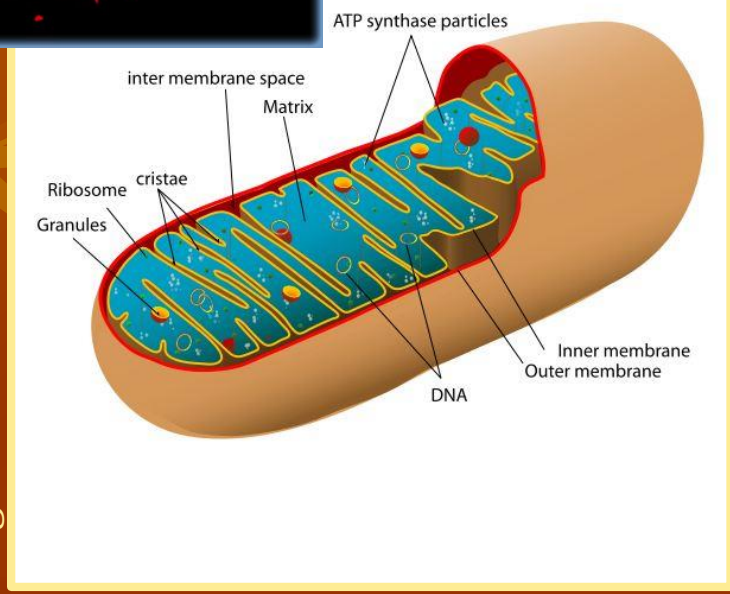
# MITOCHONDRIA (II.)

## The genetic apparatus

# Mitochondrial DNA (I.)


olympusmicro.com




brighthub.com

„extrachromosomal" DNA

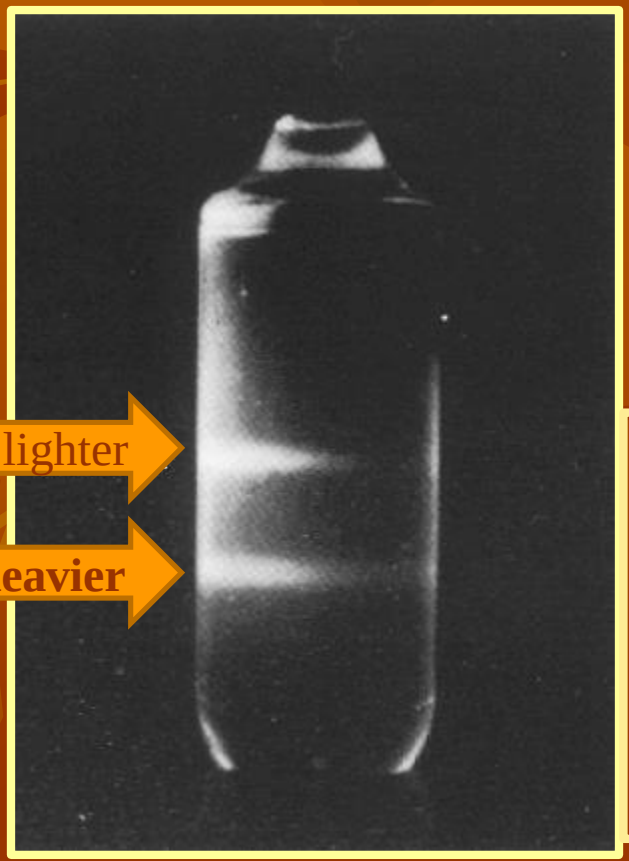
size varies
~16.5 kb in humans
(largest / 367 kb in Arabidopsis,
smallest / 6 kb in the protist
Plasmodium falciparum)

cells are polyploid / mtDNA
(2-10 „identical" copies
attached to inner membrane
in „mitochondrial nucleoid"

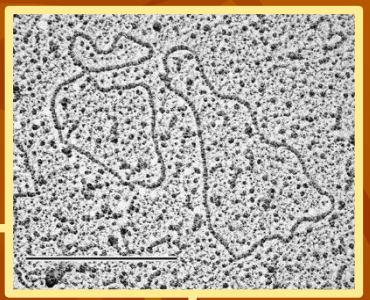
hundreds of mitochondria in every cell
∑ several x 1000 mtDNA copies in a cell)

# Mitochondrial DNA (II.)

www.mun.ca




CsCl centrifugation of DNA



ec.asm.org

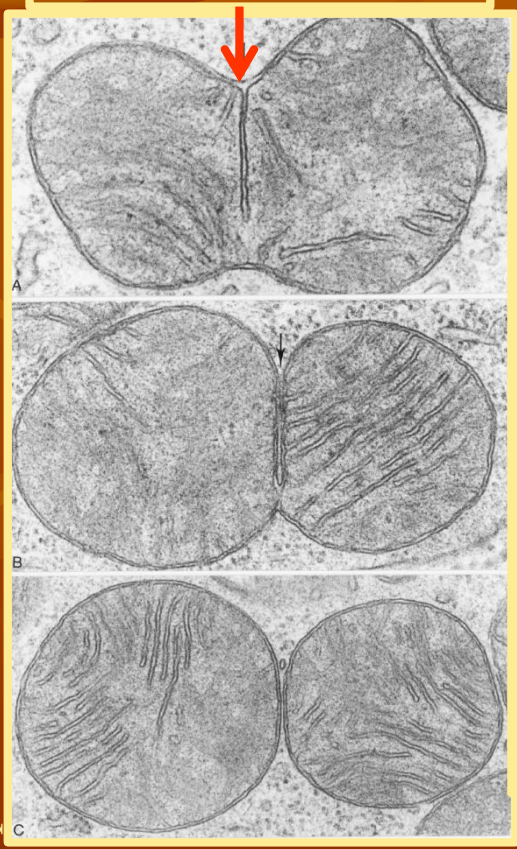
supercoiled structure
poorly associated with proteins

- less dense L- (light)
  and
- denser H- (heavy/richer in G)
  mtDNA chains in humans
  (base composition of chains differs )

- entire sequence of hu-mtDNA is known

# Duplication of mitochondria

inward furrowing of inner membrane



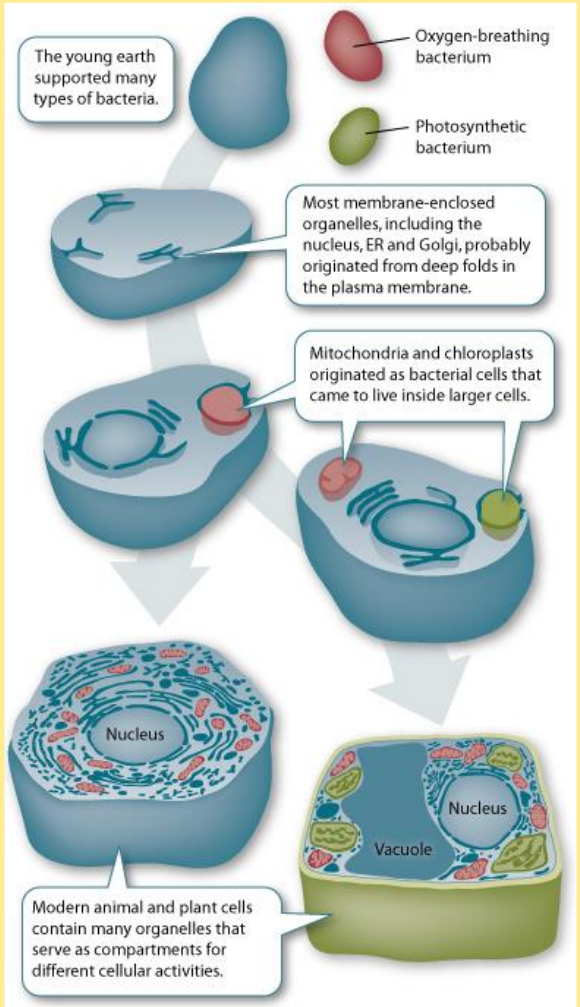

Mitochondria are never made „de novo”.

multiplication / growth + fission
(~ like prokaryotes)

number ↑ eg. / physical excercise

(They can also fuse with one another.)

# The endosymbiosis theory




learn.genetics.utah.edu

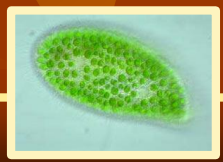
**Primitive anaerobic eukaryote engulfed an aerobic prokaryote → instead of digesting it they „learned" to live together → mutually advantageous relationship in the eukaryote's cytoplasm = endosymbiosis.**

**Host: provides nutrients + shelter**
**receives plenty of energy (ATP)**

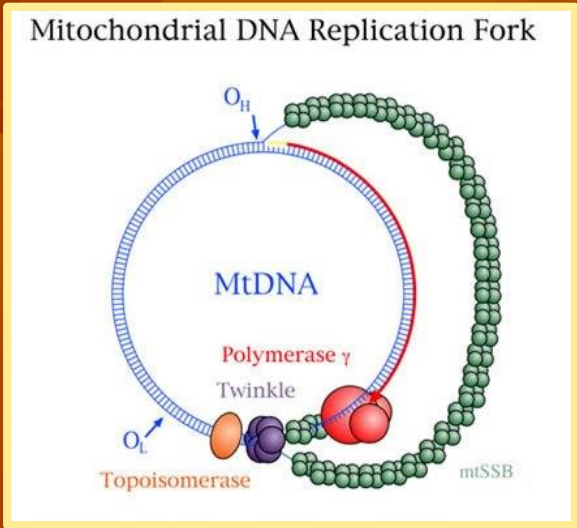
**most mt-genes transferred into the nuclear genome**

**Nucleic acid and protein synthesis can be inhibited by various antibacterial compounds (eg. antibiotics).**

**(Similar relationship between amoeboid unicellular organisms and cyanobacteria!)**



# Replication of mtDNA (I.)




nih.gov

**mtDNA replication is:**

**asymmetrical; 2 replic. origins OH and OL (2/3 complete leading strand synthesis from OH reveals OL to start lagging strand synthesis).**

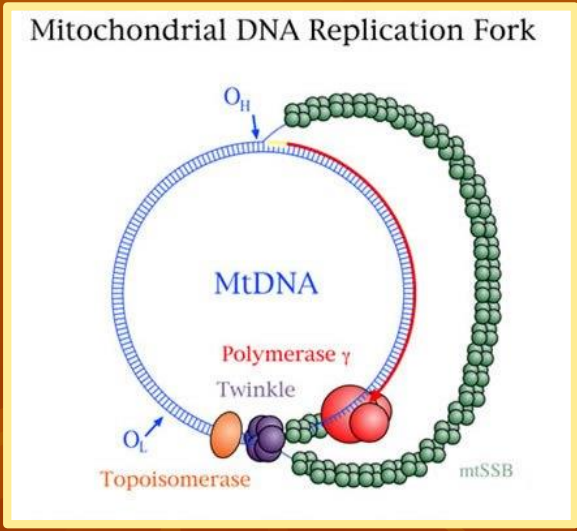
**continuous throughout the entire cell cycle with no distinct phase specificity**

**performed by enzymes: -coded by nuclear sequences**

**-synthesized on free cytoplasmic ribosomes**

**-imported from the cytoplasm by posttranslational transport**

# Replication of mtDNA (II.)




nih.gov

**Why have mtch. retained part of their genome with only few genes ↔ elaborate enzymatic machinery to replicate it??**

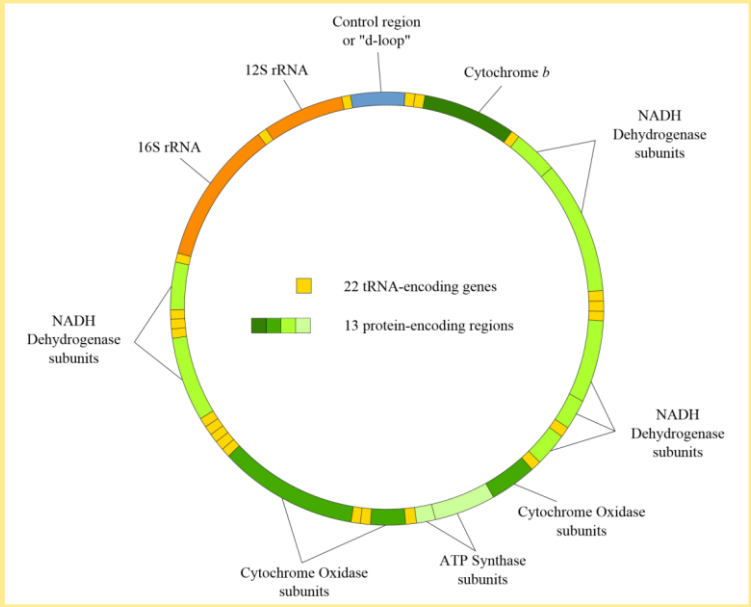
**(- hydrophobic protein products??**
**- possibility of local = faster regulation of ATP production?!)**

**(mtTWINKLE helicase - often mutated in Progressive External Ophtalmoplegia (PEO) :**

**in adults between ages 18 and 40**
**eye muscle weakness**
**drooping eyelids (ptosis)**

**exercise intolerance, muscle weakness**
**difficulty swallowing (dysphagia))**

# Transcription of mtDNA (I.)




wikimedia.org

**2 rRNAs**

**13 mRNAs**

**22 tRNAs**

**by proteins imported from the cytoplasm**

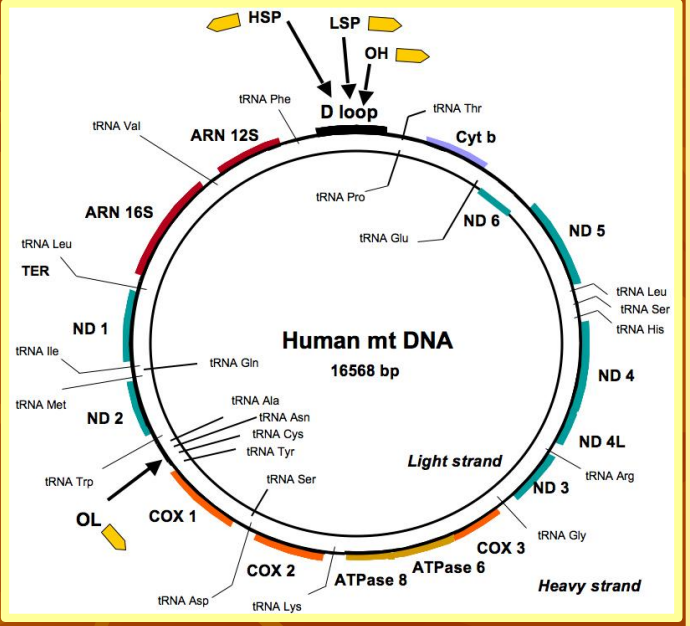
**No RNA trafficking in mammals**

**No introns in mammalian mtDNA**

**13 mRNAs → some hydrophobic inner membrane proteins of respiratory chain**
**+**
**ATP synthase subunits ↔ (more than 70 different polypeptides interact on the inner mitochondrial membrane to form the respiratory chain)**

**Monocystronic mRNAs with short or no 5'-UTR in many species SD and**
**55 nucleotide long polyA tail right after the STOP signal (made by mt-polyA polymerase)**

# Transcription of mtDNA (II.)




bioscience.org

hu mtRNA polymerase (POLRMT)
a single subunit protein

noncoding D-loop with (replication and)
transcription regulatory sequences

symmetrical transcription requires mtTFs
for initiation (at HSP and LSP) on H- and L-chain

long primary transcripts
later processed into individual r-, t- and mRNAs
(interspersed tRNAs serve as processing signals)

processing involves:
tRNA 5' and 3'endonucleolytic cleavage
mRNA polyA and tRNA 3'-CCA addition
(but no 5' cap!).

# Translation in mitochondria

**Mitochondria have their own ribosomes (55S) with 12S and 16S rRNA.**

**- mt-ribosomal proteins imported from the cytoplasm**

**↕**

**- no export of proteins**

**- in certain species ribosomes bind to mRNA via SD-sequences**

**- altered genetic code (4 codons have different „meaning”)**
**- faster mutation rate of mtDNA!**

**- initiating aminoacid = N-formyl-Met**

**- antibiotic sensitivity similar to that of bacteria**
**(eg. chloramphenicol or ototoxic aminoglycosides ⊣ mitochondrial small subunit)**

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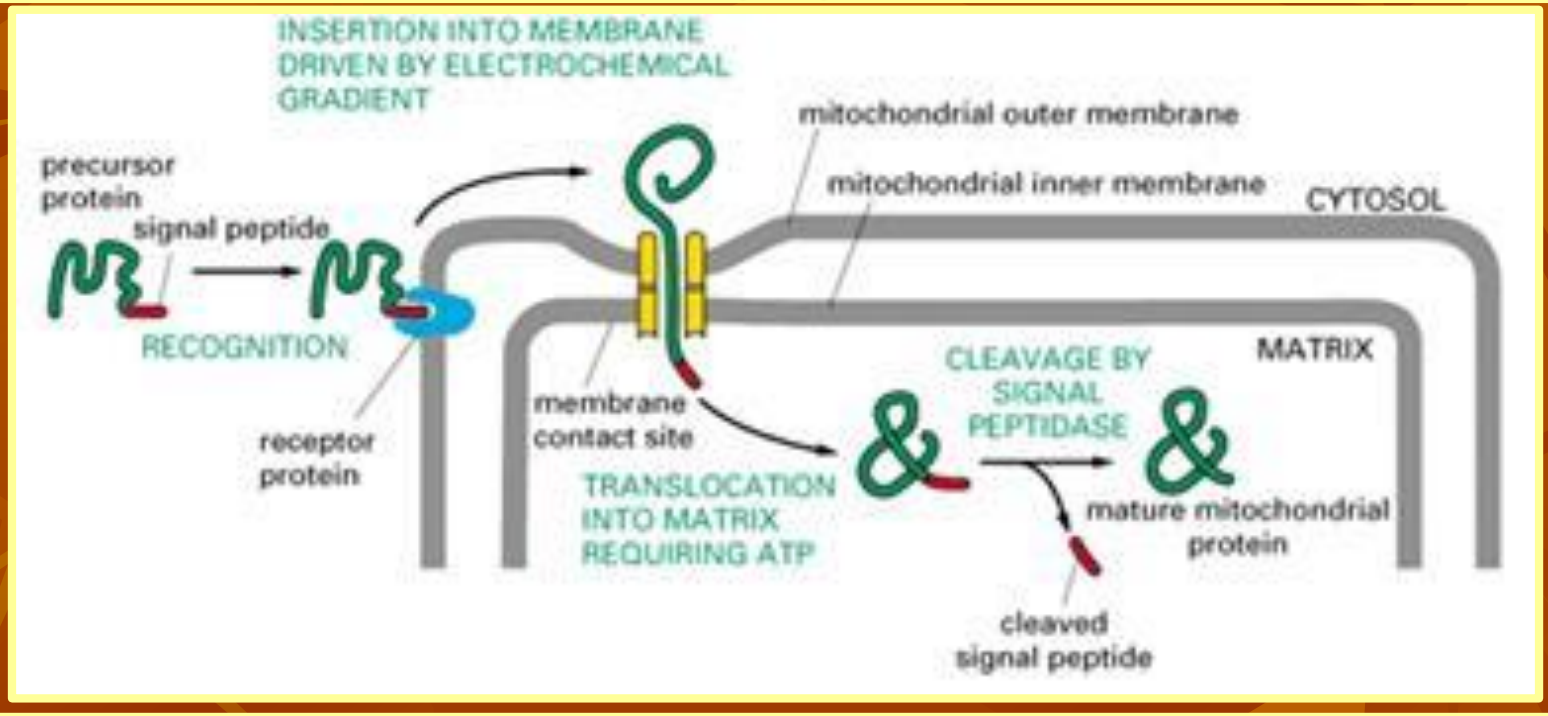
**(In cycloheximid-treated eukaryotic cells only mitochondria synthesize proteins.)**

# Posttranslational protein import (I.)

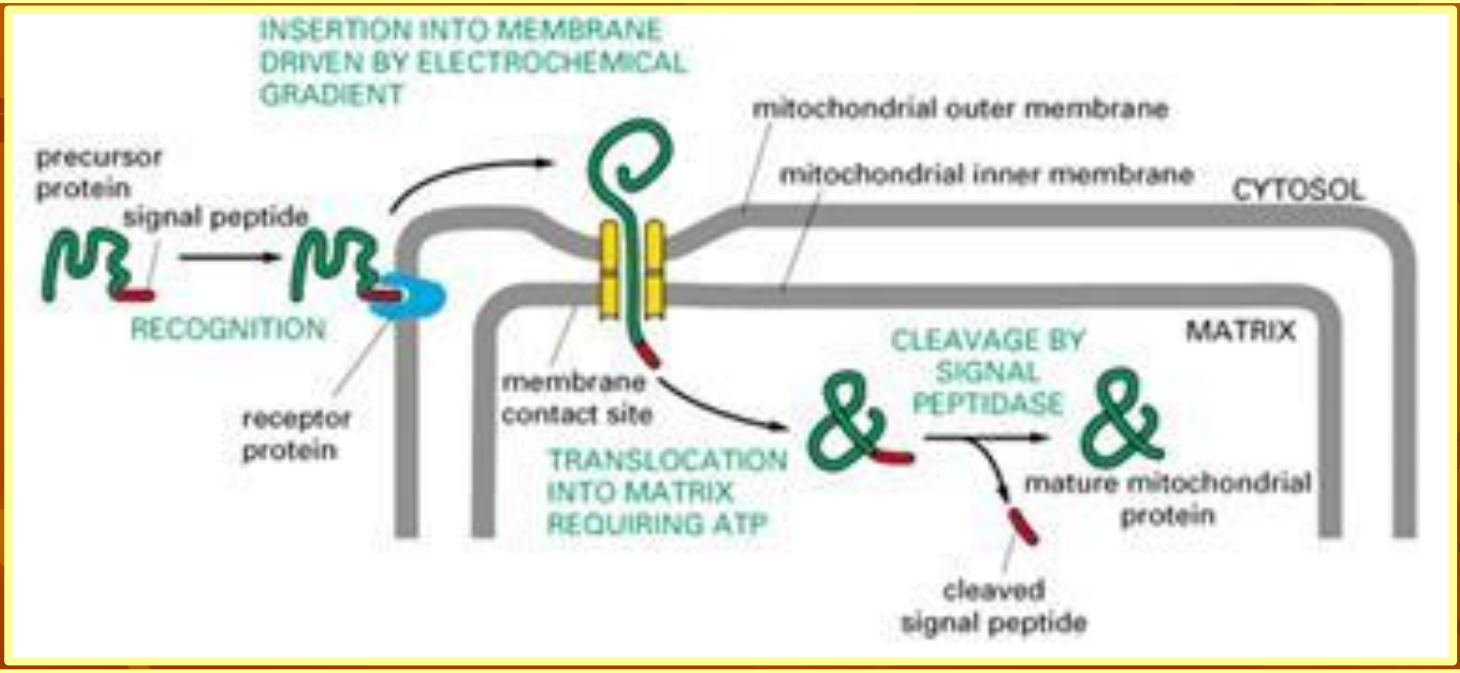



grkraj.org

Mitochondrial proteins synthesized on free ribosomes in the cytoplasm.

N-terminal uptake targeting sequence (rich in positively charged aminoacids)→ binds to chaperone and its receptor on the outer membrane → finds translocation channel (yellow) → cytoplasmic chaperones (cHsp70-ATP) keep the transported protein unfolded

# Posttranslational protein import (II.)




**Positively charged N-terminus passes through the proton-tight channel via „electrophoresis" (~ 3-60 sec/protein).**

**N-terminal signal sequence bound by inner membrane receptor → mitochondral chaperones (mHsp70-ATP) bind to and pull protein in → protease cleaves signal sequence off → protein's final conformationing / chaperones help, then dissociate**

# Faster mutation rate in mtDNA

**ROS generation / oxidative phosphorylation.**

**No histone cover of mostly coding mtDNA.**

**Weak repair capacity and proofreading → error prone mtDNA replication.**

**Steady but faster mutation rate (~ > 15x) and evolution than that of nuclear DNA.**

**→ Some (4) triplets of the mt-genetic code are different.**

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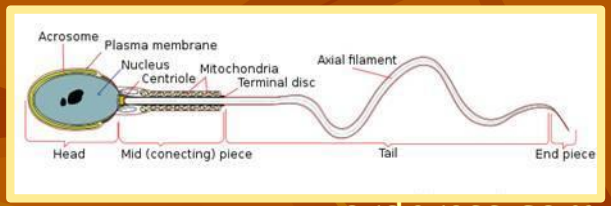
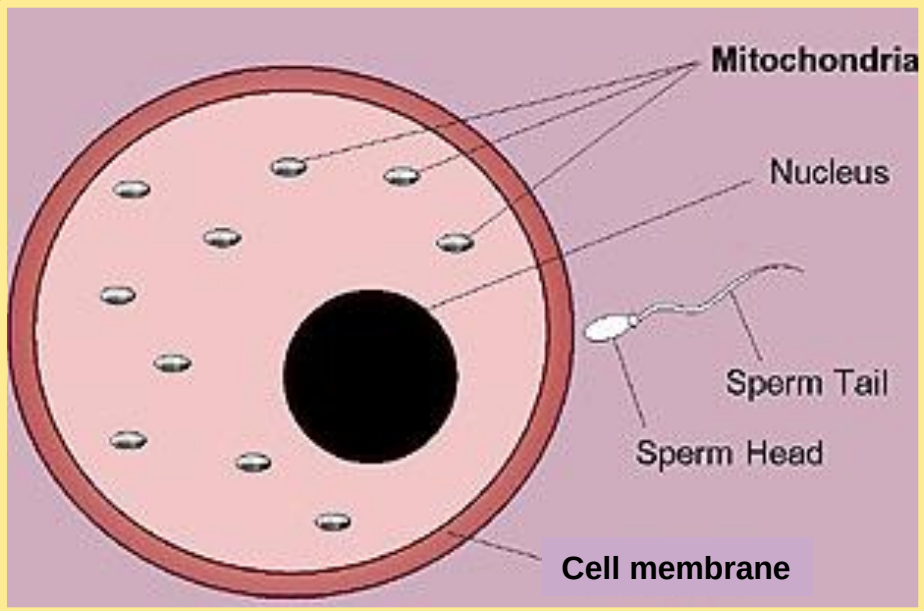
**→ The lack of mtDNA recombination allows the tracing back of mutations in order to reveal the relatedness of biological samples containing the mtDNA and to calculate the approximate time of their separation („molecular clocks”).**

# The „mitochondrial Eve” hypothesis

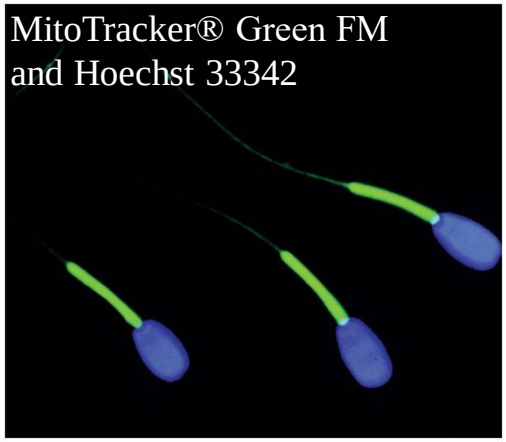



wordpress.com




dnasolutions.com.au

**~ 100 000 mtDNA copies can be present in a human egg cells.**
**(max. 0.01% mtDNA comes from the father)**




lifetechnologies.com

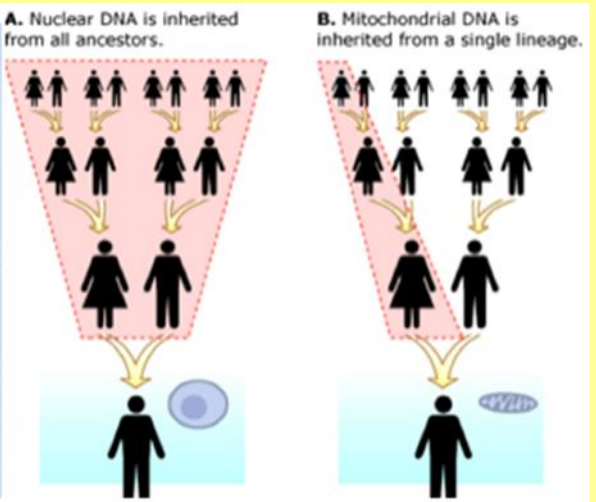
# Molecular clocks

**If mutations within a particular genetic system occur at a statistically uniform rate →**
**→ the rate at which mutations have been accumulating in mtDNA allows to refine the timeline of our evolution.**

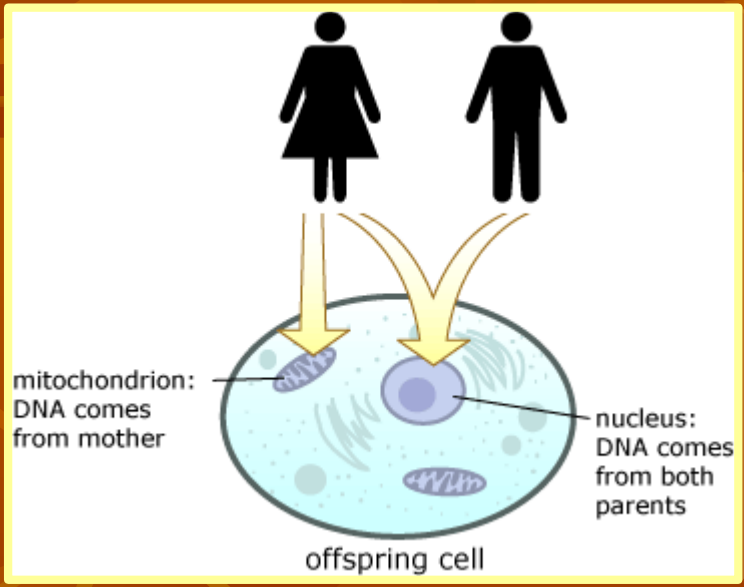
**(In reality influenced by many factors …)**

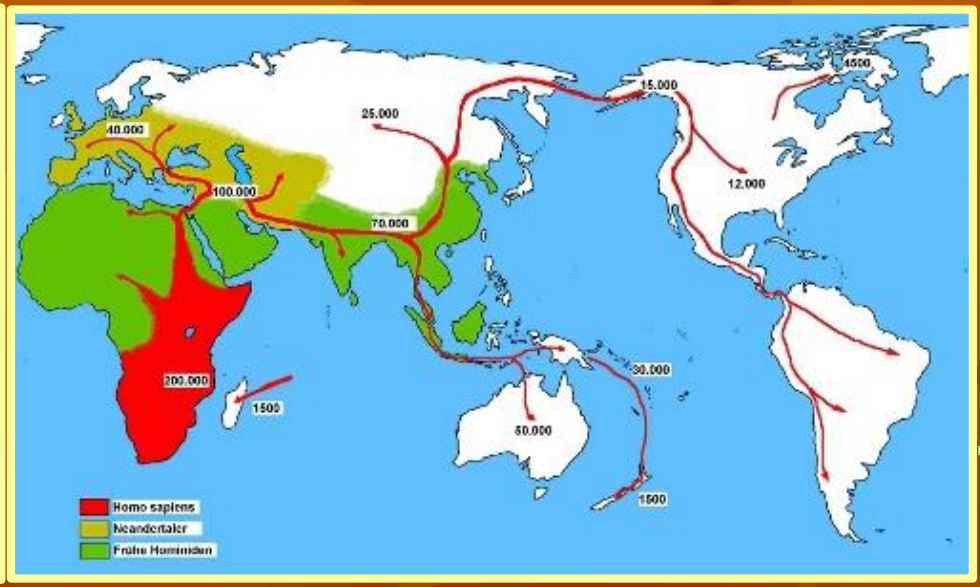
**∑ complicated science = „mitochondrial anthropology”**

3.bp.blogspot.com



# The „mitochondrial Eve” hypothesis



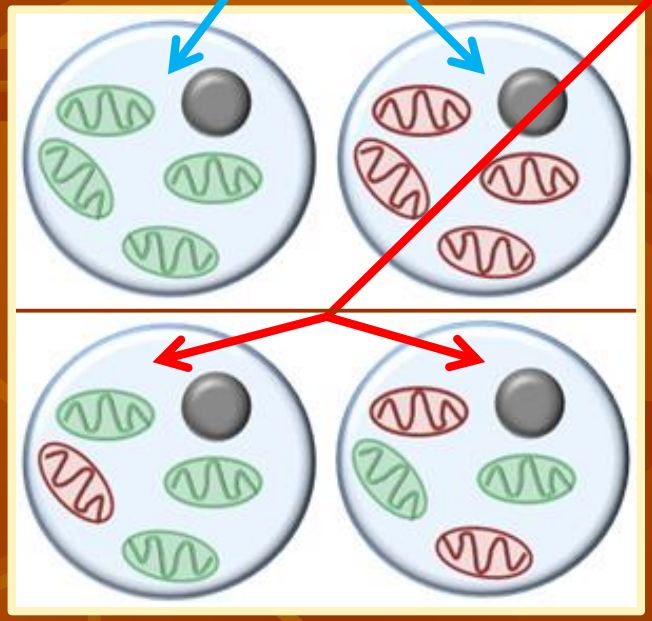






**Having compared the (placental) mtDNA of women with different ethnic background→**
**→we all are descendants of a great-grandmother who lived in East-Africa about 150-200 000 years ago („mitochondrial Eve”)**

**(theory challenged by many „stone and bone” anthropologists)**
**BUT! time scale also supported by similar genetic analysis of the Y-chromosome**

# Homoplasmy versus heteroplasmy



newcastle-mitochondria.com

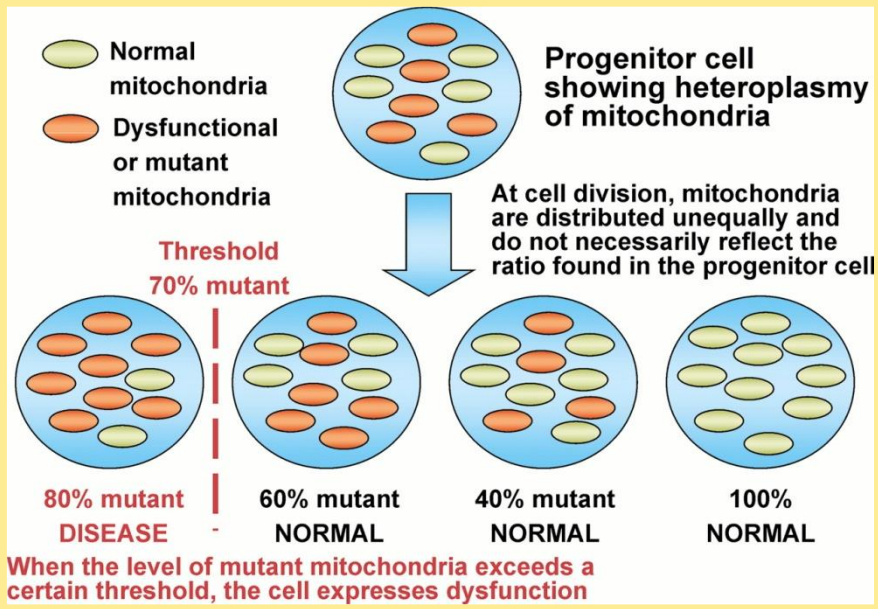
- mtDNA molecules are mutated and selected for replication randomly

- mutant mtDNA molecules segregate during the division of mitochondria

- mitochondria segregate during cell divisions

- possible pathological consequences within cells/tissues above a threshold level

# Heteroplasmy versus homoplasmy




sti.bmj.com

normal and mutated mitochondria segregate randomly among produced daughter cells at every division

„biological lottery”/varying severity of mitochondrial genetics already at the moment of conception

in women who carry the disease, one egg can be very different from another
in some eggs, 90% of the mitochondria might be defective; in others, only 10%.
Whether or not the child will have disease depends on which egg is fertilized.

# Mitochondrial diseases
# Acquired or inherited?

Accumulating mtDNA mutations

↓

reaching critical threshold

↓

first/milder symptoms

↓

additional (acquired) mutations come with time

↓

worsening condition

(Mitochondrial dysfunction is also seen as part of the aging process affecting oxidative phosphorylation.

# Mitochondrial diseases (I.)
# General picture

clinically heterogeneous group of disorders (> 100 described)

some affect a single organ (e.g., the eye in Leber hereditary optic neuropathy [LHON])

↕

many involve multiple organ systems (multisystemic)

disorders may present at any age (i ~ $1/10^4$)

many affected individuals display a cluster of clinical features =
= discrete clinical syndrome (with considerable clinical variability)

↕

many individuals do not fit neatly into one particular category

(symptoms may vary and are highly unpredictable)
tissues/organs with the highest E-demand will suffer first

# Mitochondrial diseases (II.) Affected tissues and typical symptoms

**Brain → seizures, movement disorders, stroke-like episodes, dementia**

**Sceletal muscle → weakness, fatigue, proximal myopathy**

**Heart muscle → cardiomyopathy, conductivity problems**

**Eyes → retinopathy, optic neuropathy, blindness**

**Liver → hepatopathy**

**Kidneys → glomerulopathy**

**Pancreas → diabetes mellitus**

**Inner ear → hearing impairment, deafness**

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**mid- and late pregnancy loss is a common occurrence that often goes unrecognized**

# Mitochondrial diseases (III.) Diagnosis and treatment

**characteristic clinical picture in some forms**
**(can be confirmed by molecular genetic testing of DNA from a blood sample)**

**more structured approach needed in many others**
**- family history (maternal inheritance!)**
**- blood and/or CSF (cerebrospinal fluid) tests**
**- neuroimaging, cardiac evaluation, and muscle biopsy for histologic or histochemical evidence**
**- molecular genetic testing for a mtDNA mutation.**

**prenatal genetic testing and interpretation of test results for mtDNA disorders are difficult ← ← mtDNA heteroplasmy**

**treatment is largely supportive (eg. treatment of diabetes mellitus, cardiac pacing, etc.)**

# Mitochondrial diseases (IV.)
# Genetic counseling

**defects of nuclear DNA or mtDNA**

**Mendelian inheritance**
**autosomal recessive or dominant**

**generally occur *de novo!***
**can be transmitted by maternal inheritance**

**(The father of a proband is not at risk of having the mutation and does not transmit it to his offspring. The mother of a proband (usually) has the mutation, w/wo symptoms.)**

# Mitochondrial diseases (V.)
# Maternal inheritance

**LHON: bilateral, painless, subacute visual failure, can lead to blindness in young adults**
**male:female = 4-5:1**

# Mitochondrial diseases (VI.) Mendelian inheritance

~ 99% of mitochondrial proteins coded in nuclear genome

-mutations → ATP synthesis ↓ → symptoms similar to those seen in maternally inherited conditions

- affected respiratory chain, mtDNA-replication machinery, posttranslational protein import, etc.

# Mitochondrial diseases (VII.) and somatic mutations

↓

somatic heteroplasmy above threshold

↓

oxidative phosphorylation↓

↓

degenerative conditions

(eg. certain types of diabetes mellitus, Parkinson's disease, Alzheimer's disease)

- antioxidant prophylaxis with vitamine E, C, carotinoids

↕

- azidothymidine (AZT) in AIDS therapy damages mtDNA

# Mitochondrial diseases (VIII.) and molecular medicine

IVF / preimplantation genetic diagnosis to select embryos that are free of the disease
BUT!
some women / extrem high levels of mtDNA mutation
all their eggs → babies with mitochondrial disease:

↓

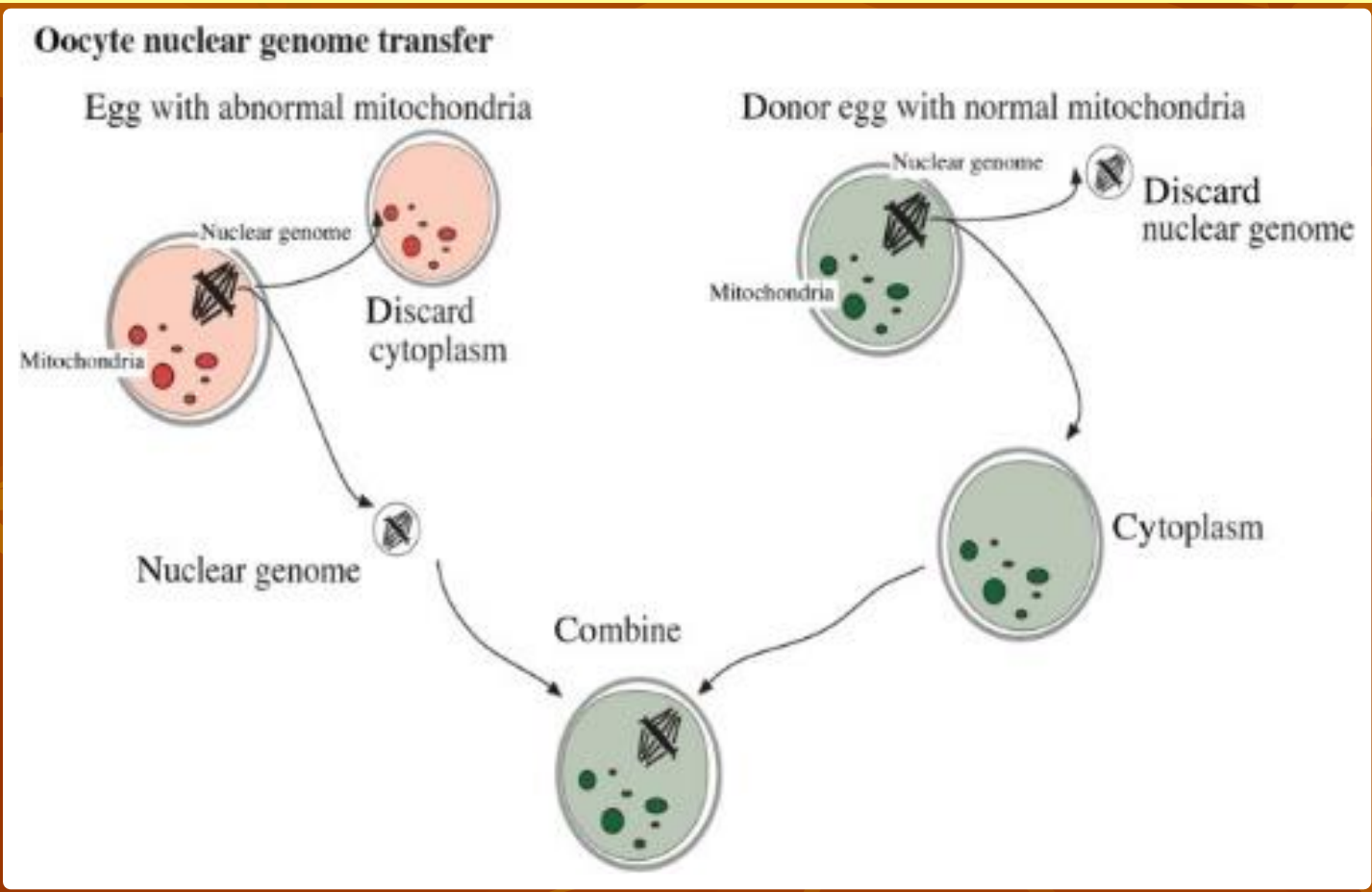
replacing the mother's faulty mitochondria wholesale with those from a healthy donor → "three-parent babies„:

a.) <u>maternal spindle transfer (MST)</u>:

standard IVF collection of eggs from the mother →
→ enucleation and transfer of plucked nucleus into enucleated healthy donor egg →
→ new egg has all the mother's chromosomes + the donor's healthy mitochondria
(apart from few faulty mitochondria carried over with the mother's nucleus)

∑ egg is fertilized with the father's sperm & embryo implanted / standard IVF
(precedent of introducing genetic changes that pass down to future generations!!)

# Mitochondrial diseases (IX.) and molecular medicine



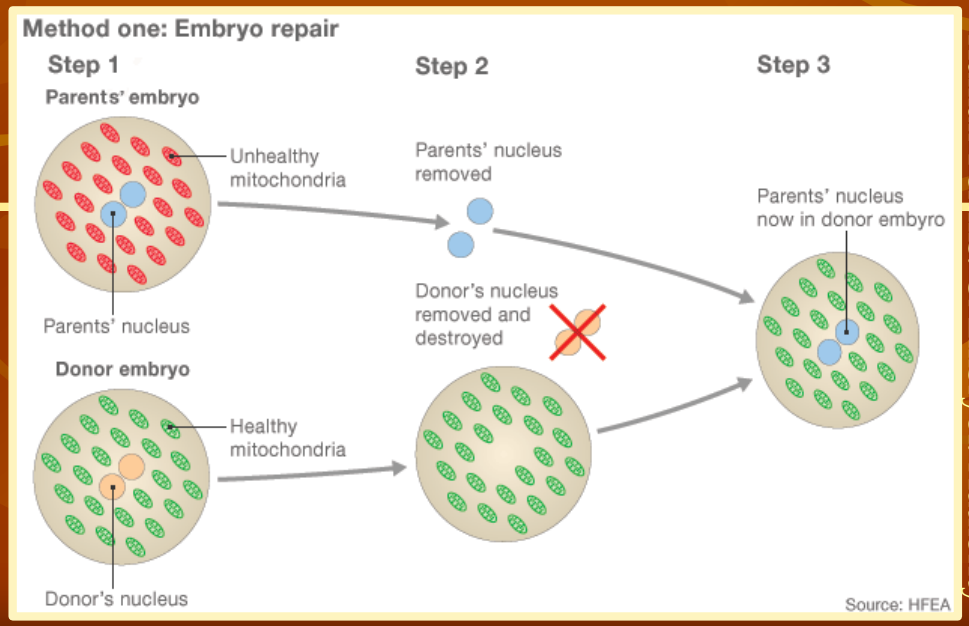

Engelstad et al., Hum Reprod. 2016 May;31(5):1058-65. doi: 10.1093/humrep/dew033. Epub 2016 Mar 2.

**Oocyte mitochondrial replacement therapy technique**

# Mitochondrial diseases (X.) and molecular medicine

**b.) <u>pronuclear transfer (PNT)</u>:**

**both eggs are fertilised with father's sperm first**
**before the eggs divide into early-stage embryos, the parents' chromosomes are removed from the mother's fertilised egg and dropped into the donor's**




Human Fertilis. & Embryol. Authority

# Mitochondrial diseases (XI.) and molecular medicine

c.) Polar body nuclear transfer (PBNT) – with even better results?):

Affected egg's polar body is transferred into healthy, enucleated donor oocyte → → smaller chance of contaminantion with sick mitochondria and easier handling of membrane bound „package" of genetic material

- still in experimental phase BUT! promising!

# Mitochondrial diseases (XII.) and molecular medicine

**Oregon National Primate Centre (2009) S. Mitalipov:**
**Birth of 4 healthy monkeys / MST.**
**Mother had no mitochondrial disease**
**BUT!**
**experiment worked + technique appeared to be safe.**

**Procedure repeated in 2012 with human embryos / letting them develop into blastocysts, then terminated**

**Following legalization of the PNT technique in the UK, in spring (2016) the first 3-parent baby was born and appears to be healthy.**
**https://www.newscientist.com/article/2107219-exclusive-worlds-first-baby-born-with-new-3-parent-technique/**

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UNIVERSITY OF PÉCS

# Thanks for the attention!