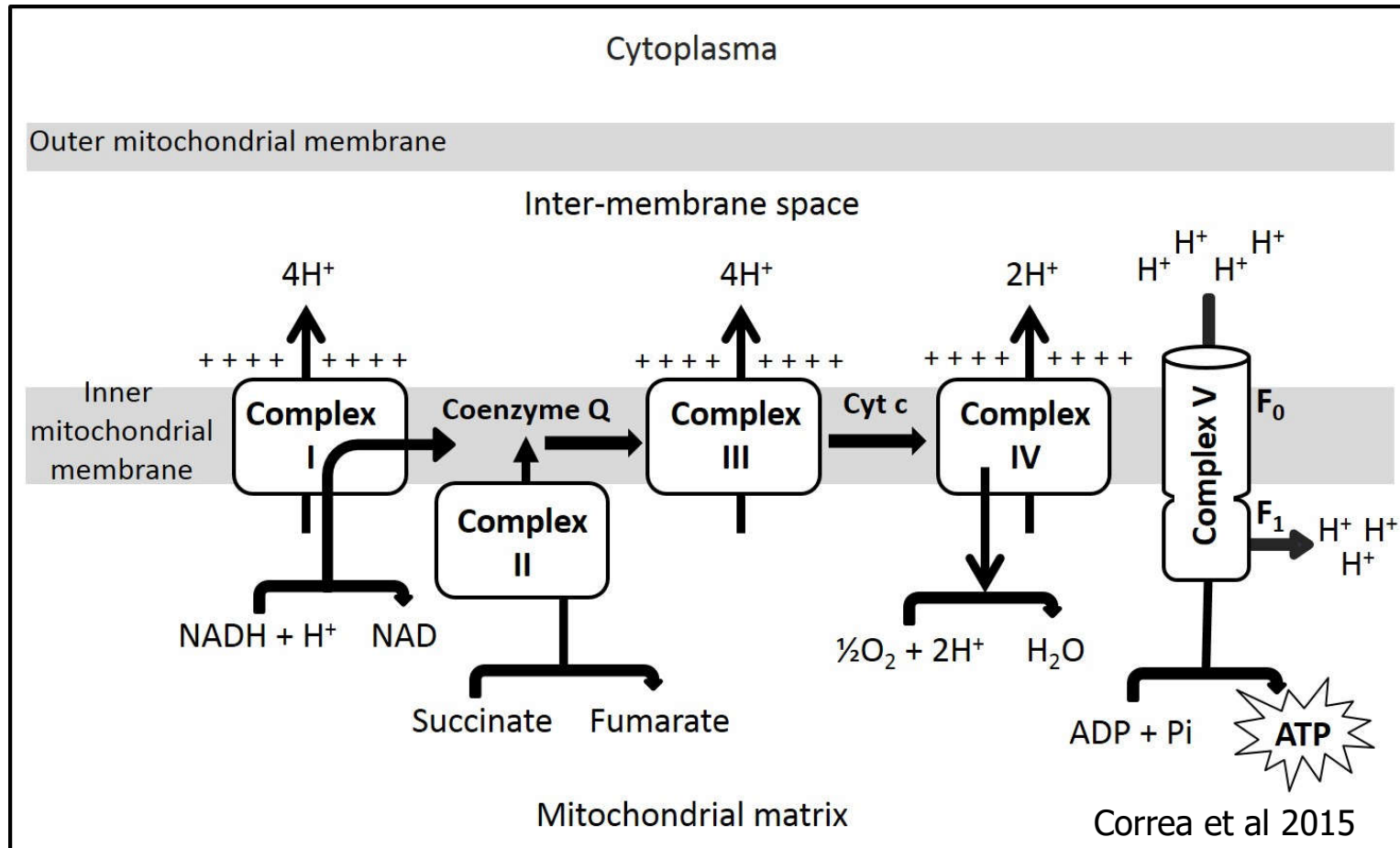
Courtesy of Stefan Jakobs

Oxidative phosphorylation in *Saccharomyces cerevisiae*

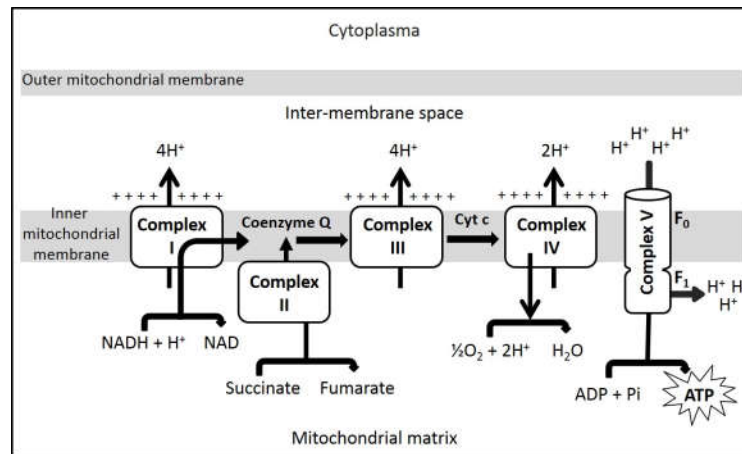
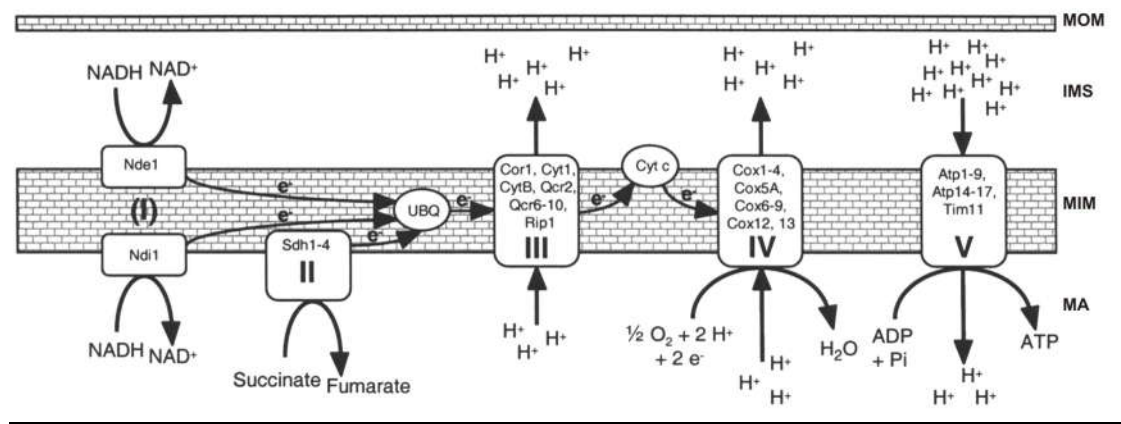


Joseph-Horne et al 2001 BBA-Bioenerg.

Oxidative phosphorylation in *Homo sapiens*



Differences/similarities between human and yeast oxidative phosp



Differences/similarities between human and yeast oxidative phosphorylation

- Similarities

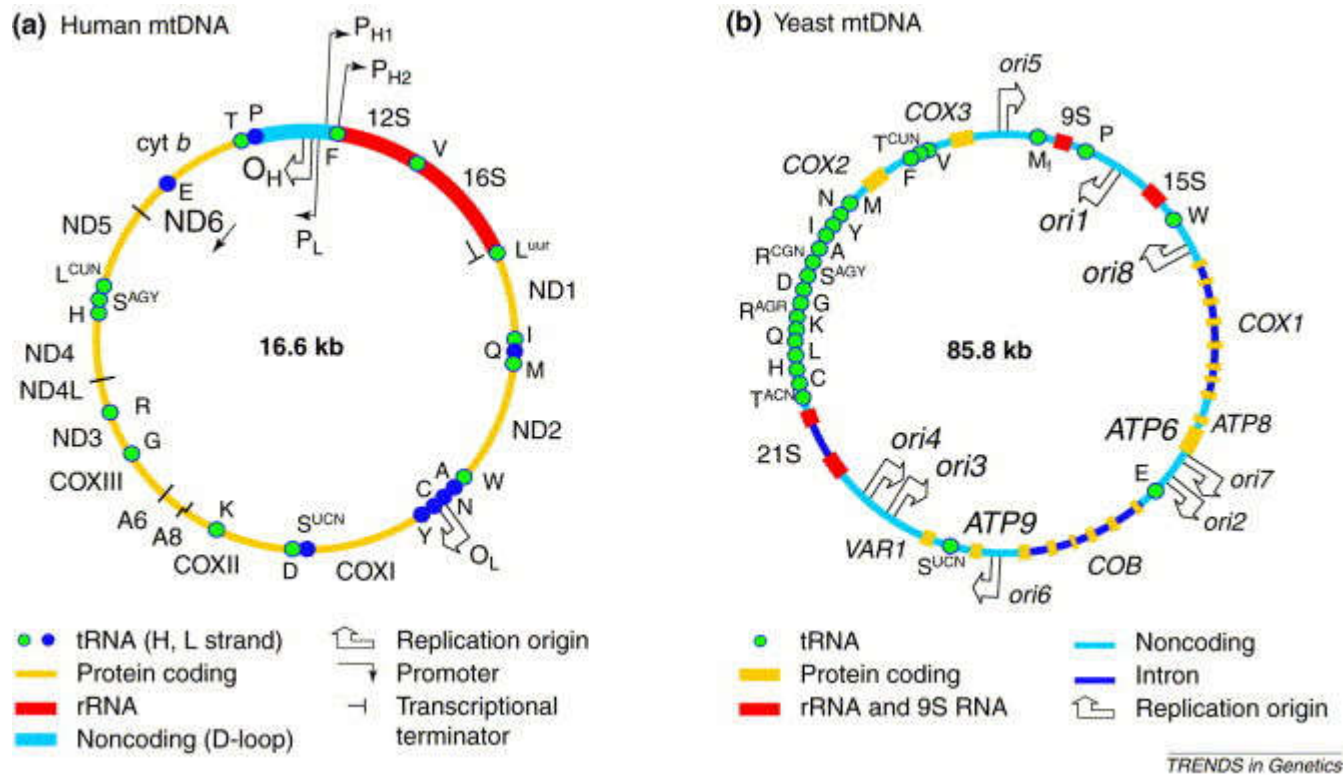
- Membrane potential across inner mitochondrial membrane generated by charge and pH
- ATP synthase uses membrane potential to generate ATP – conversion of electrochemical gradient to movement to chemical energy
- Subunits of complexes encoded by mitochondrial DNA

Differences/similarities between human and yeast oxidative phosphorylation

- Differences
 - Complex I in yeast are single subunit enzymes that do not pump protons
 - Different numbers of subunits

Generally, the same process is accomplished using similar mechanisms

Mitochondrial DNA in humans and yeast



Jacobs 2001

Differences/similarities between human and yeast mitochondrial DNA

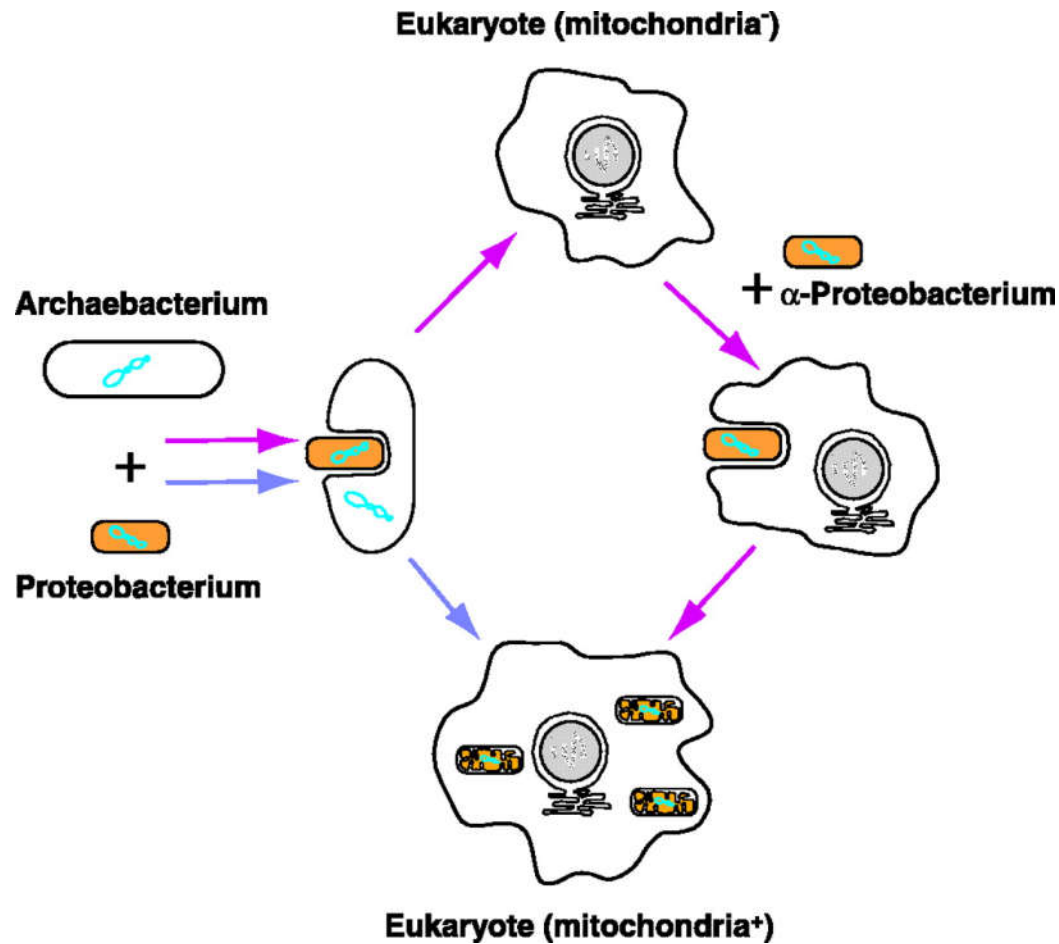
- Similarities
 - Circular
 - Genes encode proteins, tRNAs, rRNAs
 - Multiple copies per mitochondrial
- Differences
 - Size
 - Yeast mtDNA has multiple promoters, human one promoter site
 - polycistronic
 - Yeast mtDNA genes contain introns

Differences/similarities between human and yeast mitochondrial DNA

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Generally, the same process is accomplished using similar mechanisms

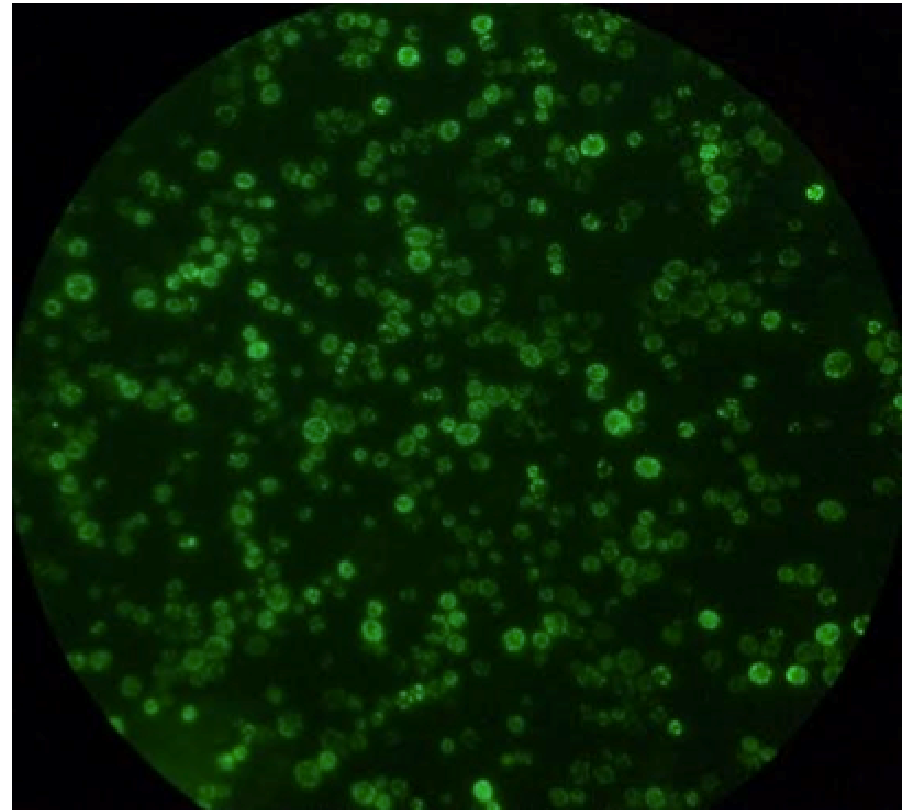
Origins of mitochondria



Grey et al 1999

Saccharomyces cerevisiae as a model to study mitochondrial biology

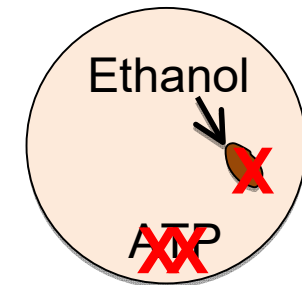
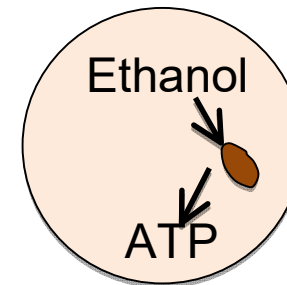
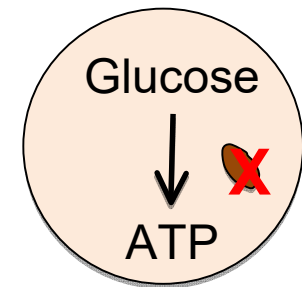
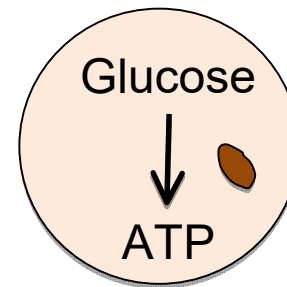
- Facultative anaerobe
 - ATP requirements can be satisfied by fermentation
 - Yeast lacking respiratory function can be propagated
 - Mitochondrial (respiratory) function can be demonstrated using metabolic substrates requiring a functional electron transport chain



Zoha Mian

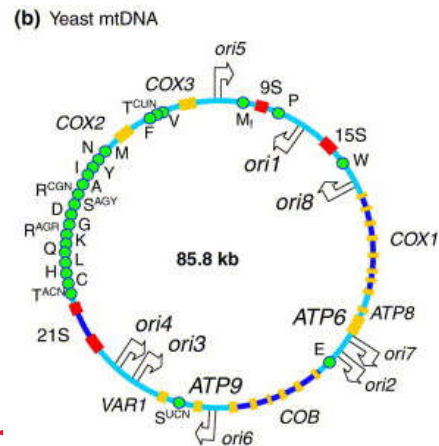
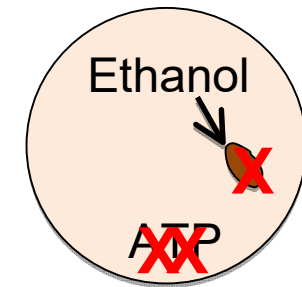
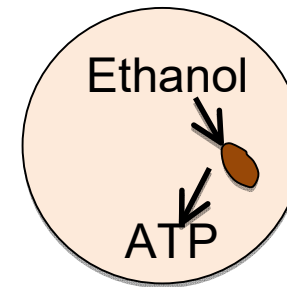
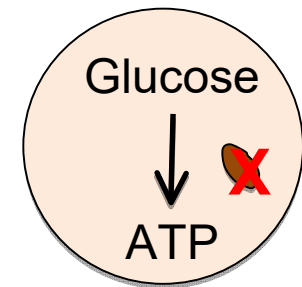
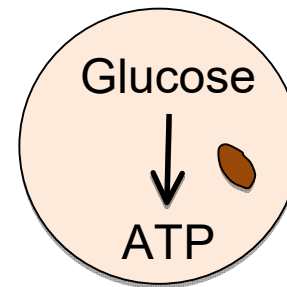
Saccharomyces cerevisiae as a model to study mitochondrial biology

- Facultative anaerobe
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Saccharomyces cerevisiae as a model to study mitochondrial biology

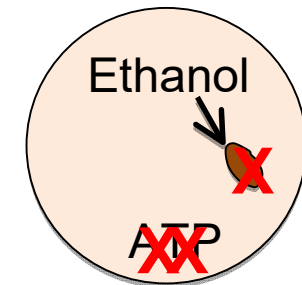
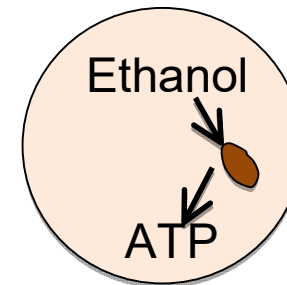
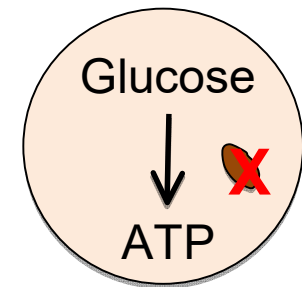
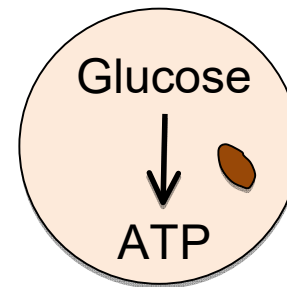
- Mitochondrial mutants
 - Pet – nuclear gene
 - Mit – mitochondrial gene
 - Spontaneous/induced
 - Single base or large deletion



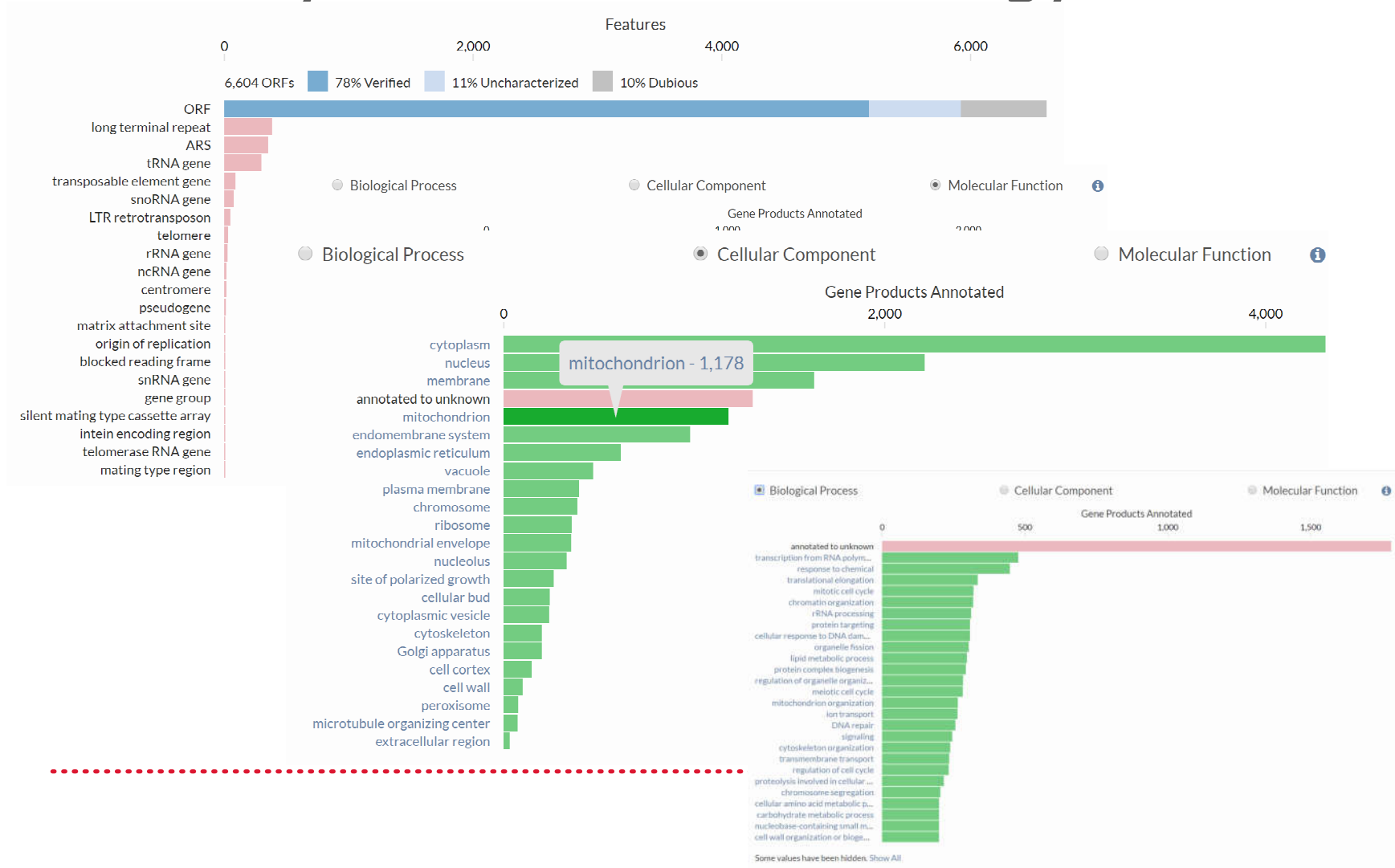
TRENDS in Genetics

Saccharomyces cerevisiae as a model to study mitochondrial biology

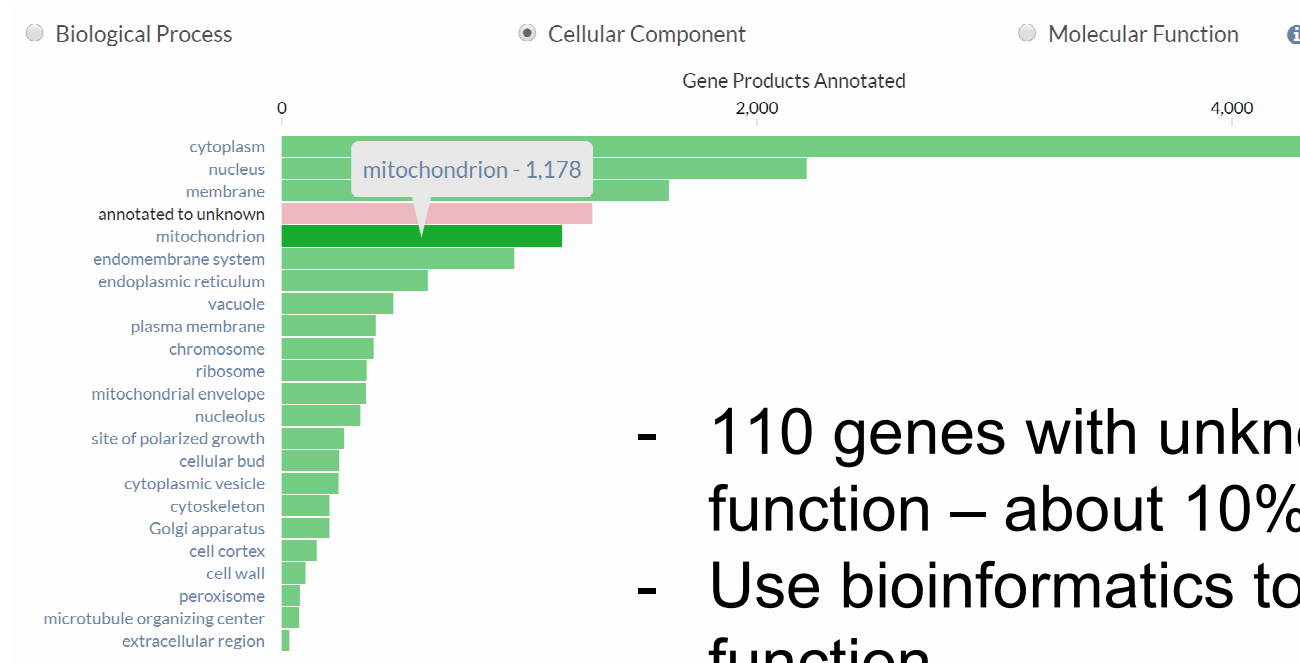
- Similar functions and organization as higher eukaryotes
- Homology in signaling pathways
- Homology in mitochondrial proteins, processes and biogenesis
- Abundance of publicly available datasets (www.yeastgenome.org)



Saccharomyces cerevisiae as a model to study mitochondrial biology

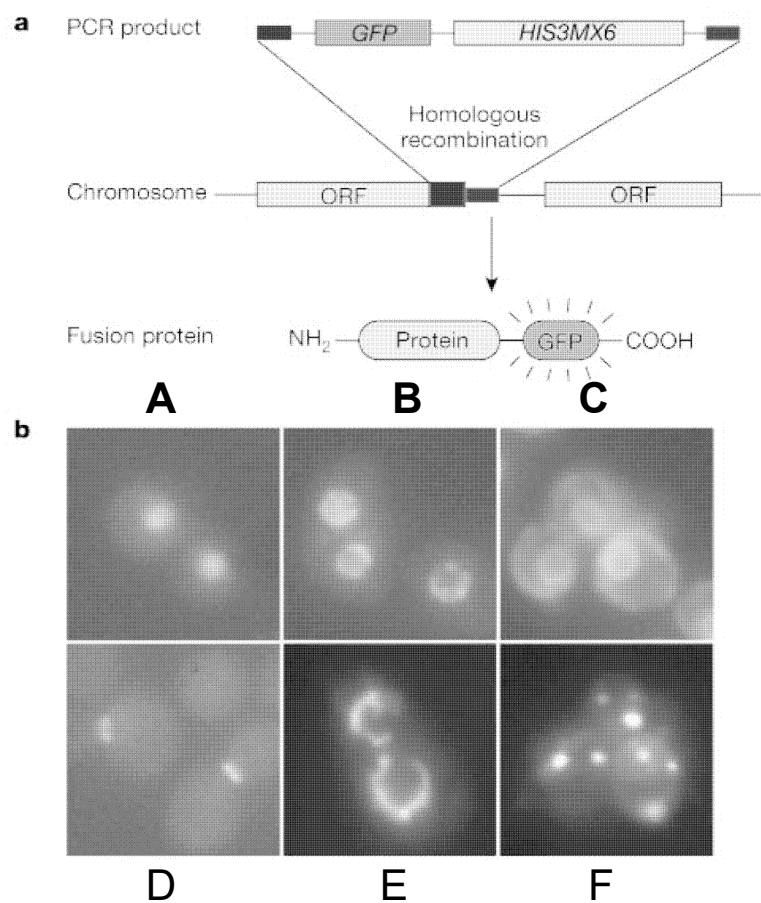


Saccharomyces cerevisiae as a model to study mitochondrial biology



- 110 genes with unknown function – about 10%
- Use bioinformatics to predict function

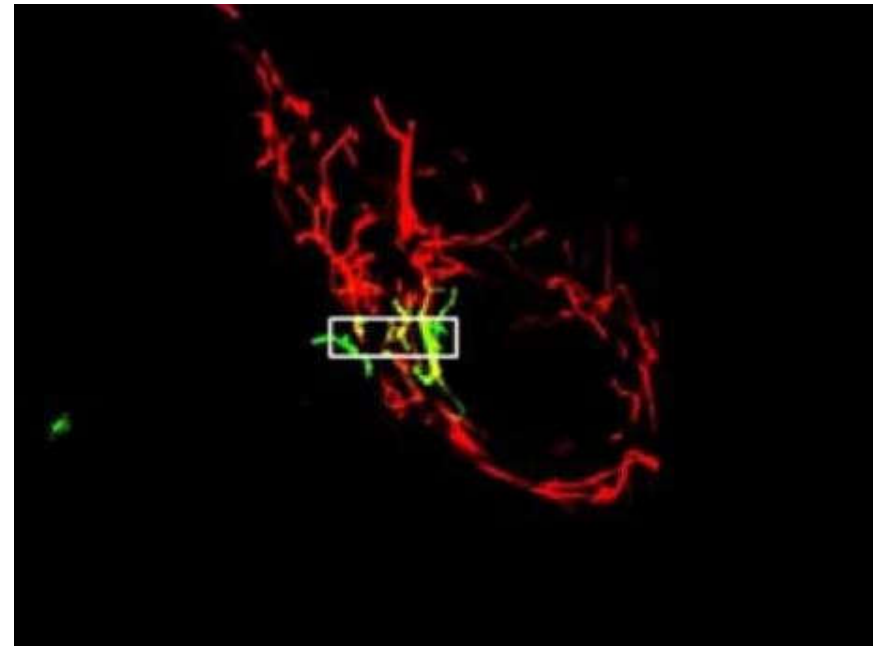
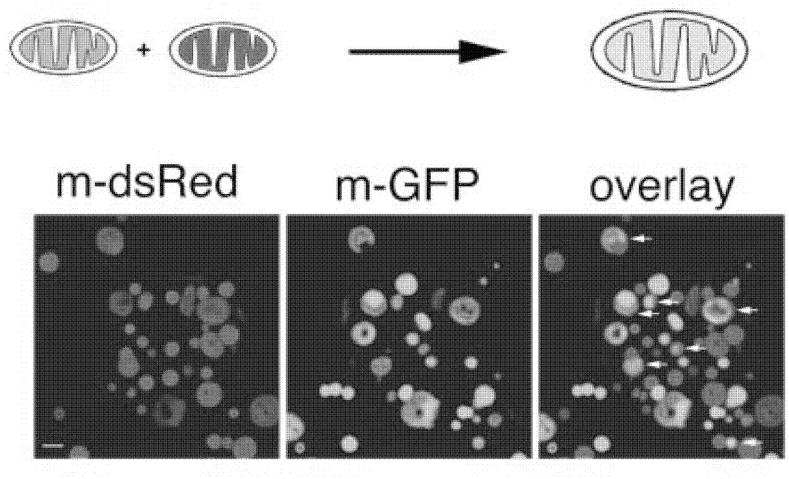
Localising genes to mitochondria in *Saccharomyces cerevisiae*



- Attach genetic “tag” that can be detected in cells to visualise location
- GFP – green fluorescent protein

Significant contributions of yeast research

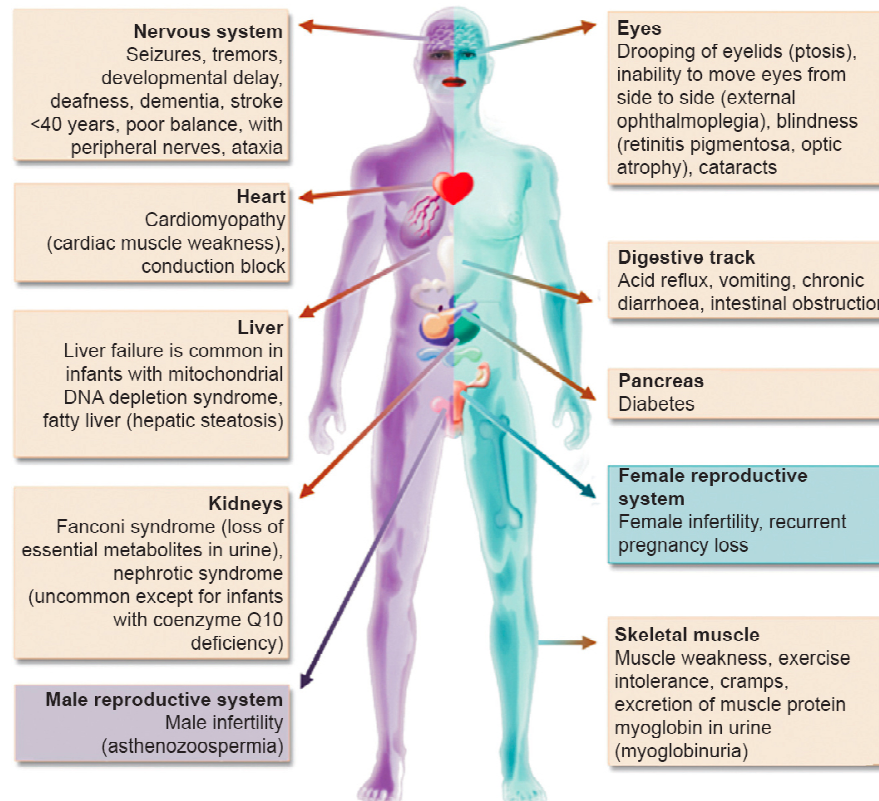
- Membrane trafficking
- Aging
- Mitochondrial biology
 - Protein import
 - Dynamics/morphology



Importance of yeast to research

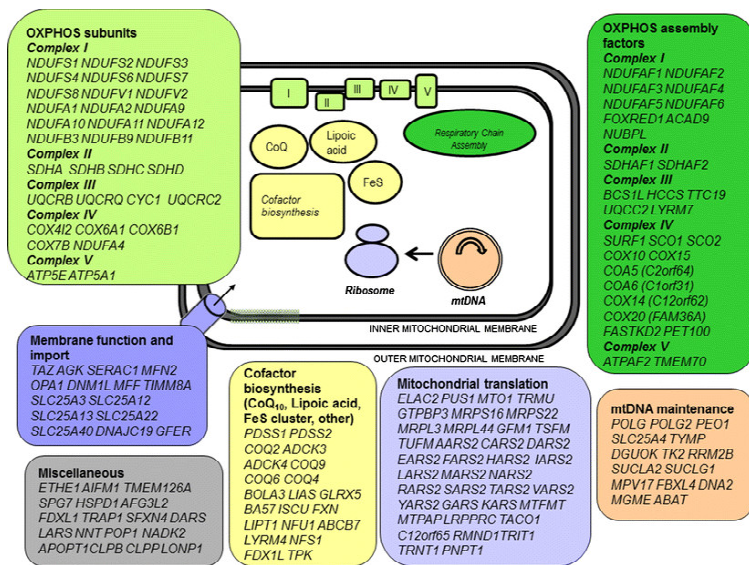
- First eukaryotic genome to be sequenced (1996)
 - Pioneered methodology to sequence human genome
- Allows for study of every gene
 - Gene deletions easy to generate through whole genome
- Many genes homologous to human genes
 - Identify function in yeast – likely to be similar in human

Mitochondria and Disease

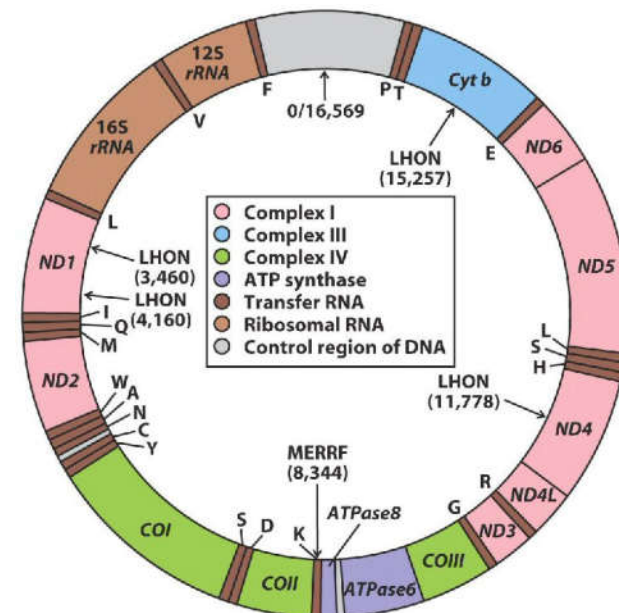


Khan et al 2015 Indian Journal of Medical Research

Mitochondria and Disease



Nuclear genes
 Mendelian inheritance



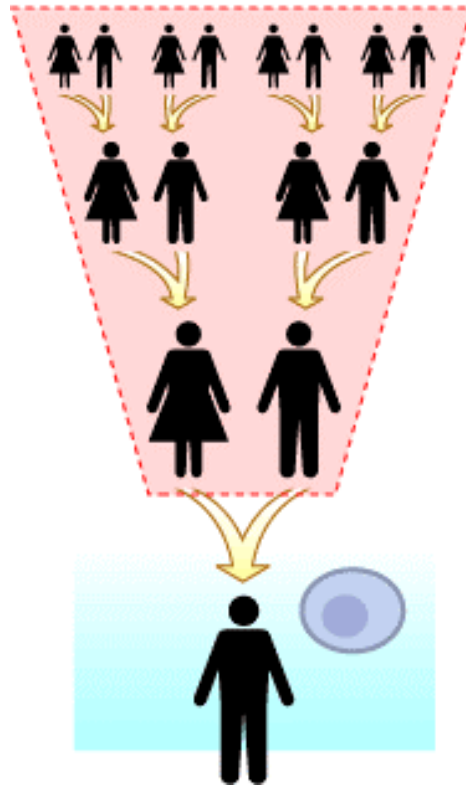
Mitochondrial genes
 Maternal inheritance

Rahman 2015 J Inherited Metabolic Diseases

<http://chemistry.umeche.maine.edu/CHY431/Code4.html>

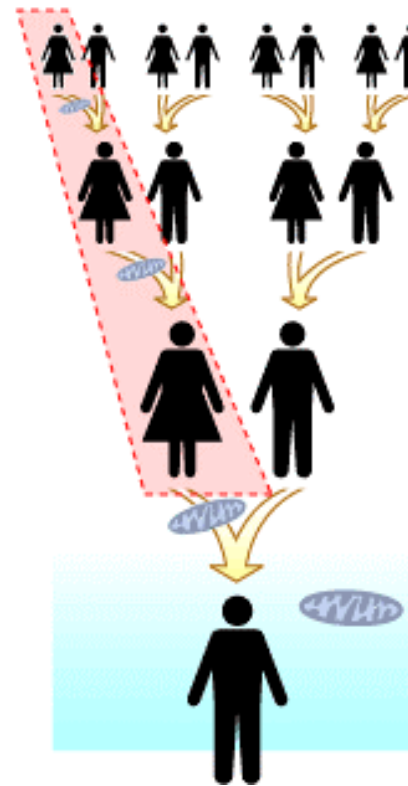
Mitochondria and Disease

Nuclear DNA is inherited from all ancestors.



Genetic disease
Both parents could pass on defective gene

Mitochondrial DNA is inherited from a single lineage.

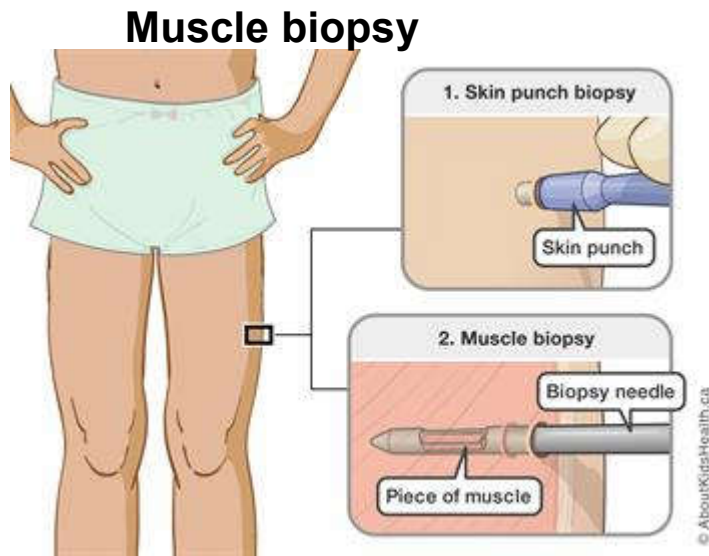


Mitochondria disease
Only mother passes on defective gene

https://undsci.berkeley.edu/search/imagedetail.php?id=175&topic_id=&keywords=dna

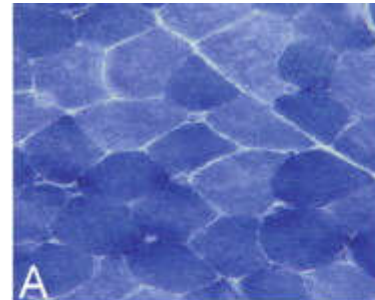
How to study mitochondrial disease

Test cells from the patient directly

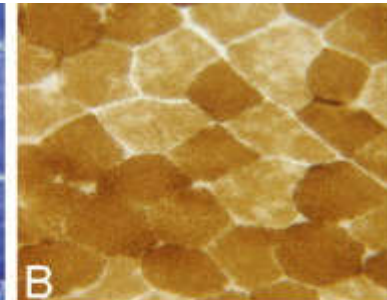


Blood test
- Lactic acidemia

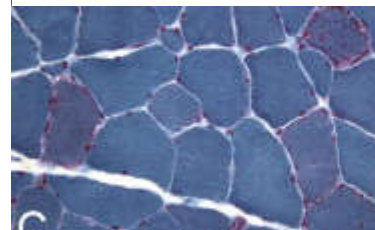
Normal SDH



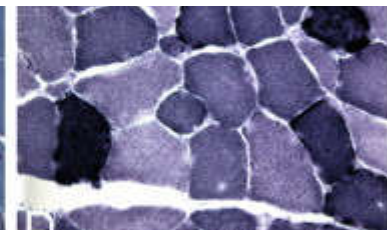
Normal COX



Patient Gomori



Patient SDH



Patient COX



Patient SDH/COX



Immunohistochemistry

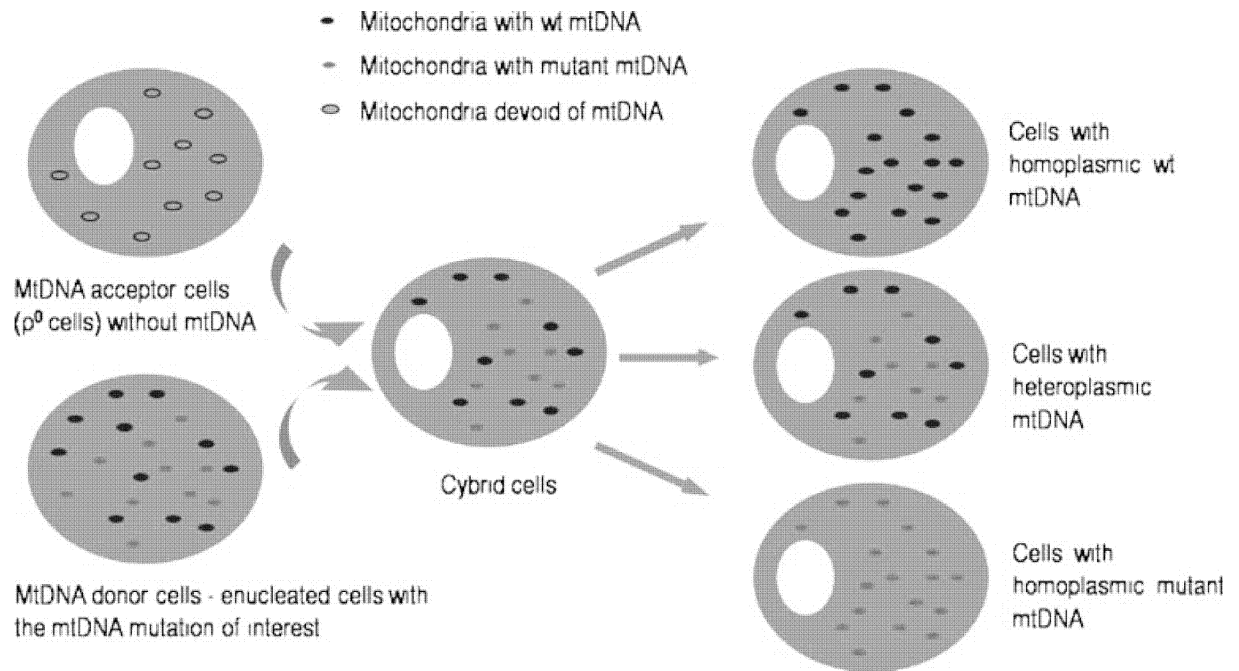
Kadenbach ed. *Mitochondrial Oxidative Phosphorylation*. New York

How to study mitochondrial disease

Create an immortal cell line with the mutation

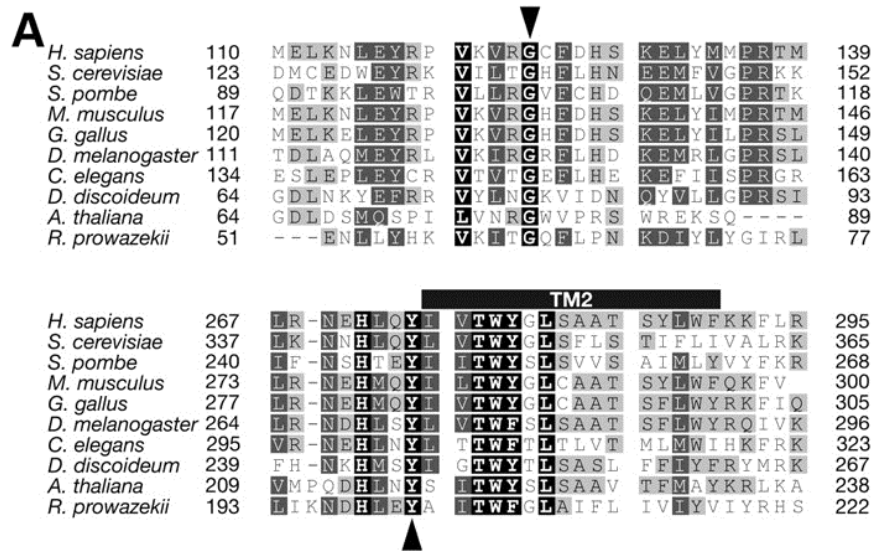
Treat with ethidium bromide to remove mtDNA in cell line

Cell from patient (platelet)

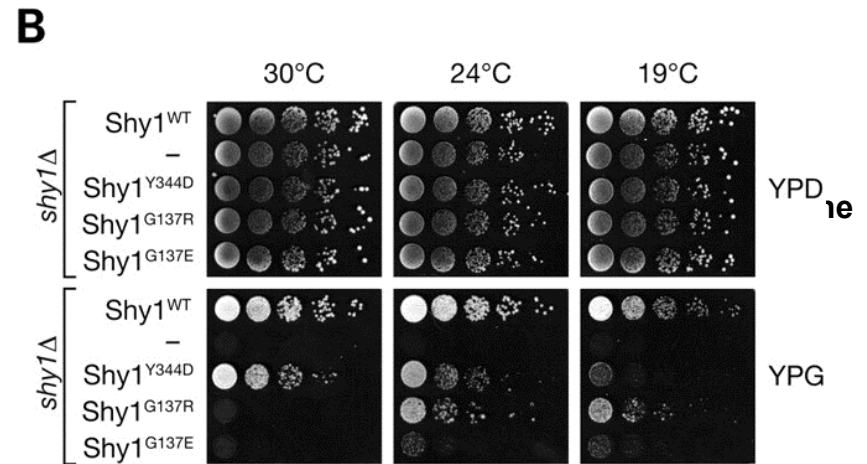


Maximo et al 2009

Modelling nuclear encoded mitochondrial disease in yeast



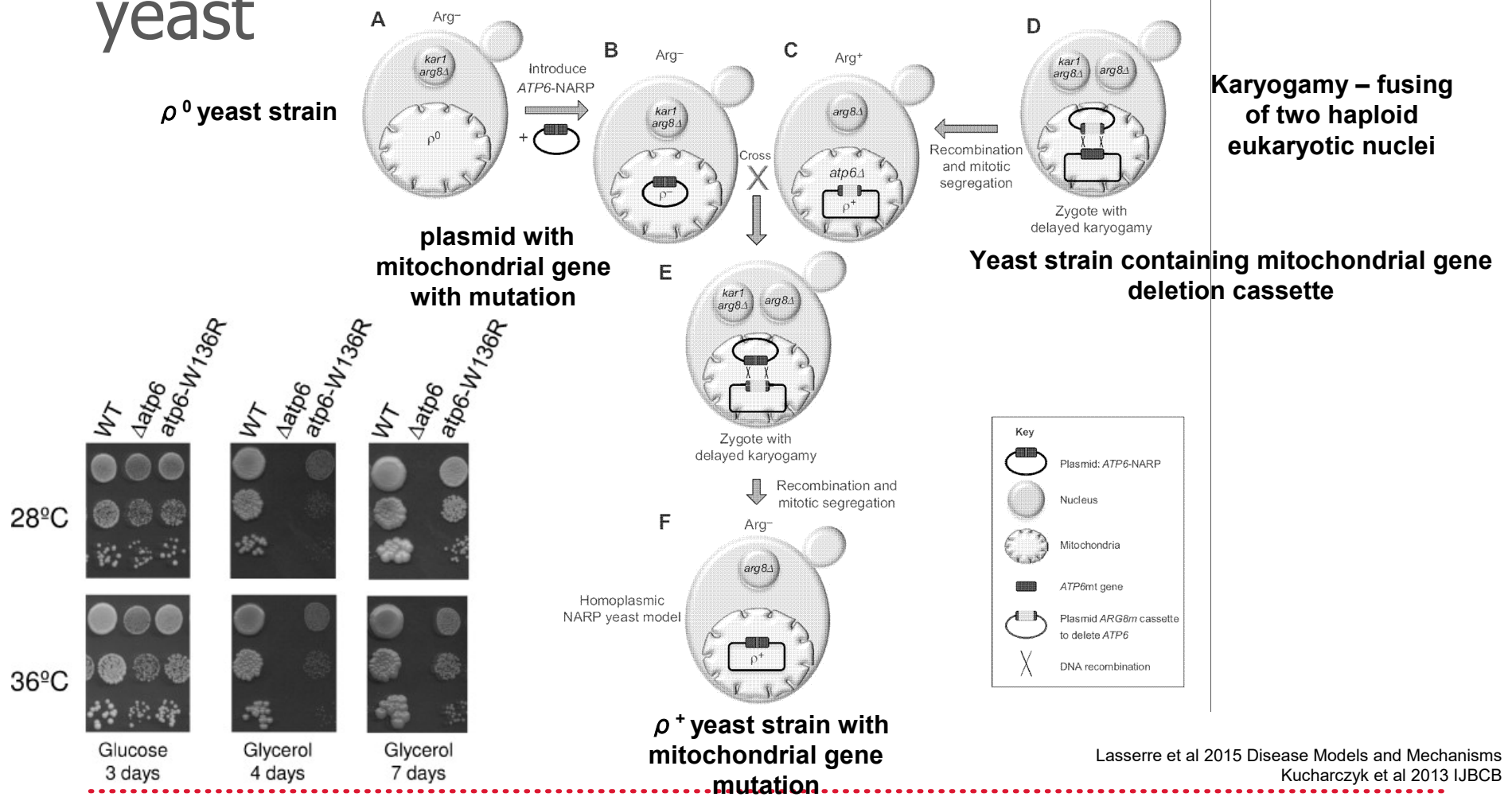
Sequence alignment of human, yeast and other species to identify conserved amino acids associated with disease



Introduce mutation into yeast in a deletion strain and test the phenotype

Reinhold et al 2011 Human Molecular Genetics

Modelling mitochondrial encoded disease in yeast



Lasserre et al 2015 Disease Models and Mechanisms
 Kucharczyk et al 2013 IJBCB

Mitochondrial DNA Nomenclature

Anderson *et al.*, Nature 290. p457 -465, 1981 first published the complete mtDNA sequence.

- This is now the reference sequence to which all mtDNA sequences are compared; the **CAMBRIDGE REFERENCE SEQUENCE (CRS)**.
- Each base around the DNA circle is numbered from 1 to 16,569. Base 1 is between HV1 and HV2.
- This numbering system is used to compare sequences and to locate differences.

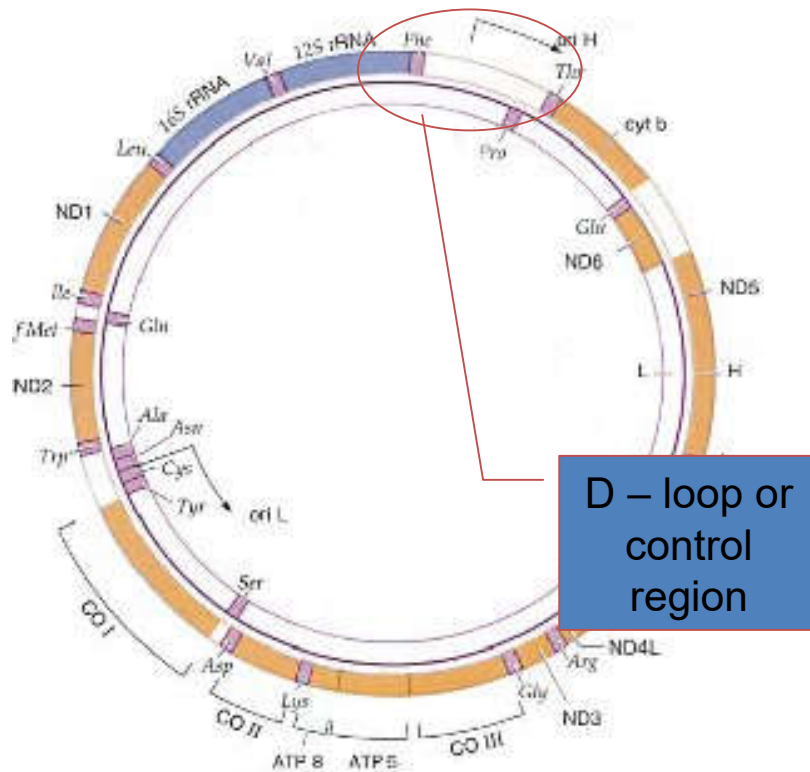
Mutations and mtDNA

- Most mutations in mtDNA are point mutations and small, one or two nucleotide insertions.
- There are no tandem repeats or large insertions/deletions.

Mutations and mtDNA

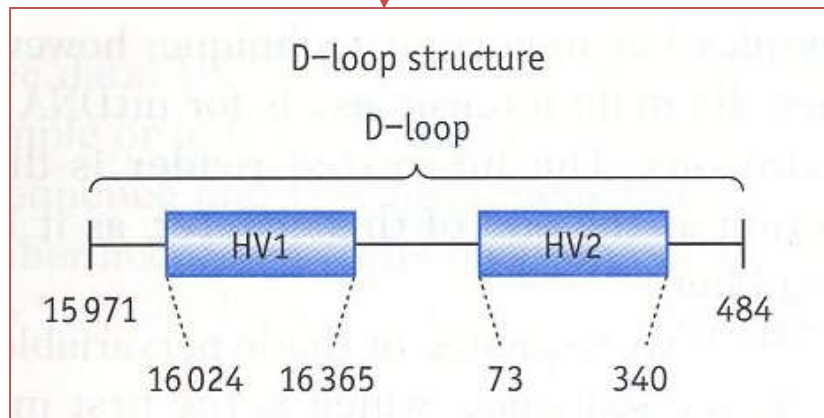
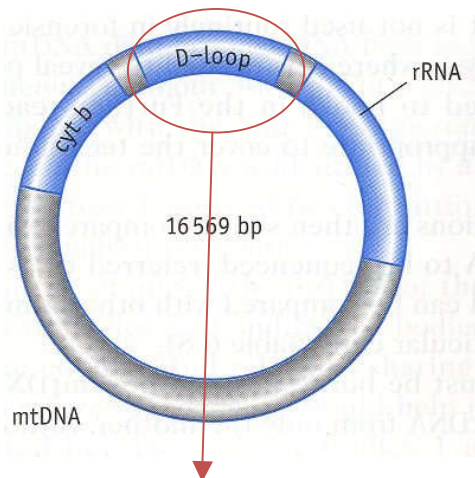
- mtDNA mutates 6 – 17 times more frequently than nuclear DNA
 - mtDNA polymerase is error prone
 - No DNA repair mechanisms in the mitochondrion

Human mtDNA – D-loop



- D – loop or control region
- Involved in control of replication.
- More variable than other regions – therefore of forensic value.
- About 1,100 bp long

Human mtDNA – D-loop



- The D-loop contains two regions which are more variable in sequence than other regions
- Hypervariable regions 1 and 2 (HV1 and HV2)
- HV1 – 340 bp
- HV2 – 267 bp

Human mtDNA – HV1 and HV2

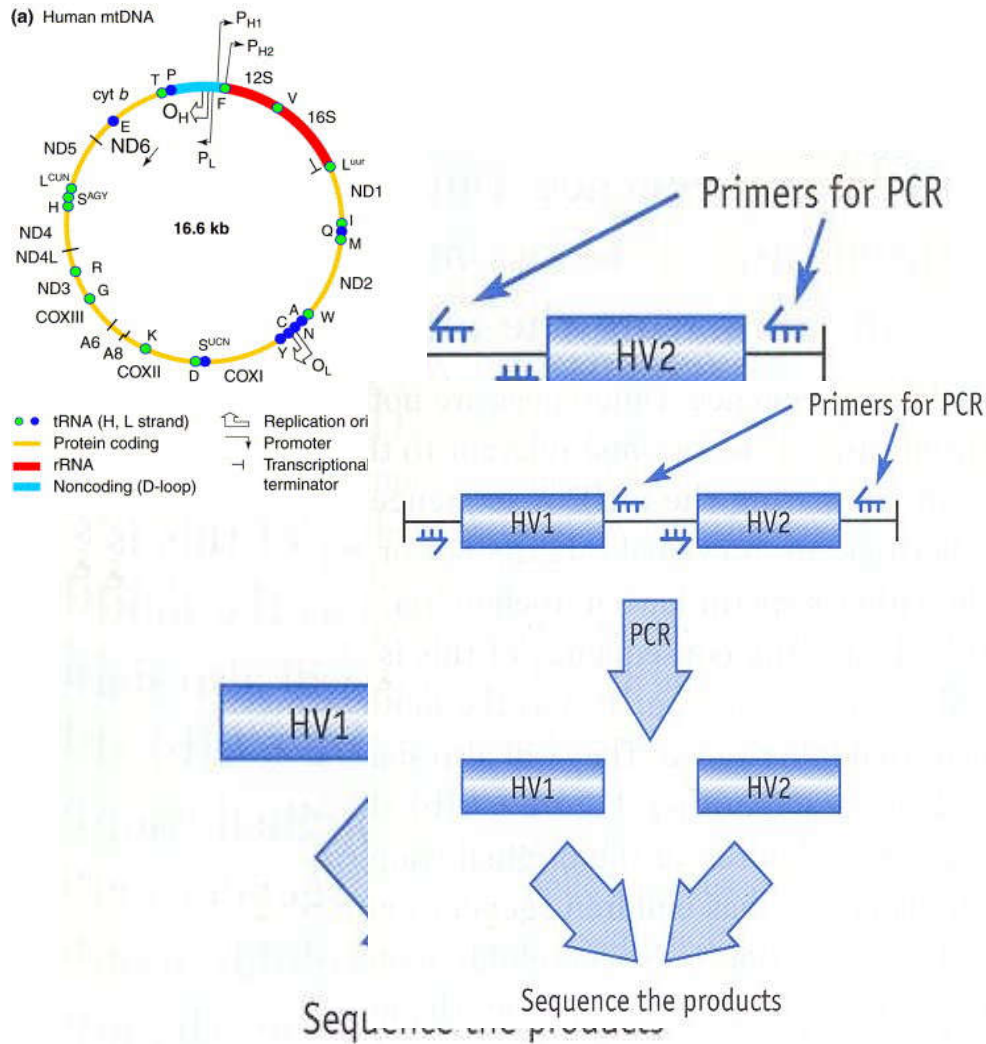
HV1

- 240 sites are variable
- variation ranges from 0 – 20 bp
- On average 8 bp difference between individuals

HV2

- 117 sites are variable
- variation ranges from 0 – 10 bp
- On average 2 bp difference between individuals

Strategy for mtDNA analysis



- As the differences are likely to be point mutations can only analyse by sequencing.
- PCR carried out on HV1 and HV2 – the products are subject to Sanger dideoxy automated sequencing

mtDNA

People with identical mtDNA sequences (or identical over part of the molecule) share the same HAPLOTYPE.

Certain parts of mtDNA (not necessarily in HV1 and HV2) show similar sequences in certain populations
- widely used in anthropology

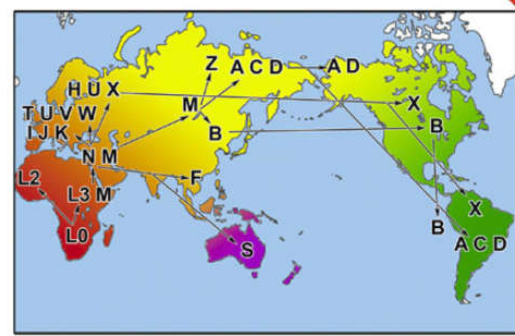
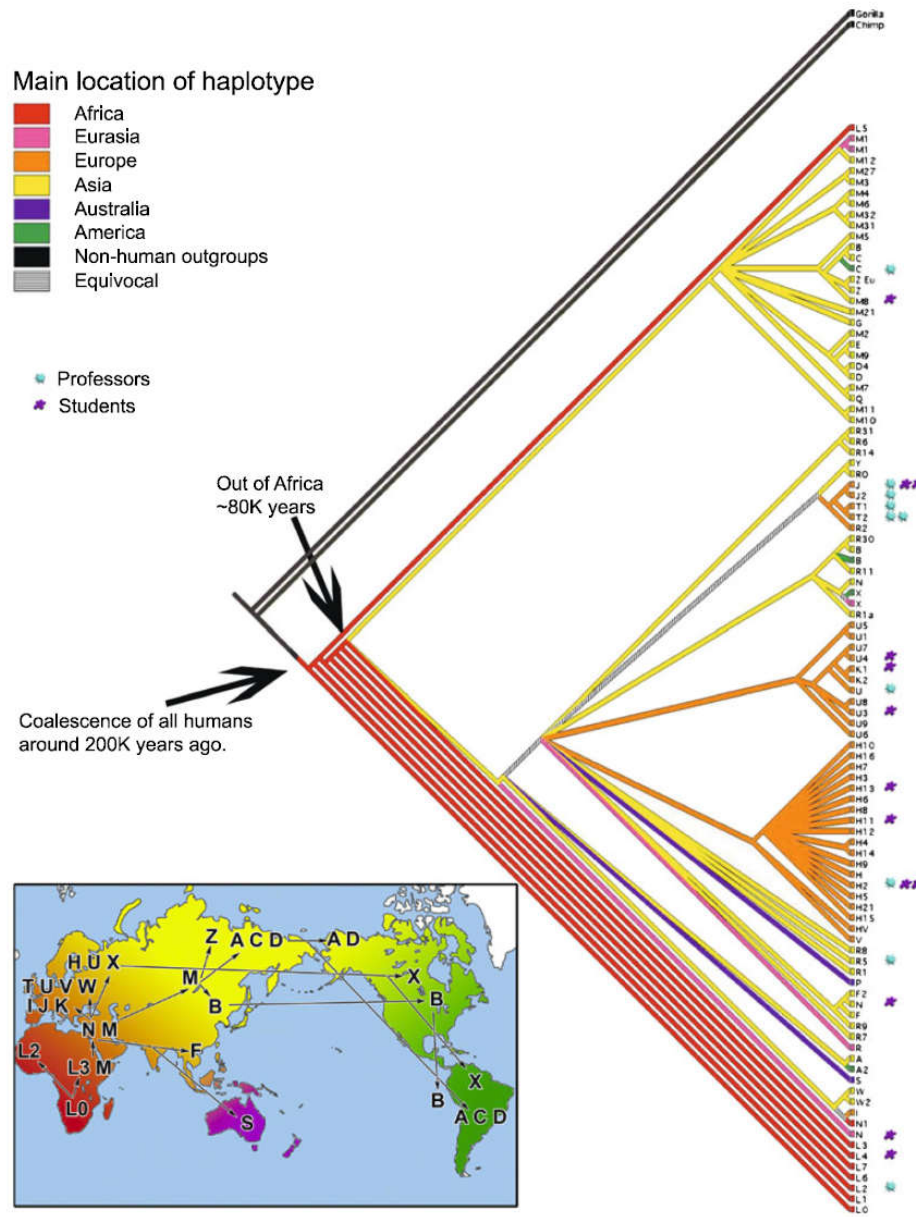
Classify humans according to mtDNA haplotype.

mtDNA

Main location of haplotype

- Africa
- Eurasia
- Europe
- Asia
- Australia
- America
- Non-human outgroups
- Equivocal

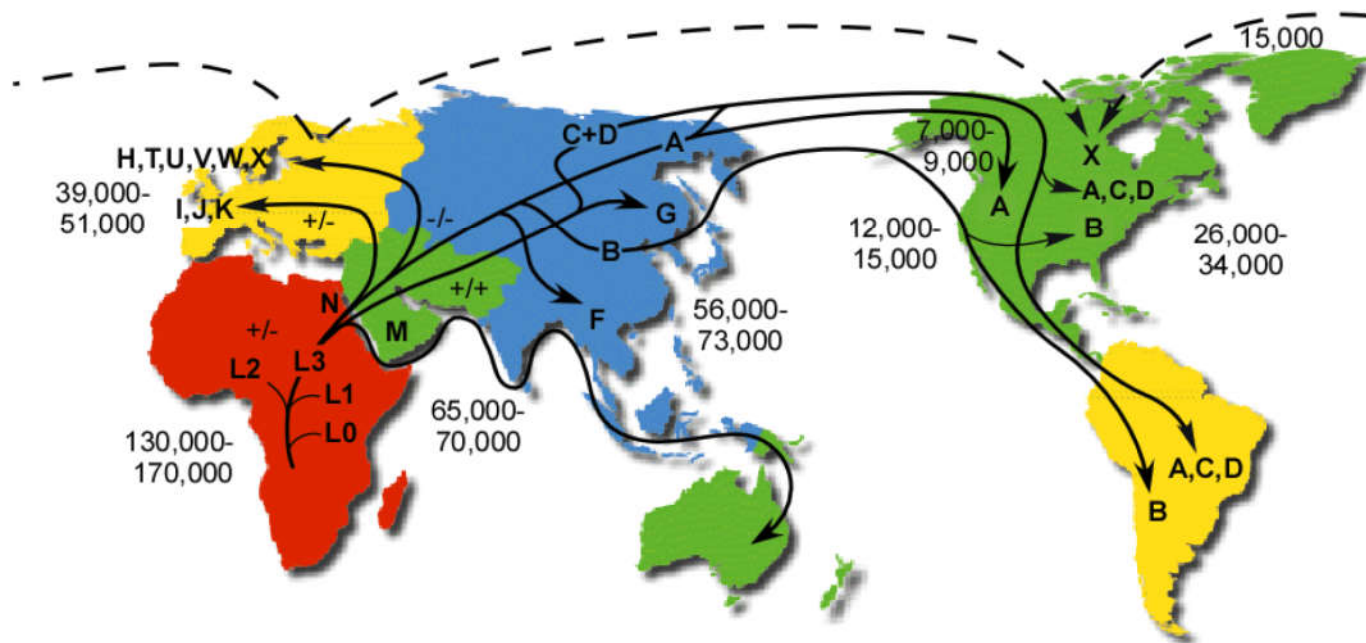
- Professors
- ★ Students



Mitochondrial phylogeny to demonstrate human migration

Human mtDNA Migrations

From <http://www.mitomap.org>



Symbols +/-, +/+, and -/- represent RFLP status for Dde I 10394 / Alu I 10397

Mutation rate = 2.2 - 2.9 % / MYR
Time estimates are YBP

Analysis

hv1	A	A	T	A	T	T	G	T	A	C	G	G	T	A	C	C	A	T	A	A	A	-	T	A	C
trim_1	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	-	T	A	C
trim_2	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	A	T	A	C
trim_3	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	-	T	A	C
trim_4	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	A	T	A	C
trim_5	A	A	T	A	T	T	G	T	A	C	G	G	T	A	C	C	A	T	A	A	A	A	T	A	C
trim_6	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	-	T	A	C
trim_7	A	A	T	A	T	T	G	T	A	C	A	G	T	A	C	C	A	T	A	A	A	-	T	A	C
trim_8	A	A	T	A	T	T	G	T	A	C	G	G	T	A	C	C	A	T	A	A	A	A	T	A	C
trim_9	A	A	T	A	T	T	G	T	A	C	G	G	T	A	C	C	A	T	A	A	A	-	T	A	C

hv1	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_1	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_2	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_3	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_4	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_5	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_6	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_7	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_8	T	T	G	A	C	C	A	C	C	T	-	G	T	A	G	T	A	C	A	T	A	A	A	A	A
trim_9	T	T	G	A	C	C	A	C	C	T	A	G	T	A	G	T	A	C	A	T	A	A	A	A	A

- CLUSTAL W analysis of part of HV1.
- Most of sequence is the same – identical sequences are of no value
- Base pair differences are highlighted
- Maternal relatives will show the same differences
- Cambridge Reference Sequence (HV1) identification used to give numbers to the bases that are altered.

Which samples are from relatives?
Which relatives?

Mutations and mtDNA sequencing

- Establishes relatives
- Over many many generations

Mitochondria summary

- An organelle required to produce ATP and carry out essential metabolic pathways
- Two genomes required for functional mitochondria
- Study required to understand and treat disease
- Human migration by mtDNA sequencing
- Forensic applications by mtDNA sequencing