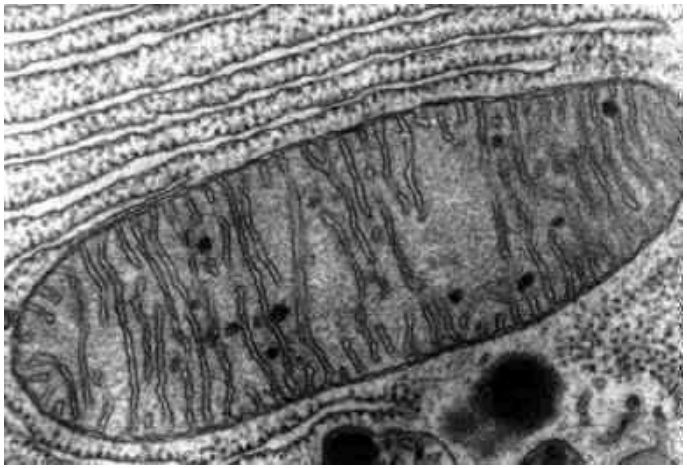
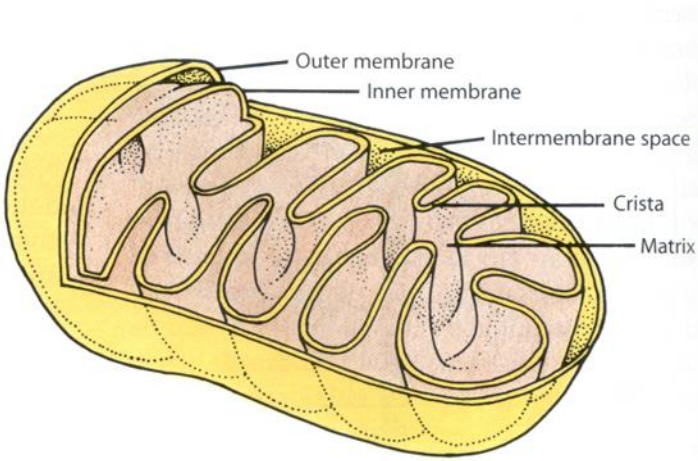
Mitochondrial Genome and Cytoplasmic inheritance

Introduction

- membrane-bound organelle (eukaryotic only!).

- Each cell contains hundreds to thousands of mitochondria.
- Site of Krebs cycle and oxidative phosphorylation (the electron transport chain, or respiratory chain).
- two membranes: outer and inner.
- Folds of the inner membrane, where most of oxidative phosphorylation occurs, are called cristae.
- Inside inner membrane = matrix
- Between membranes = intermembrane space
- Mitochondrial DNA is inside the inner membrane.

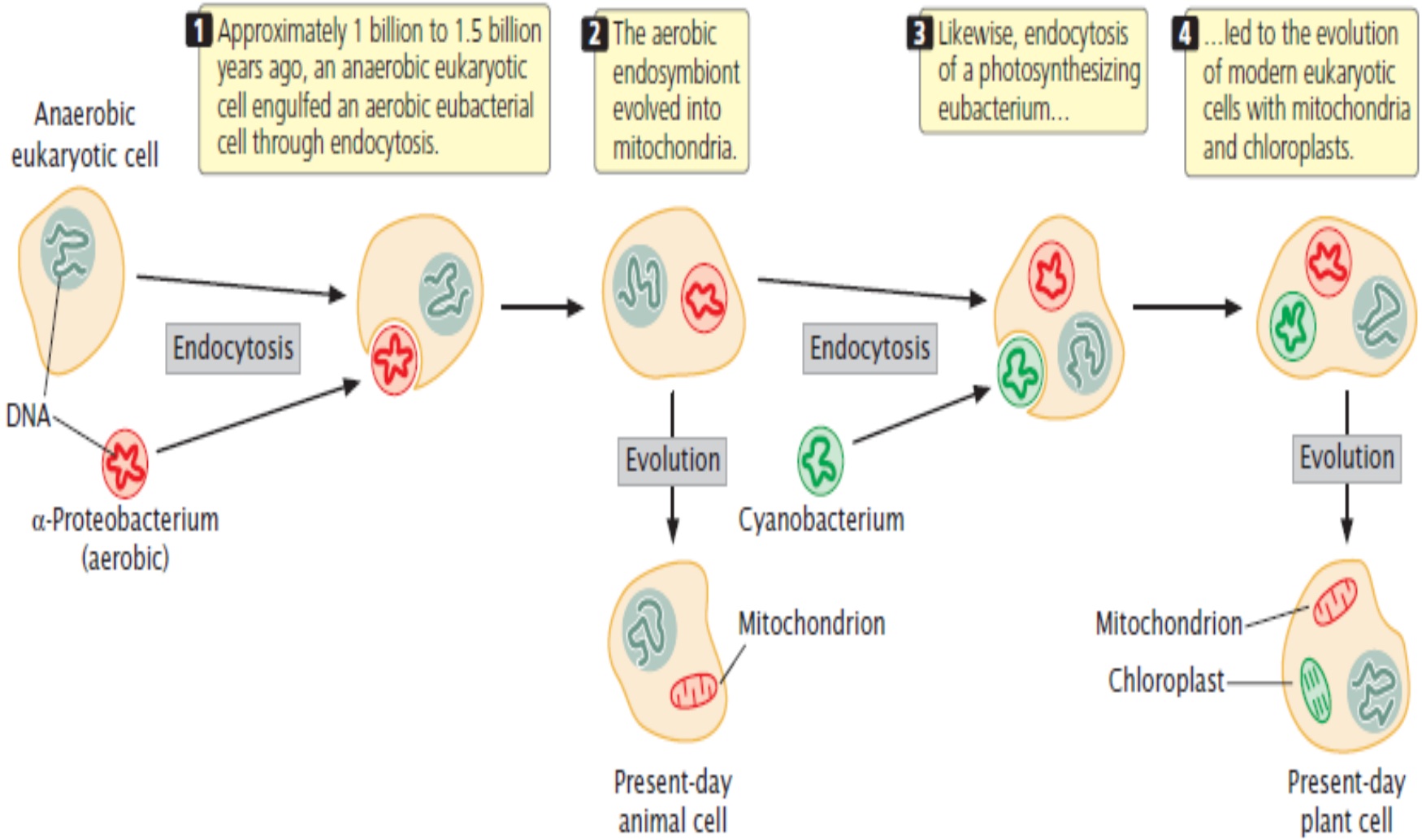




Endosymbiont Hypothesis

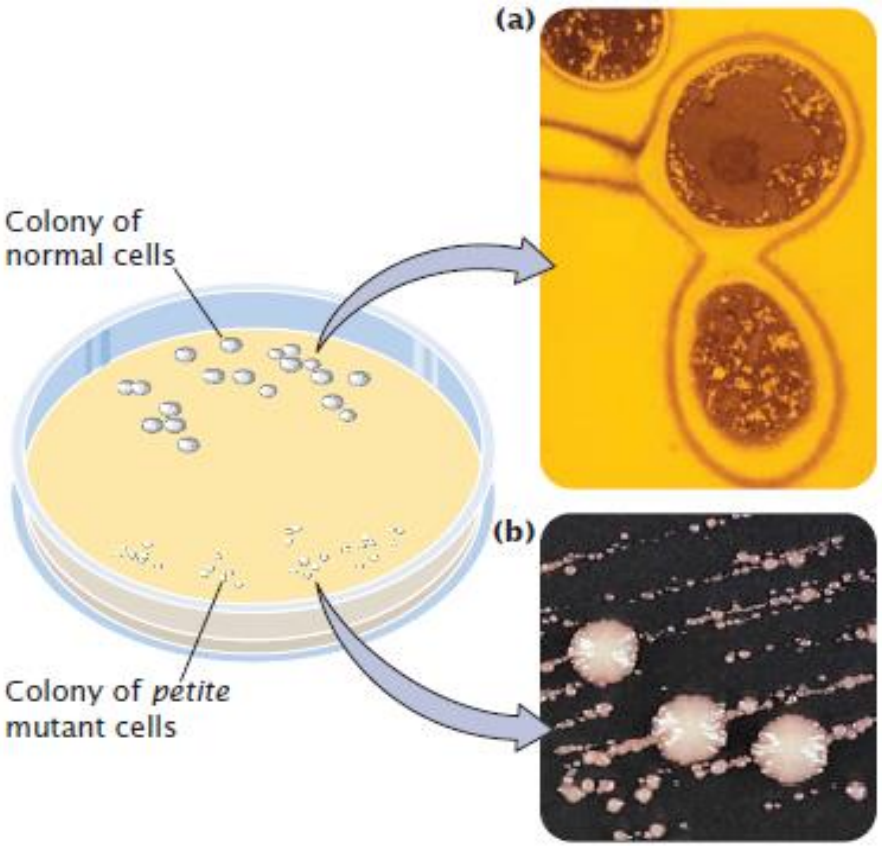
- **endosymbiont hypothesis:** originally proposed in 1883 by Andreas Schimper, but extended by Lynn Margulis in the 1980s.
- Mitochondrial ribosomal RNA genes and other genes show that the original organism was in the alpha-proteobacterial family (similar to nitrogen-fixing bacteria)

- **Evidence:**
  - mitochondria have their own DNA (circular)
  - the inner membrane is more similar to prokaryotic membranes than to eukaryotic. By the hypothesis, the inner membrane was the original prokaryotic membrane and the outer membrane was from the primitive eukaryote that swallowed it.
  - mitochondria make their own ribosomes, which are of the prokaryotic 70S type, not the eukaryotic 80S type.
  - mitochondria are sensitive to many bacterial inhibitors that don't affect the rest of the eukaryotic cell, such as streptomycin, chloramphenicol, rifampicin.
  - mitochondrial protein synthesis starts with N-formyl methionine, as in the bacteria but unlike eukaryotes.



21.6 The endosymbiotic theory proposes that mitochondria and chloroplasts in eukaryotic cells arose from eubacteria.

Traits encoded by mtDNA



21.5 The *petite* mutants have large deletions in their mtDNA and are unable to carry out oxidative phosphorylation. (a) A normal yeast cell and (b) a *petite* mutant. [Part a: David M. Phillips/ Visuals Unlimited. Part b: Courtesy of Dr. Des Clark-Walker, Research School of Biological Sciences, the Australian National University.]

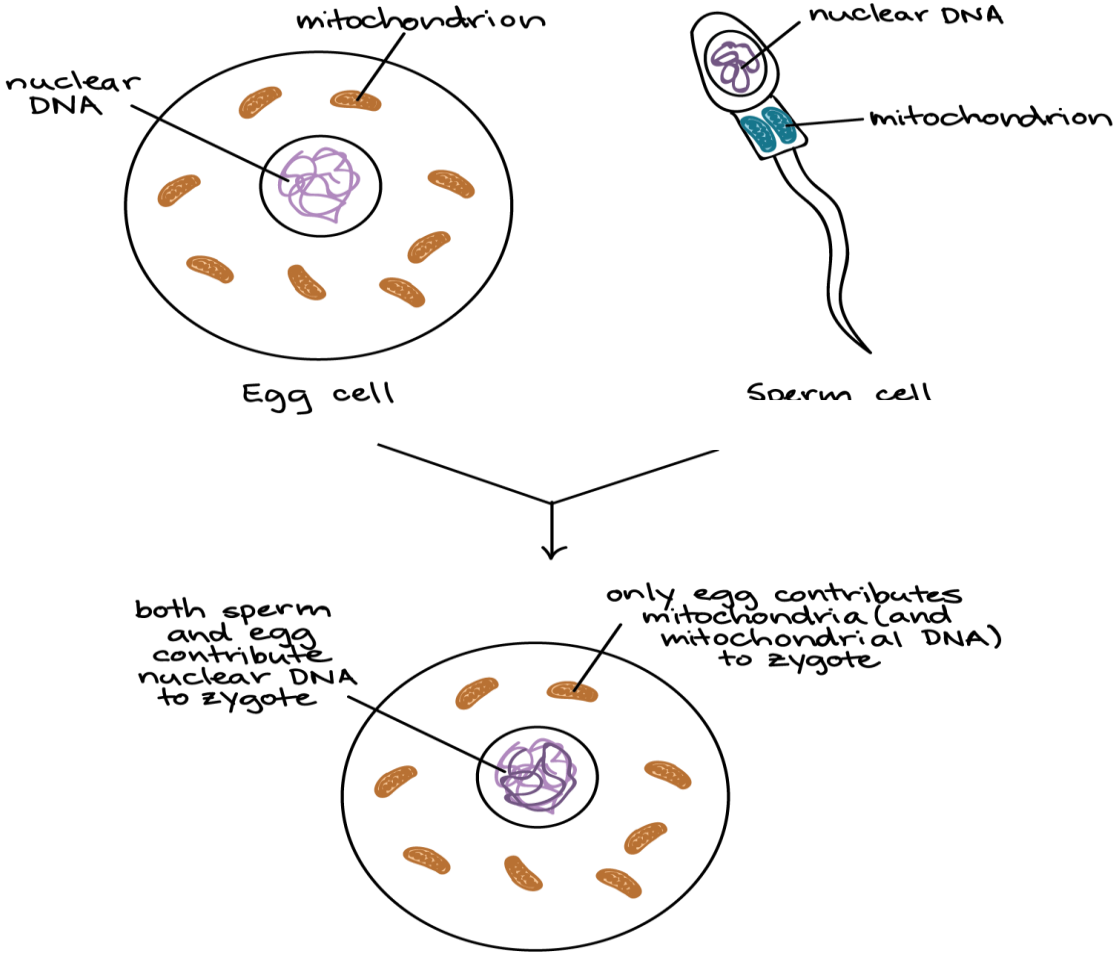
Some petite mutations are defects in nuclear DNA,
but most petite mutations occur in mitochondrial DNA.
Mitochondrial petite mutants often have large deletions
in mtDNA or, in some cases, are missing mtDNA entirely.
Much of the mtDNA encodes enzymes that catalyze aerobic respiration, and therefore the petite mutants are unable to carry out aerobic respiration and cannot produce normal quantities of ATP, which inhibits their growth

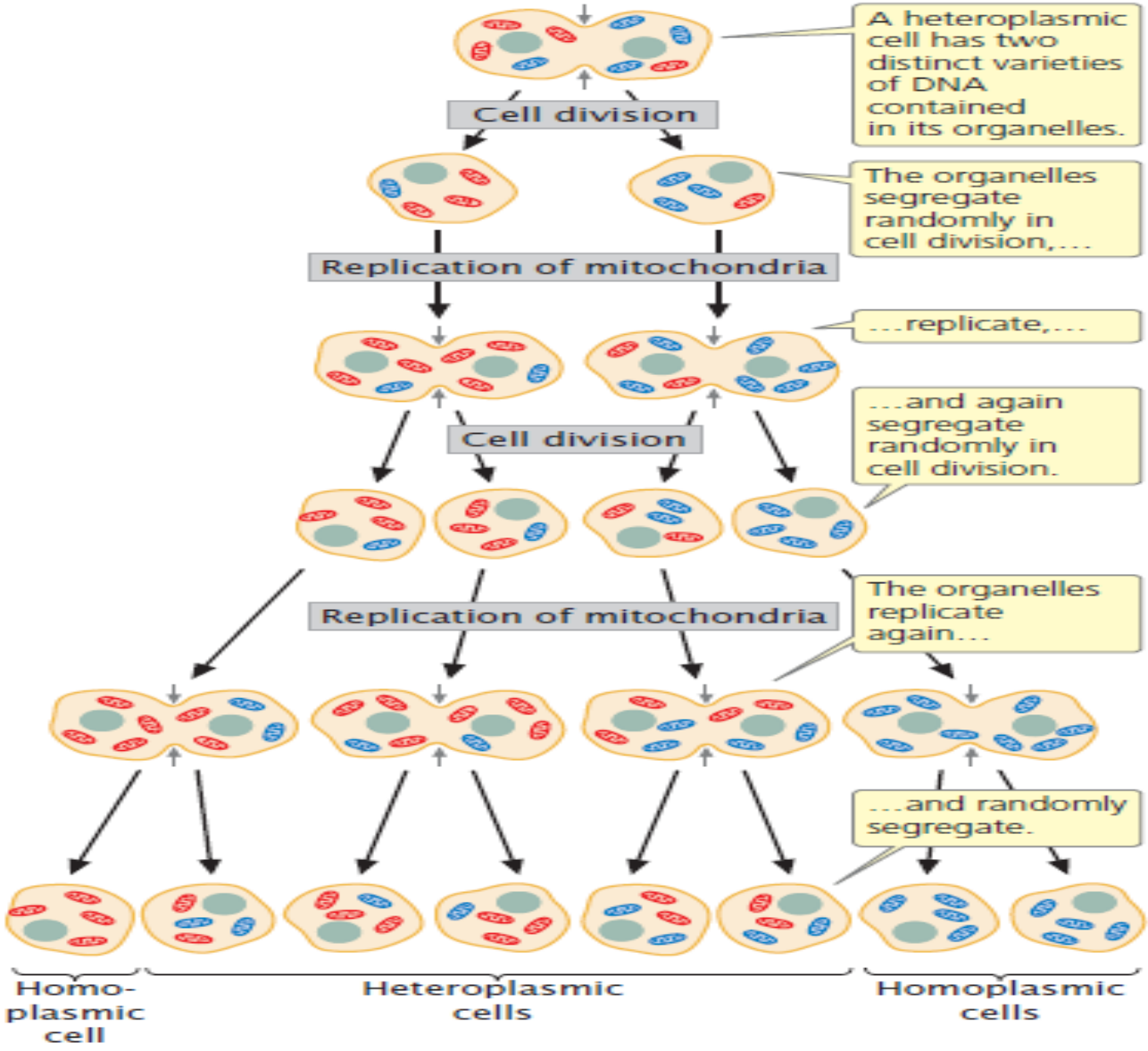
Maternal (Organelle) Inheritance

DNA contained in mitochondria or chloroplasts determines the phenotype of the offspring.

These phenotypes arise due to the source of organelles—only from the egg—such that there is only a maternal influence on phenotype.

Cytoplasmic inheritance





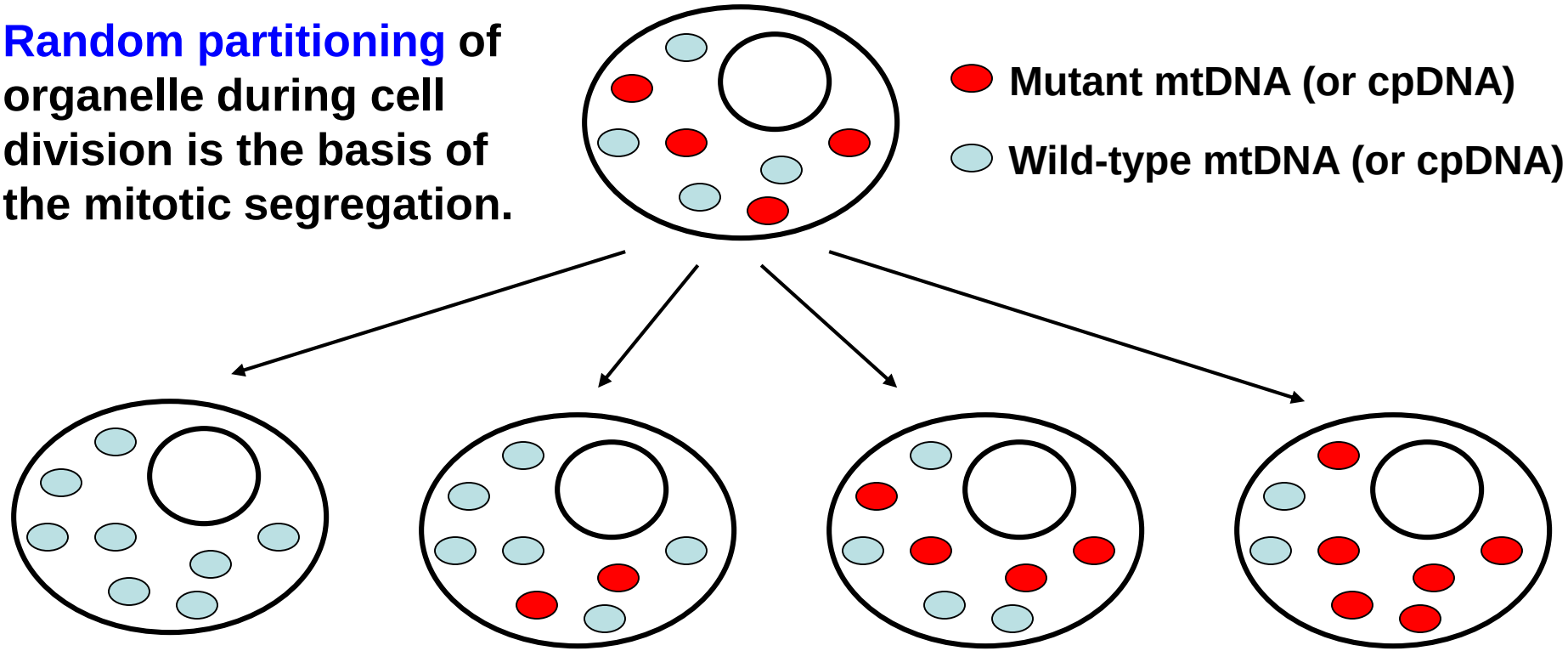
21.4 Organelles in a heteroplasmic cell divide randomly into the progeny cells. This diagram illustrates replicative segregation in mitosis; the same process also takes place in meiosis.

Replicative segregation

Heteroplasmic cells:
Cells contain a mixture of organelle DNA.

Homoplasmic cells:
Cells carry only one type of organelle DNA.

Random partitioning of organelle during cell division is the basis of the mitotic segregation.



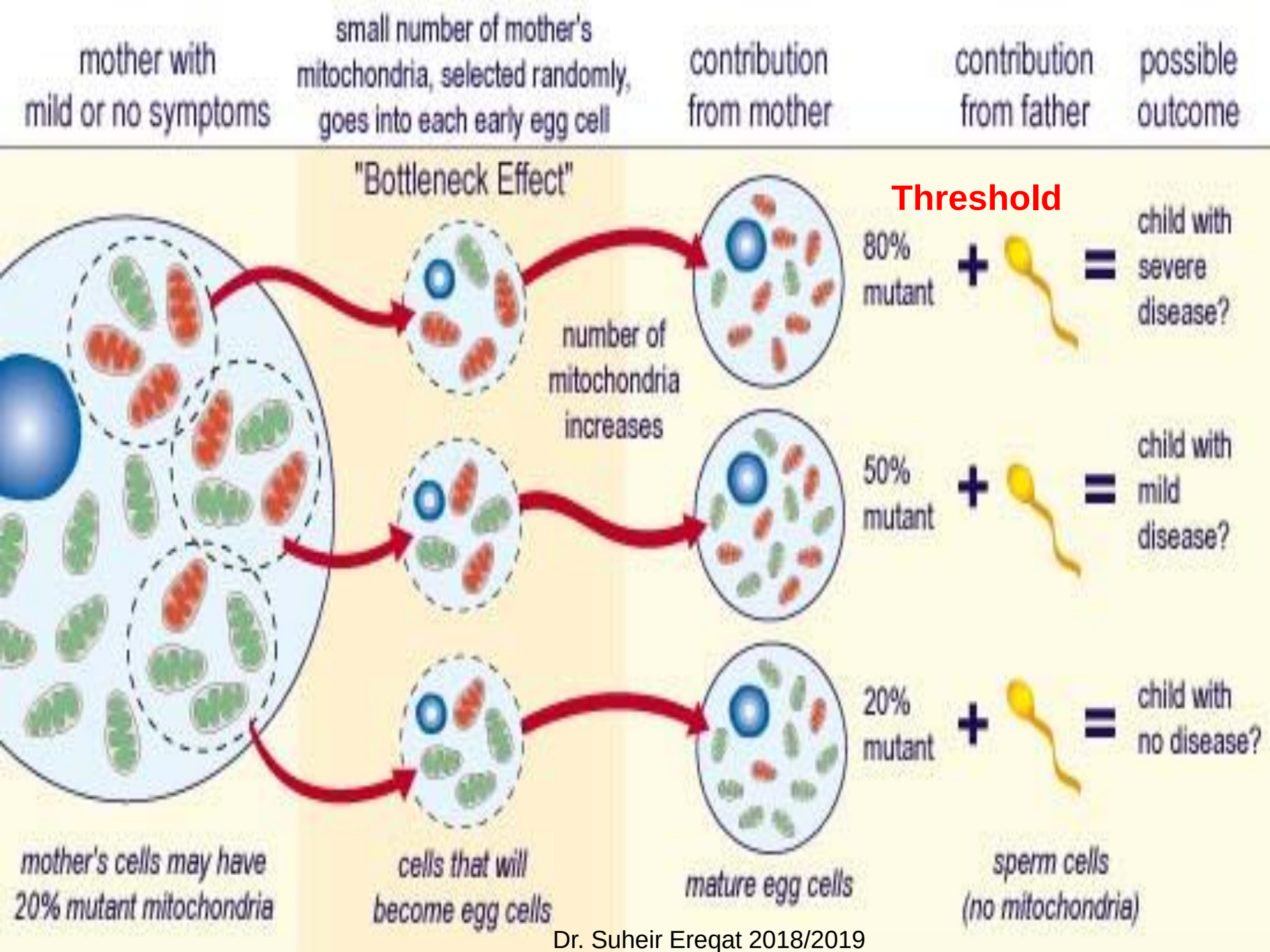
the severity of the disease is frequently related to the proportion of mutant mtDNA sequences inherited at birth.

Mitochondrial Syndromes

The severity of the condition is dependent on the number of disabled mitochondria present in the egg.

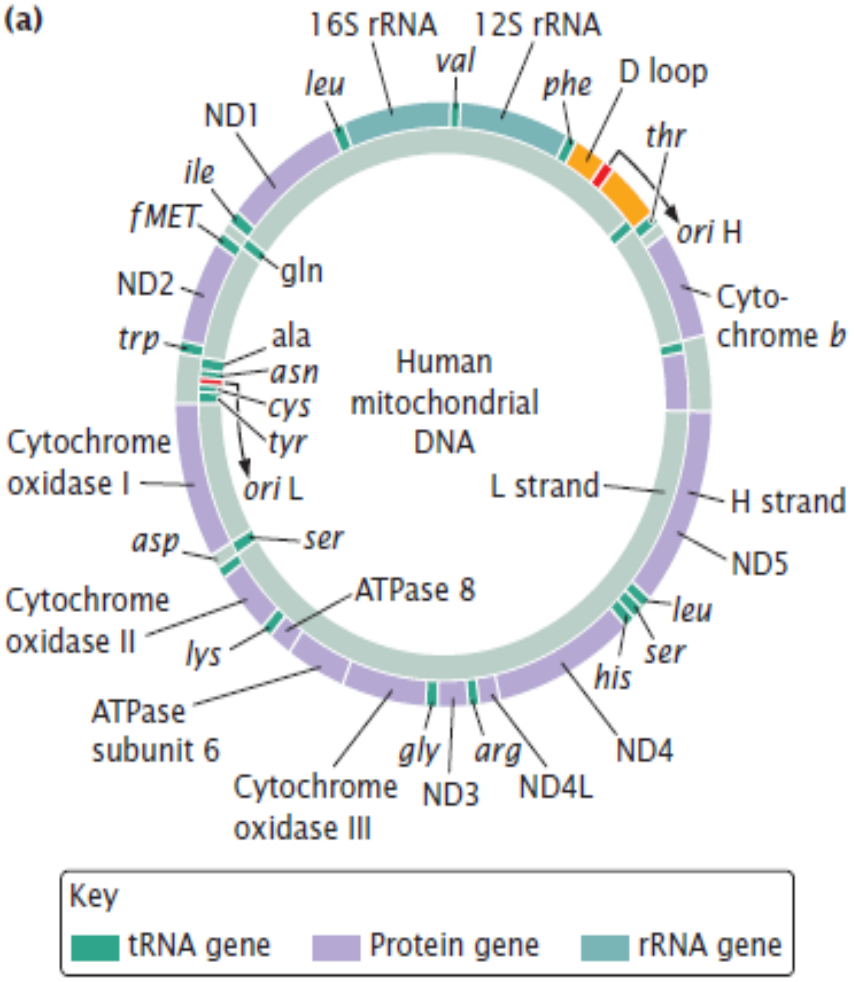
An egg with a large number of disabled mitochondria would result in a child with severe abnormalities

An egg with only a few disabled mitochondria would result in an individual only mildly affected.


mother with mild or no symptoms
small number of mother's mitochondria, selected randomly, goes into each early egg cell
contribution from mother
contribution from father
possible outcome
"Bottleneck Effect"
Threshold
number of mitochondria increases
80% mutant
child with severe disease?
50% mutant
child with mild disease?
20% mutant
child with no disease?
mother's cells may have 20% mutant mitochondria
cells that will become egg cells
mature egg cells
sperm cells (no mitochondria)

Genome Structure

- The mitochondrial genome is a circle, 16.6 kb of DNA 37 genes (2 rRNA, 22 tRNA, 13 polypeptides).
- The two strands are notably different in base composition, leading to one strand being “heavy” (the H strand more G’s) and the other light (the L strand: more c’s).
- The H strand is the template for both rRNAs, 14 of the 22 tRNAs, and 12 of the 13 proteins, whereas the L strand serves as template for only 8 of the tRNAs and 1 protein.
- The D loop (D = “displacement” is 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).



Genetic Code

- The mitochondrial genetic code has drifted from the universal code: there are so few polypeptides that changes in the code are tolerated.
- Human mitochondrial code is different from other groups such as plants or fungi.
- Uses 2 of the 3 universal stop codons, but also uses 2 other codons as stop codons. Also, UGA codes for tryptophan in the mitochondrial, while it is a stop codon in the universal code. AUA gives methionine in the mitochondria instead of isoleucine.

Second letter

First letter	U	C	A	G	Third letter
U	UUU, UUC Phenylalanine UUA, UUG Leucine	UCU, UCC, UCA, UCG Serine	UAU, UAC Tyrosine UAA Stop codon UAG Stop codon	UGU, UGC Cysteine UGA Stop codon UGG Tryptophan	U C A G
C	CUU, CUC, CUA, CUG Leucine	CCU, CCC, CCA, CCG Proline	CAU, CAC Histidine CAA, CAG Glutamine	CGU, CGC, CGA, CGG Arginine	U C A G
A	AUU, AUC, AUA Isoleucine AUG Methionine; start codon	ACU, ACC, ACA, ACG Threonine	AAU, AAC Asparagine AAA, AAG Lysine	AGU, AGC Serine AGA, AGG Arginine	U C A G
G	GUU, GUC, GUA, GUG Valine	GCU, GCC, GCA, GCG Alanine	GAU, GAC Aspartate GAA, GAG Glutamate	GGU, GGC, GGA, GGG Glycine	U C A G

First letter

Third letter

First letter	Second letter U	Second letter C	Second letter A	Second letter G	Third letter
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr *Stop* *Stop*	Cys Cys *(Stop)* Trp Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile (Met) Ile (Ile) Met Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser (Arg) *Stop* (Arg) *Stop*	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Table 21.2 Nonuniversal codons found in mitochondrial DNA

Codon	Universal Code	mtDNA		
		Vertebrate	*Drosophila*	Yeast
UGA	Stop	Tryptophan	Tryptophan	Tryptophan
AUA	Isoleucine	Methionine	Methionine	Methionine
AGA	Arginine	Stop	Serine	Arginine

Source: After T. D. Fox, *Annual Review of Genetics* 21:69, 1987.

Replication and Transcription

- 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.

- 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.

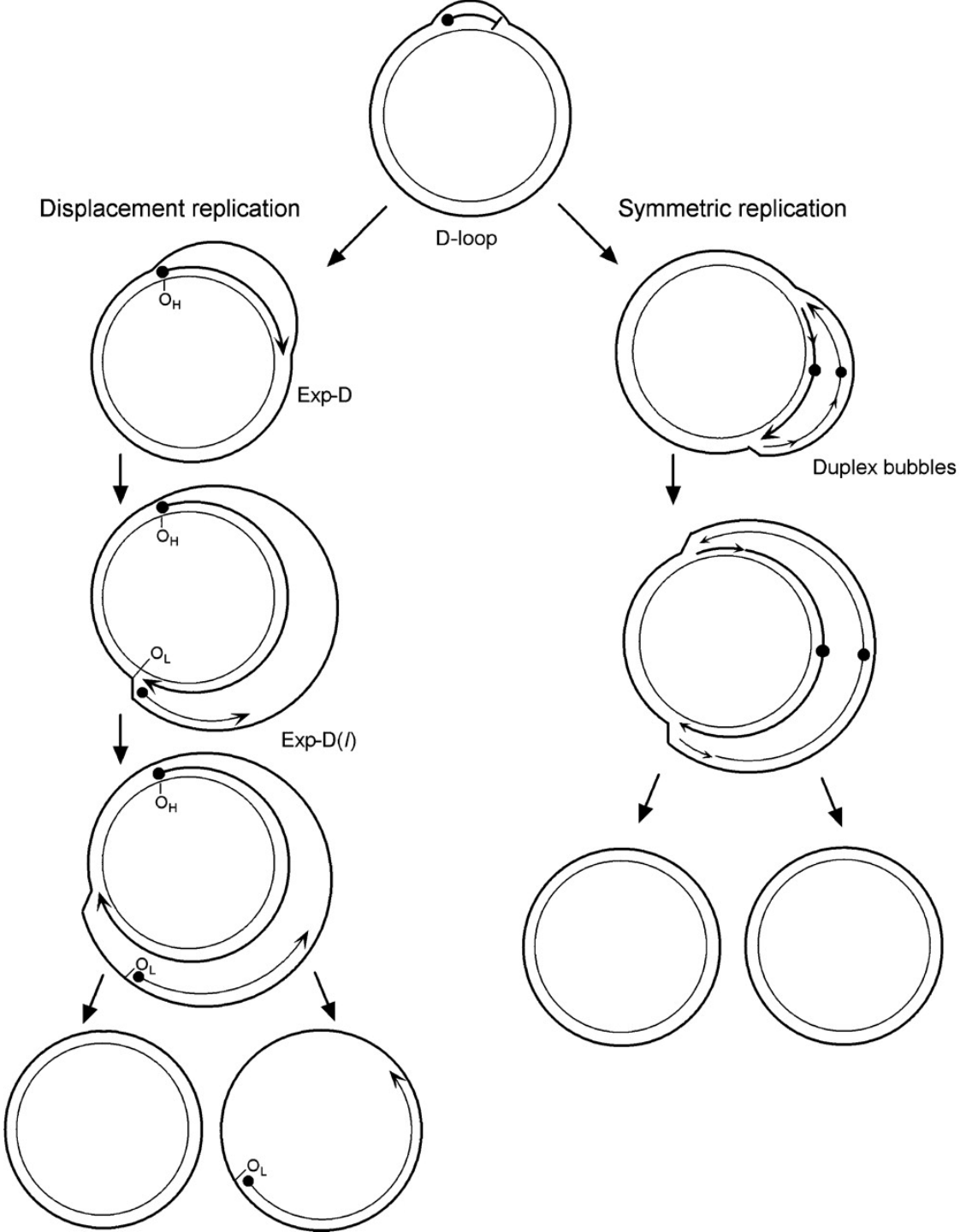

D-loop
Displacement replication
Symmetric replication
O_H
Exp-D
O_L
Exp-D(*l*)
Duplex bubbles

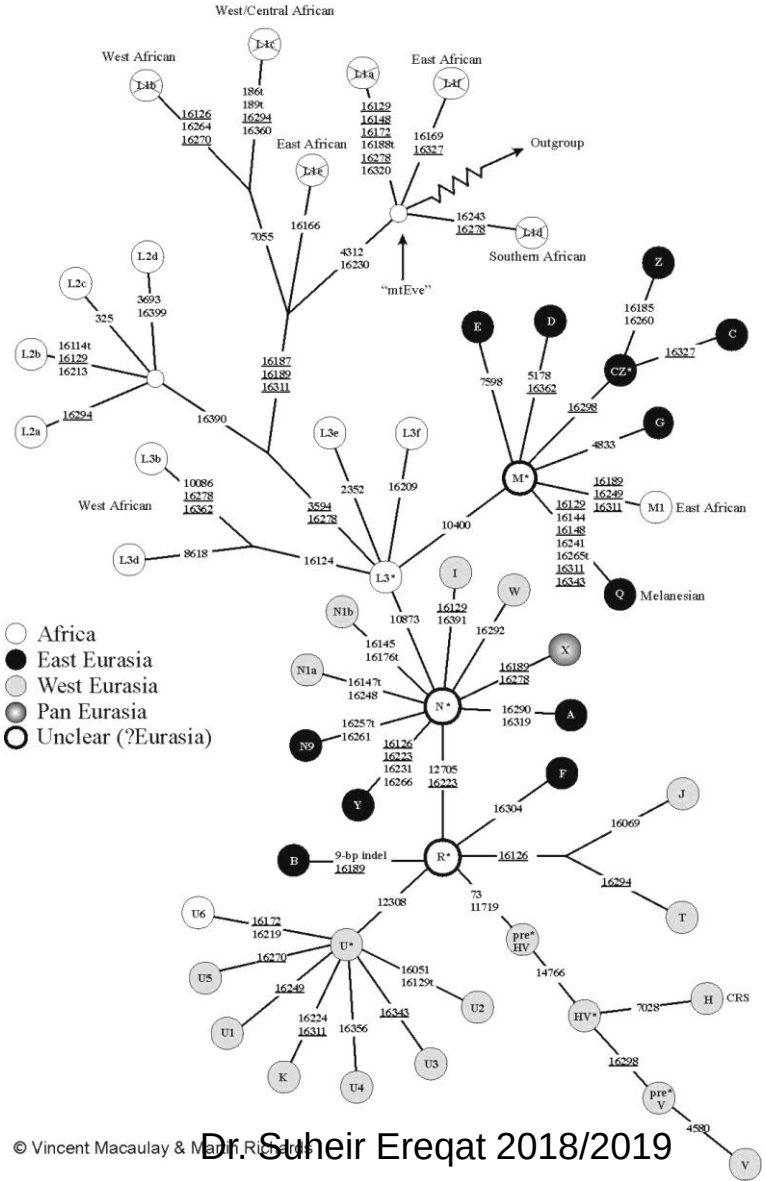
Table 21.4 Comparison of nuclear eukaryotic, eubacterial, mitochondrial, and chloroplast genomes

Characteristic	Eukaryotic Genome	Eubacterial Genome	Mitochondrial Genome	Chloroplast Genome
Genome consists of double-stranded DNA	Yes	Yes	Yes	Yes
Circular	No	Yes	Most	Yes
Histone proteins	Yes	No	No	No
Size	Large	Small	Small	Small
Number of molecules per genome	Several	One	One in animals; several in some plants	One
Pre-mRNA introns	Common	Absent	Absent	Absent
Group I introns	Present	Present	Present	Present
Group II introns	Absent	Present	Present	Present
Polycistronic mRNA	Uncommon	Common	Present	Common
5′ cap added to mRNA	Yes	No	No	No
3′ poly(A) tail added to mRNA	Yes	No	Some in animals	No
Shine–Dalgarno sequence in 5′ untranslated region of mRNA	No	Yes	Rare	Some
Nonuniversal codons	Rare	Rare	Yes	No
Extended wobble	No	No	Yes	No
Translation inhibited by tetracycline	No	Yes	Yes	Yes

Mitochondrial Genetics

- <u>Maternal inheritance</u>: Inherited through the mother (egg) only. Allows tracing female line back in time.
- A few sperm mitochondria enter the egg, but they are degraded and lost.

- <u>Mutation rate</u> in mtDNA is very high: 10 times the nuclear rate. mtDNA is associated with the inner membrane, the site of oxidative phosphorylation. Large amounts of “reactive oxygen species” (peroxide and superoxide) are present, and they are quite mutagenic. The D loop has an especially high rate of mutation. Part of the effects of aging have been attributed to the gradual loss of mitochondria due to accumulated mutations in individual cells.



Genetic Diseases

in general: malfunctions of respiratory chain, so affects high metabolism tissues the most: nervous system, muscles, kidney, liver.

Genetic Diseases

from mutations in mtDNA have been identified in humans

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1-Leber hereditary optic neuropathy **(LHON**), which typically leads to sudden loss of vision in middle age, results from mutations in the mtDNA genes that encode electron-transport proteins.

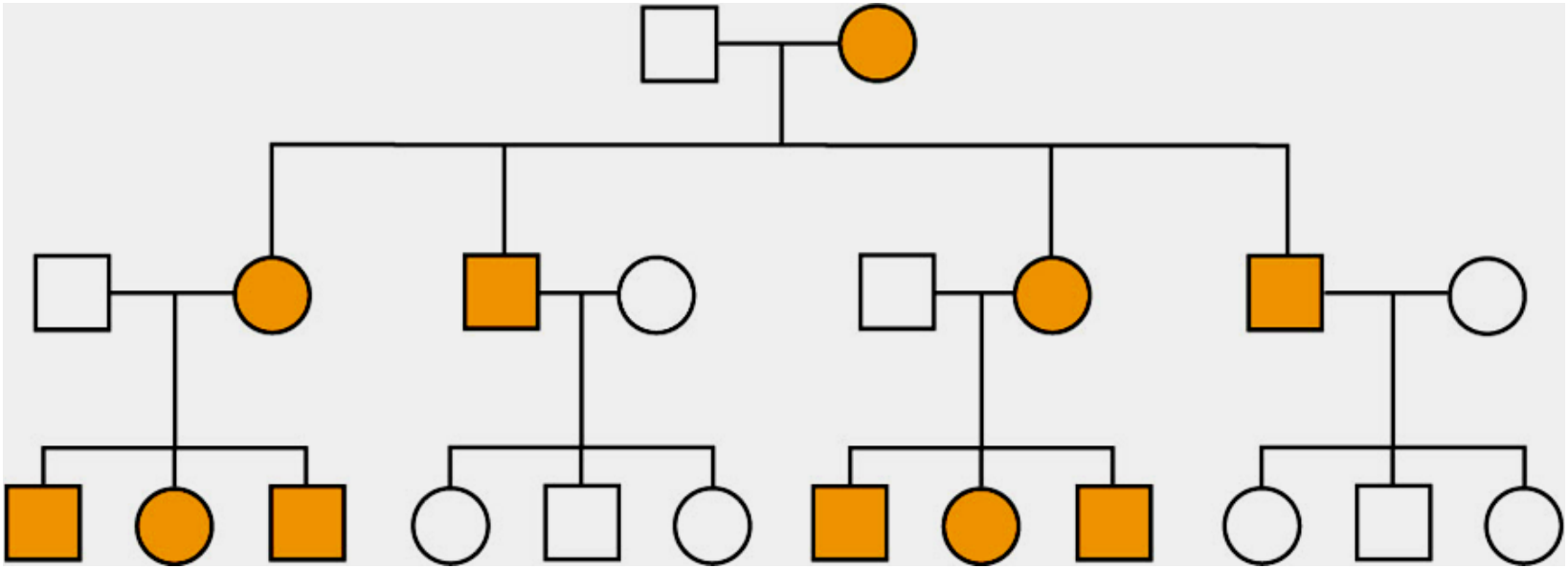
2- neurogenic muscle weakness, ataxia, and retinitis pigmentosa (**NARP),** which is characterized by seizures, dementia, and developmental delay.

3-Kearns–Sayre syndrome **(KSS**) and chronic external opthalmoplegia (**CEOP**), both of which result in paralysis of the eye muscles, droopy eyelids, and, in severe cases, vision loss, deafness, and dementia.

4- Myoclonic epilepsy and ragged red fiber disease (**MERRF**). CNS symptoms: epilepsy, deafness, dementia. Skeletal and heart muscles abnormal, mitochondria appear abnormal. Multiple enzyme defects in respiratory chain

All of these diseases exhibit cytoplasmic inheritance and variable expression

Hypothetical example of LHON pedigree



LHON (Leber's hereditary optic neuropathy)
- A disease in which defects in the mitochondria's electron transport chain lead to optic nerve degeneration and blindness.

- Mutation in the NADH dehydrogenase subunit 4 gene.

Dr. Suheir Ereqat 2018/2019

Genetics, from Genes to Genomes, Hartwell et al., 2nd edition.

Inheritance of Mitochondrial Mutations

In general, only egg cells contribute mitochondria to offspring. Mutant mtDNA are not typically inherited from a male.

Thus, mitochondrial mutations exhibit **maternal inheritance**, or **mitochondrial inheritance.**

If a mother is homoplasmic for an mtDNA mutation, then all of the mitochondria she passes to her children will also be homoplasmic for the mutation.

In the pedigree illustrated, note that all of an affected female's children are affected, but none of the affected male's children inherited the condition.

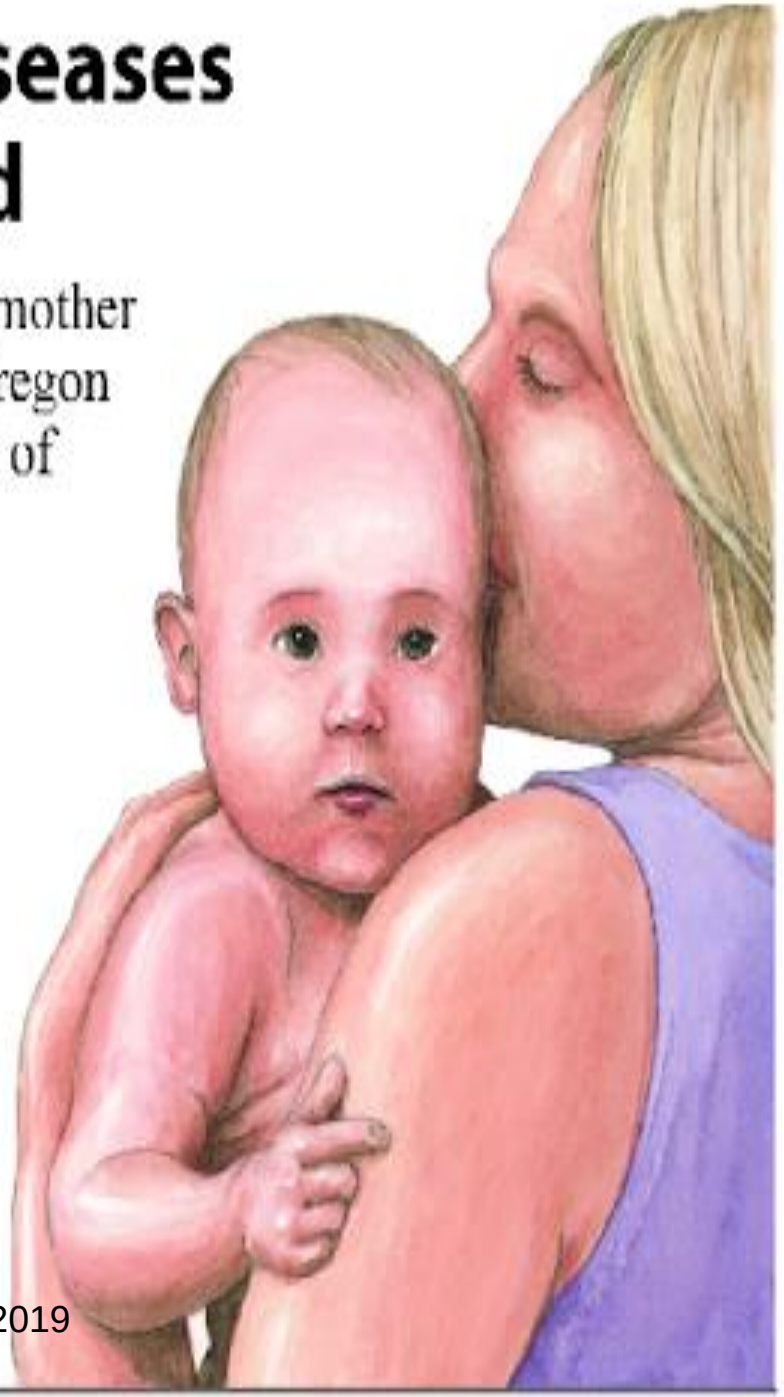
If a mother is heteroplasmic for an mtDNA mutation, then the chance she will pass on the mutation is reduced due to the random assortment of both mtDNA and mitochondria during replication and division. Therefore, the higher the proportion of mutant mtDNA, the higher the chance of passing the mutation, and, therefore, the condition, on to one's offspring.

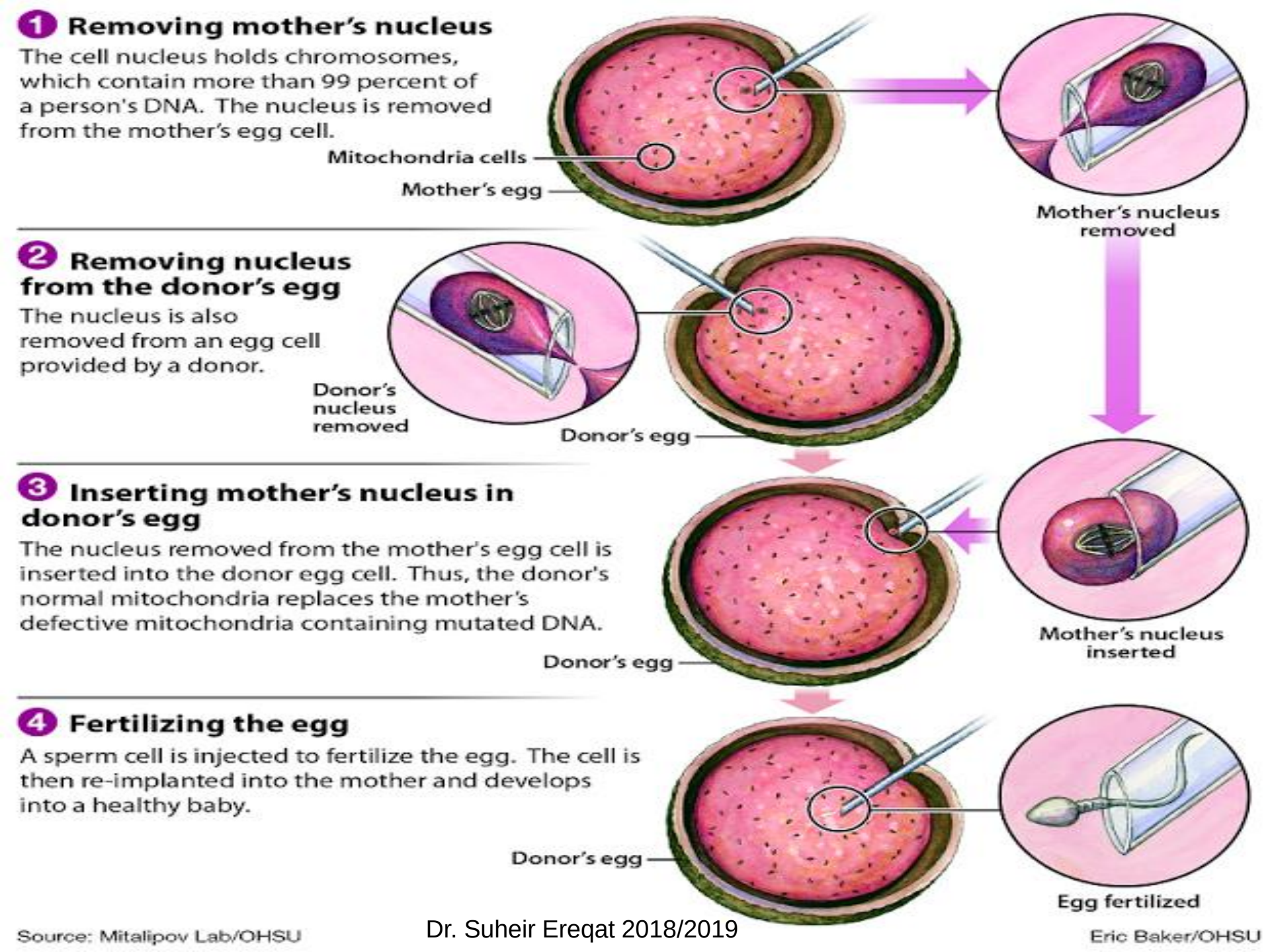
Gene therapy to prevent diseases passed from mother to child

More than 300 genetic diseases can be passed from mother to child because of mutated genes. Researchers at Oregon Health & Science University have developed a form of gene therapy to prevent these diseases.

The mitochondria

Mitochondria are the powerstations of a cell, providing it with the energy to function. A mother's egg cell contains thousands of mitochondria, each containing its own DNA. If defective, the DNA in these cells can pass diseases from mother to child. Here's how researchers hope to use gene therapy to prevent these diseases:




1 Removing mother's nucleus
The cell nucleus holds chromosomes, which contain more than 99 percent of a person's DNA. The nucleus is removed from the mother's egg cell.
Mitochondria cells
Mother's egg
Mother's nucleus removed
2 Removing nucleus from the donor's egg
The nucleus is also removed from an egg cell provided by a donor.
Donor's nucleus removed
Donor's egg
3 Inserting mother's nucleus in donor's egg
The nucleus removed from the mother's egg cell is inserted into the donor egg cell. Thus, the donor's normal mitochondria replaces the mother's defective mitochondria containing mutated DNA.
Donor's egg
Mother's nucleus inserted
4 Fertilizing the egg
A sperm cell is injected to fertilize the egg. The cell is then re-implanted into the mother and develops into a healthy baby.
Donor's egg
Egg fertilized
Source: Mitalipov Lab/OHSU
Eric Baker/OHSU

Why does oxidative phosphorylation capacity decline with age?