Chapter 14

Organellar Genetics

Sections to study

14.1 Mitochondria and their genomes

14.2 Chloroplasts and their genomes

14.3 The relationship between organellar and nuclear genomes

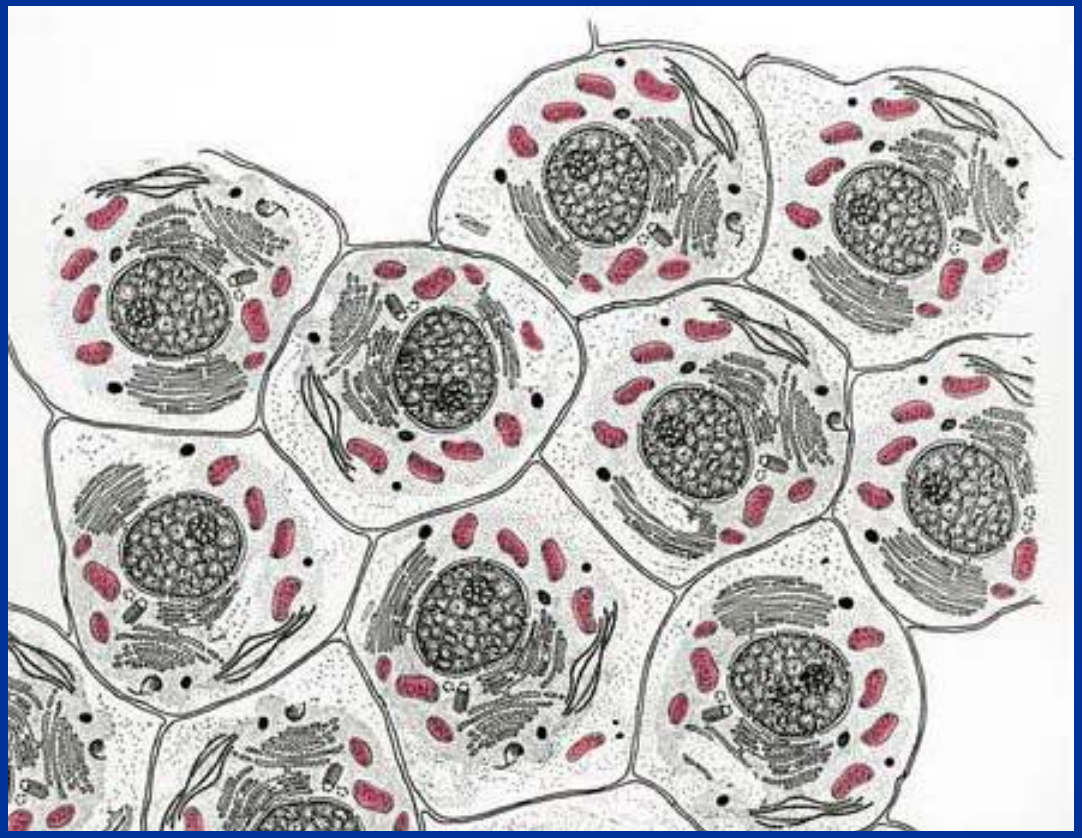
14.4 Non-Mendelian inheritance of mitochondria and chloroplasts

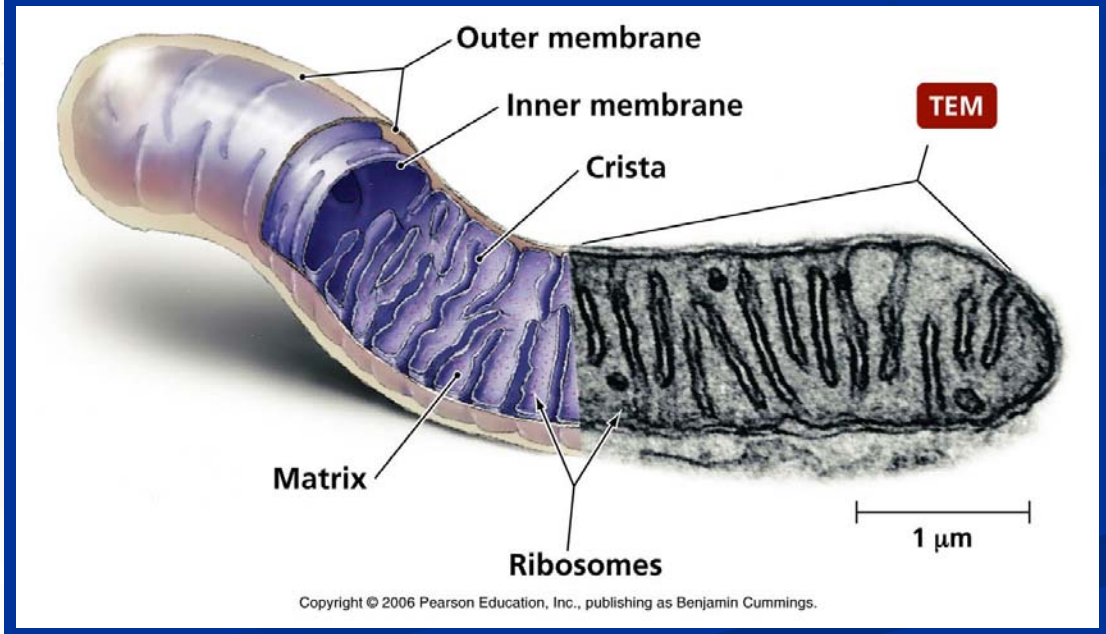
14.5 Mutant mitochondria and human disease

14.1 Mitochondria and their genomes

Mitochondria are sites of energy production.

- Present in eukaryotes.
- Number of mitochondria in each cell varies.





- **Produce ATP through the oxidative phosphorylation of ADP.**

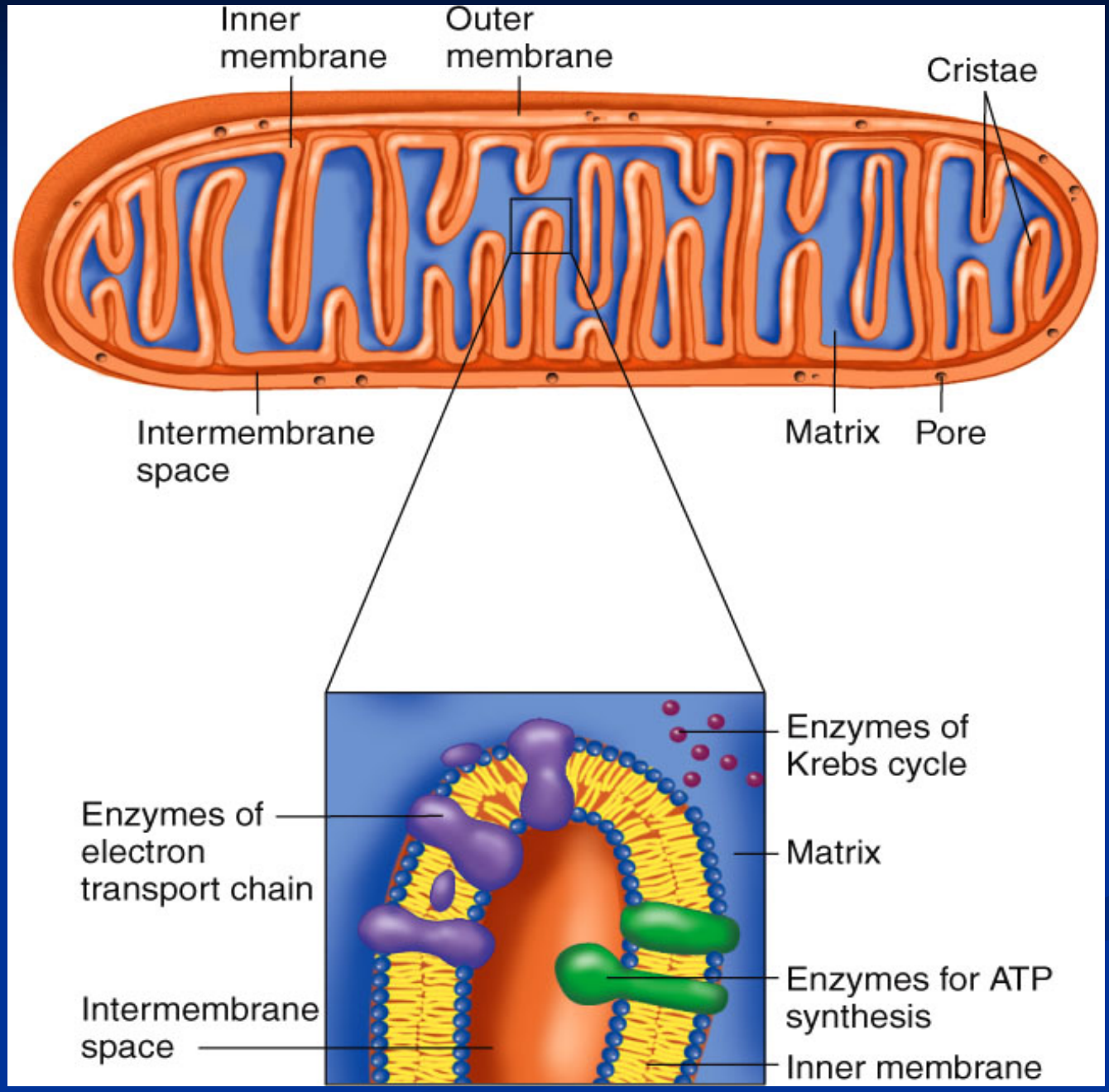
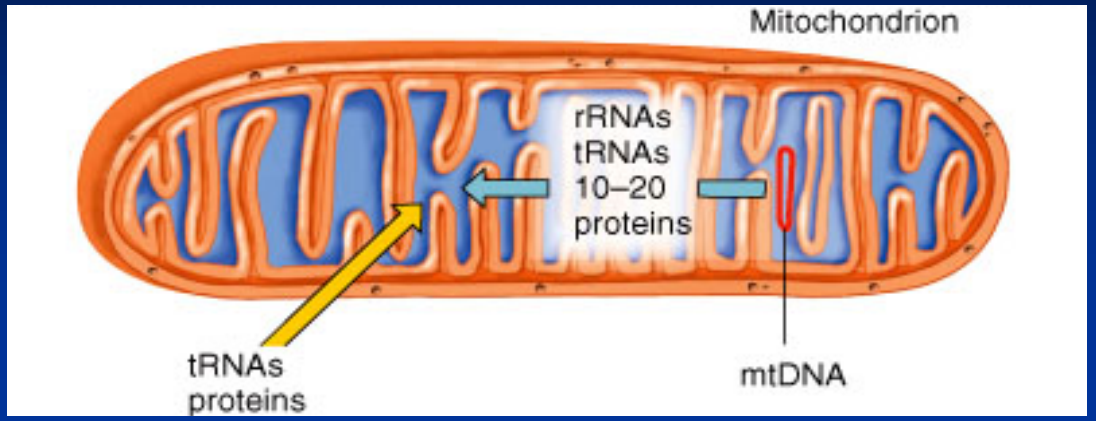


Fig 14.2

The genomes of mitochondria



- Shape: circular in most species; linear in a few species.
- mtDNA lies within matrix of the organelle in structures called **nucleoids**.
- Number of mtDNA in each mitochondria can change.
- Replication is independent of the cell cycle and occurs randomly.

The size and gene content of mtDNA vary from organism to organism

Size: 6 - 2400 kb.

TABLE 16.1 Mitochondrial DNA Sizes

Organism	Size (kb)
Plasmodium	6
Yeast	75
Drosophila	18
Pea	110
Human	16.5

TABLE 16.2 Comparison of Some Functions Encoded in mtDNA

Organism	Oxidative Phosphorylation Genes	tRNAs	Genome Size (kb)
Yeast	7	25	75
Marchantia (liverwort)	14	29	186.0
Human	13	22	16.5

Human mtDNA carries closely packed genes

- 37 genes
  - 13 encode polypeptide subunits that make up oxidative phosphorylation apparatus
  - 22 tRNA genes
  - 2 genes for large and small rRNAs
- Compact gene arrangement
  - No introns
  - Genes abut or slightly overlap.

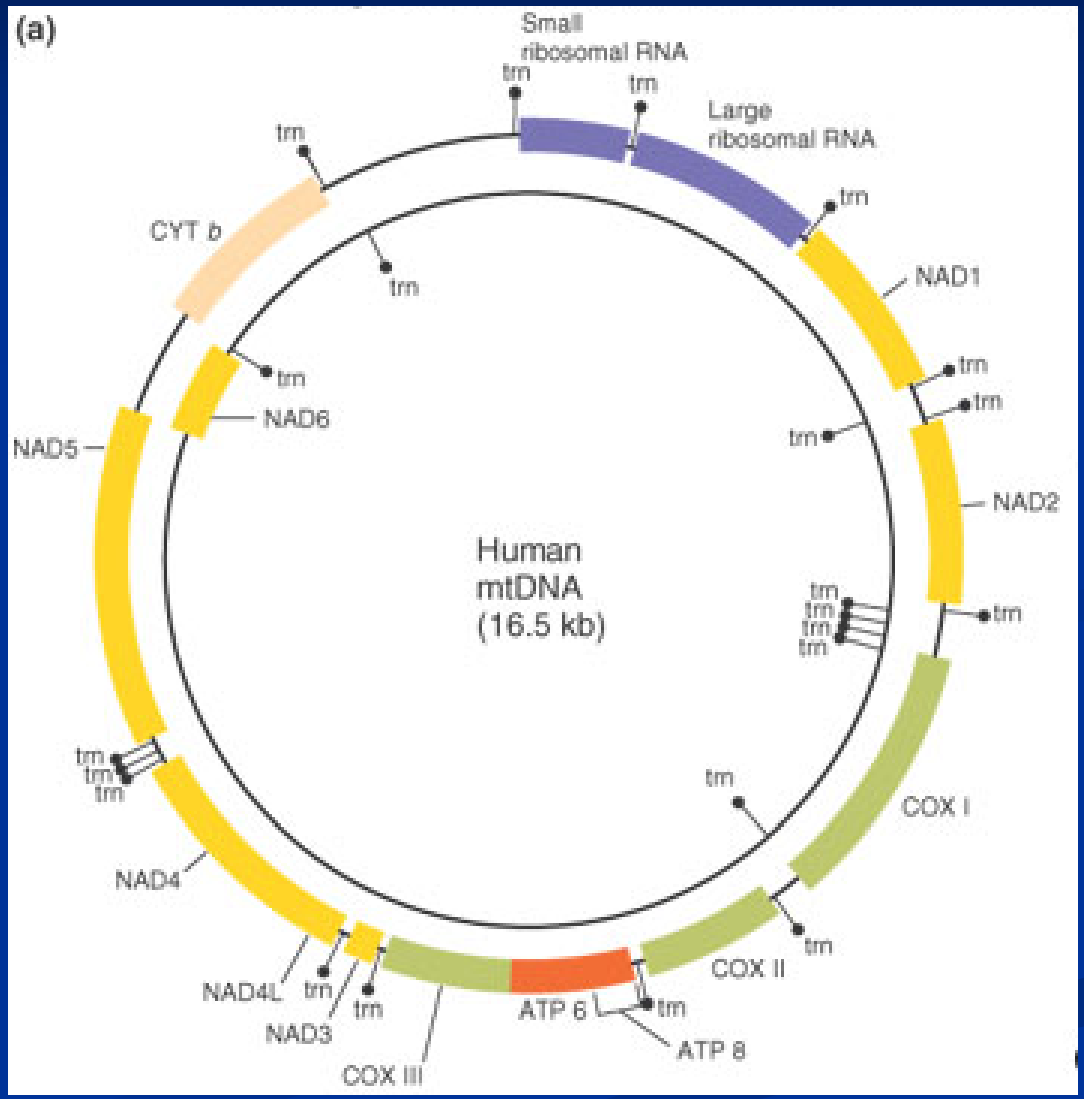
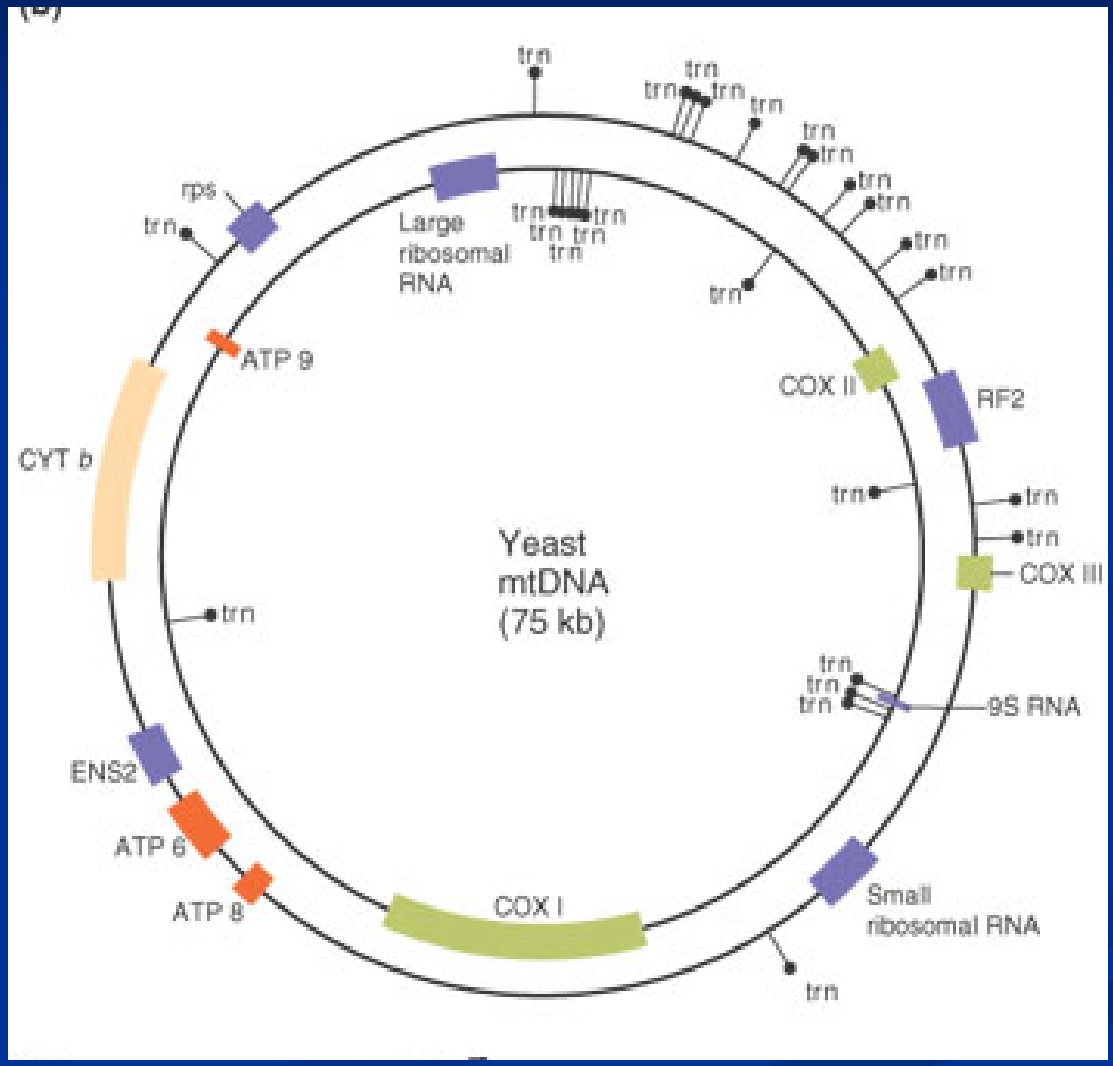


Fig 14.3

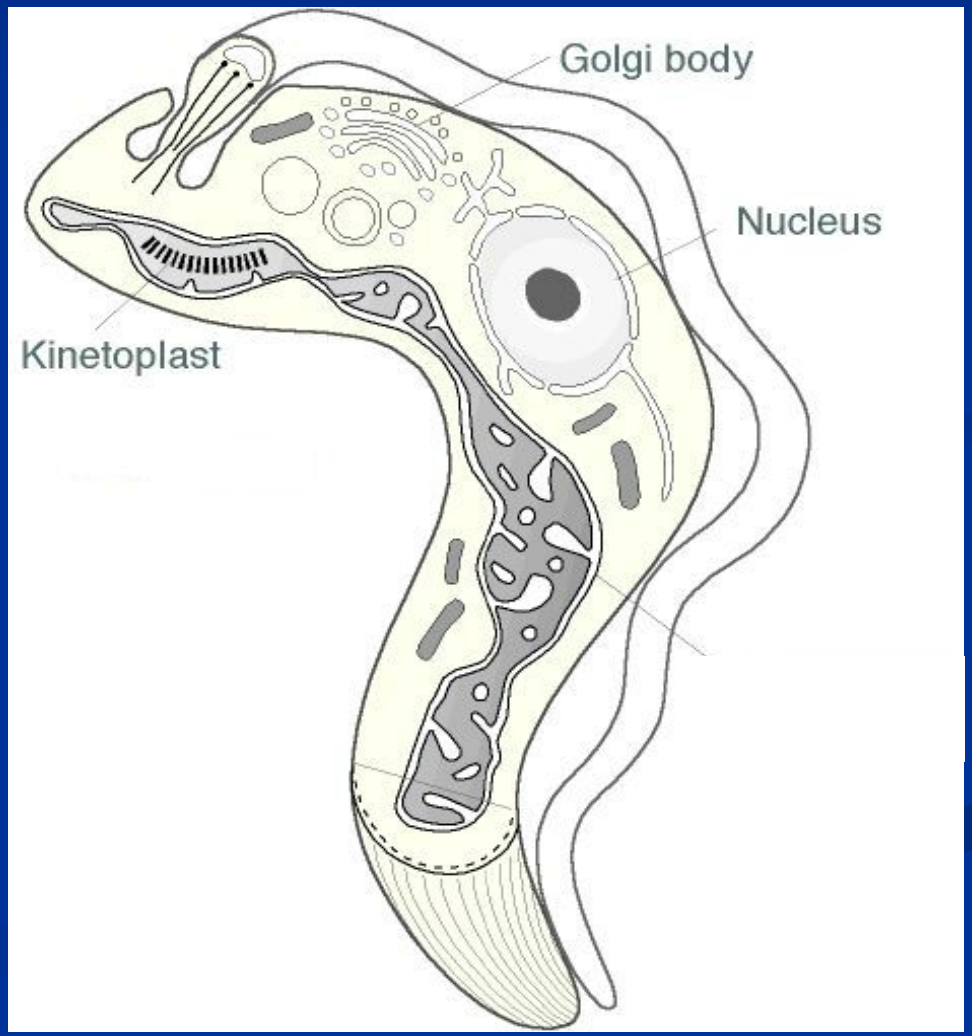
Yeast mtDNA contains spacers and introns

- **Four times longer than human mtDNA.**
  - **Long intergenic sequences called spacers separate genes accounting for more than half of DNA.**
  - **Introns form about 25% of mtDNA.**



Unusually organized mtDNAs of protozoan parasites of the genera *Trypanosoma, Leishmania,* and *Crithidia*

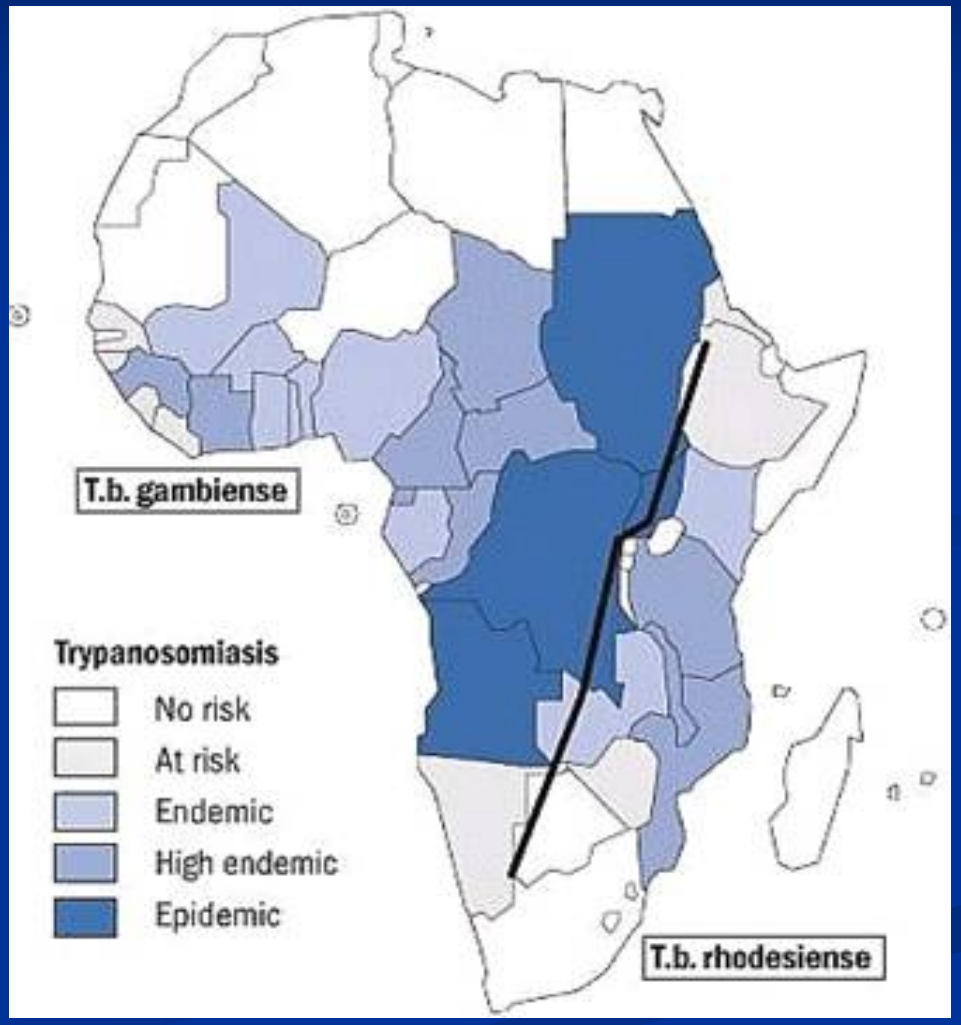
- Protozoan parasites contain a single mitochondrion called **kinetoplast (动基体).**



Protozoan parasites of the genera *Trypanosoma* (锥虫), *Leishmania* (利什曼原虫), and *Crithidia* (短膜虫)

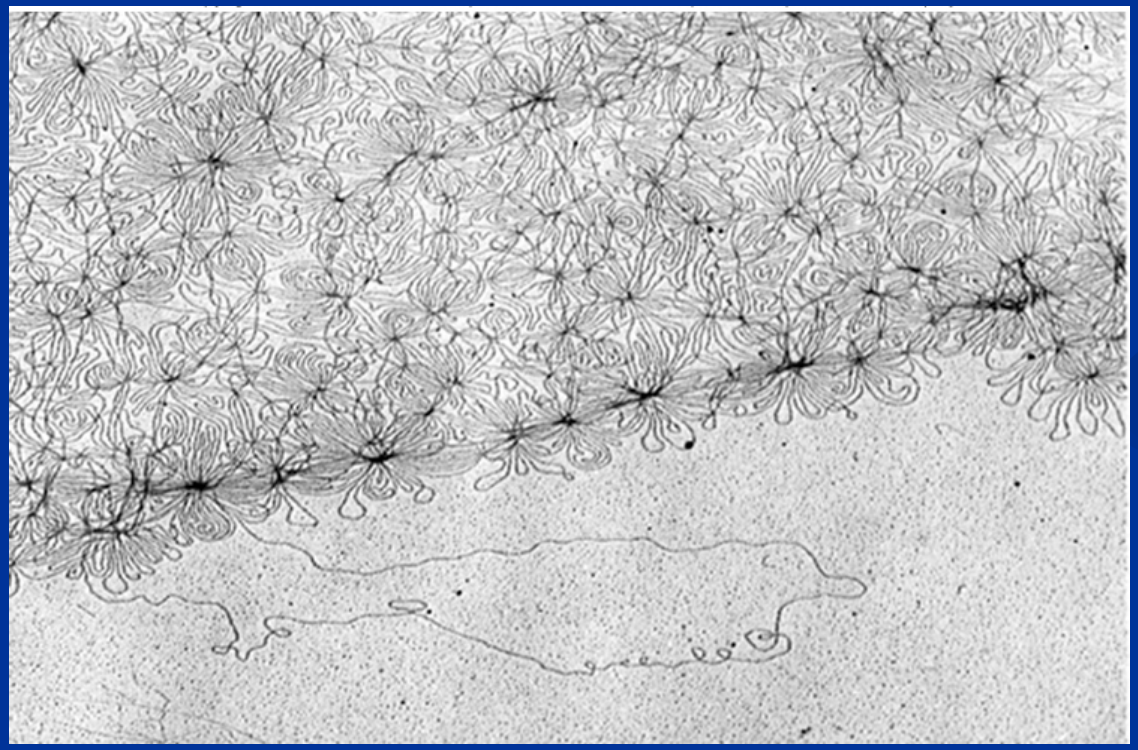
Trypanosomes in the blood

Tsetse fly



African sleeping sickness

- **mtDNA exists in one place as a large network of interlocking minicircles and maxicircles.**
  - **Large network of 10-25,000 minicircles 0.5 – 2.5 kb in length interlocked with 50-100 maxicircles 21-31 kb long.**



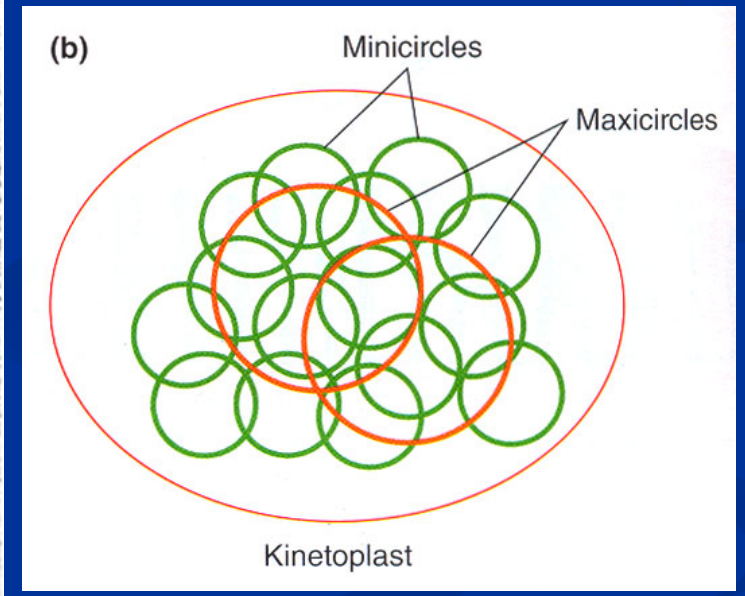
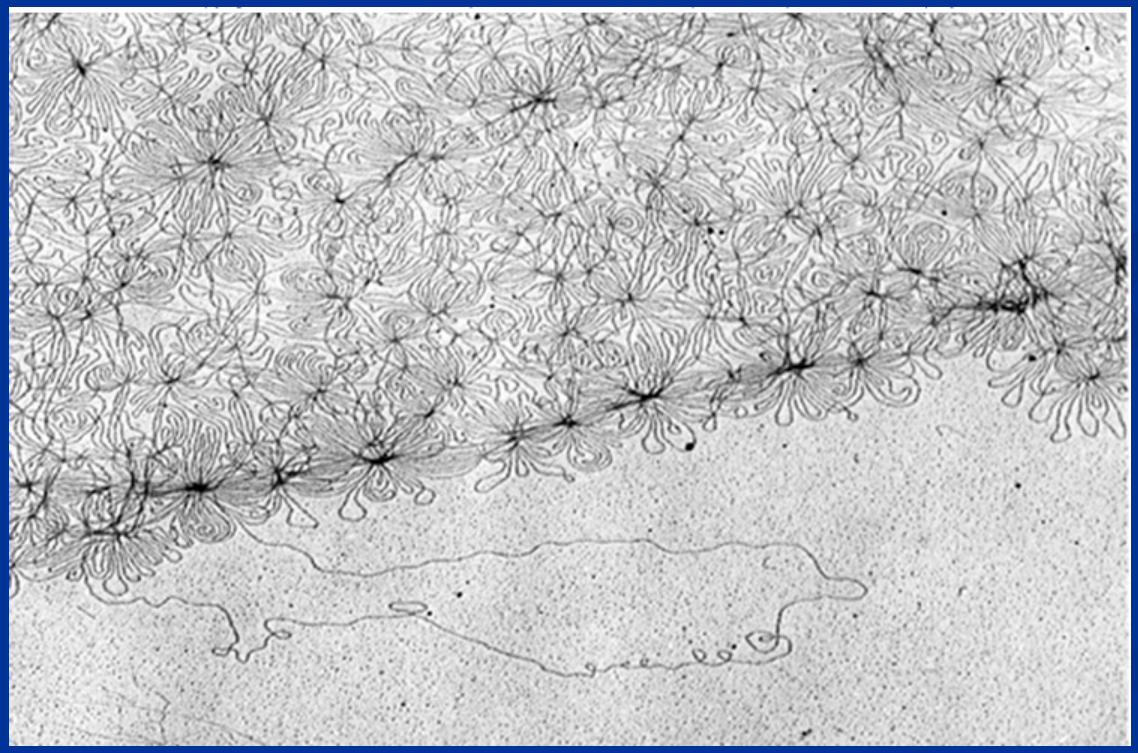


Fig. 14.4

- **Maxicircles contain genes.**
  - **Maxicircle DNAs carry only short, recognizable gene fragments instead of whole genes.**
  - **Two types of RNAs in kinetoplast: short fragments and mature mRNAs.**
- **Minicircles carry no genes, involved in RNA editing.**



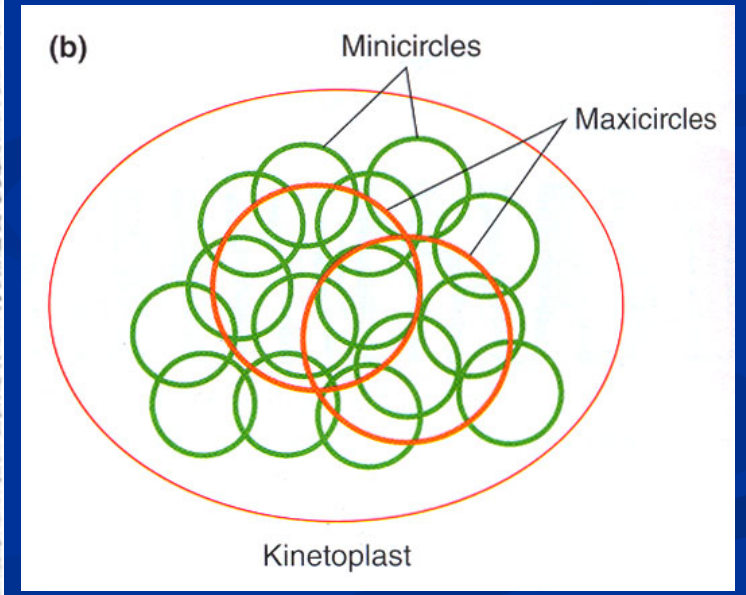


Fig. 14.4

RNA editing, a rare variation on the basic theme of gene expression

- **RNA editing** – A post-transcriptional modification process that converts pre-mRNA to mature mRNA by adding or deleting nucleotides.
- Discovered in the mitochondria of trypanosomes, also found in the mitochondria of some plants and fungi.
  - Adds or deletes uracils in trypanosomes.
  - Adds or deletes cytosines in plants.

RNA editing in trypanosomes: addition or deletion of uracil

- Pre-mRNA pairs with guide RNA. (atypical G-U pairing)
- Unpaired G and A in guide RNA initiate the addition of U in pre-mRNA.
- Unpaired U's in pre-mRNA are deleted.

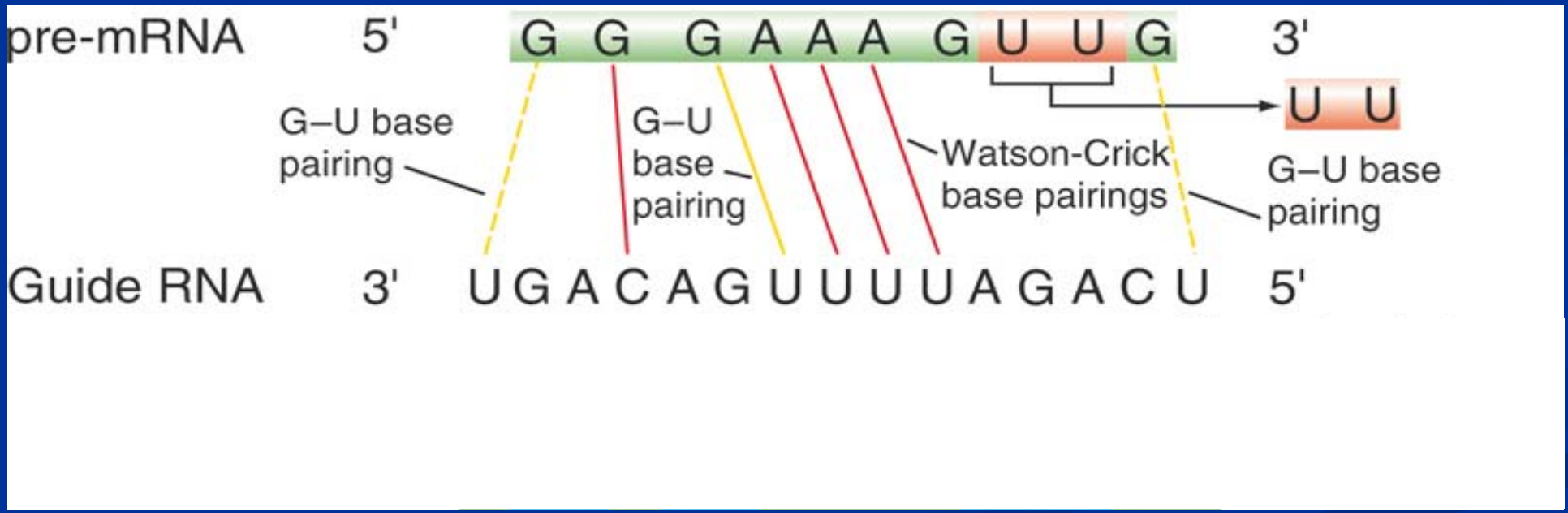


Fig. 14.5

mRNA translation in mitochondria

- **Mitochondria has its own translational machinery.**
  - **tRNAs**
  - **Ribosomes: different from bacterial and eukaryotic ribosomes.**
  - **Translation initiation, elongation factors.**
- **Mitochondrial translation resembles prokaryotic translation.**
  - **First amino acid is N-formyl methionine.**
  - **Inhibited by bacterial translation inhibitors, such as chloramphenicol and erythromycin.**

Altered genetic code in mitochondria shows that the genetic code is not universal

TABLE 16.3 Variations in the Genetic Code of Human Mitochondria

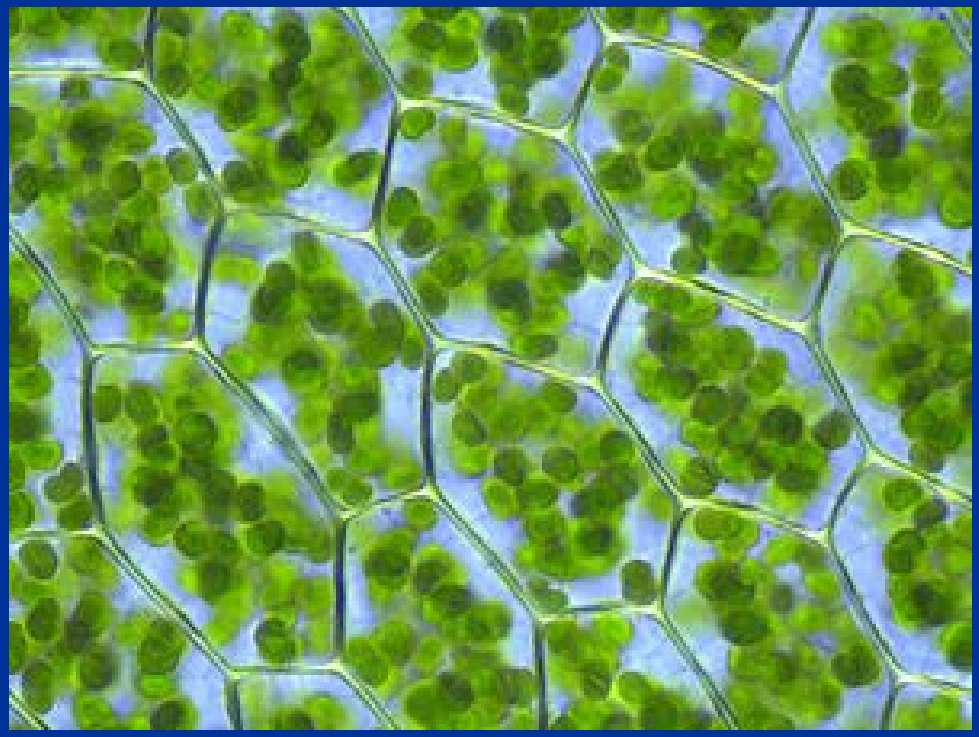
Characteristic	Universal Code	mtDNA Code
Number of tRNAs	32	22
UGG	Trp	Trp
UGA	Stop	Trp
AGG	Arg	Stop
AGA	Arg	Stop
AUG	Met	Met
AUA	Ile	Met

Altered genetic code. The human mtDNA genetic code is simplified such that a modified U in the tRNA "wobble" position can read all four codons in a codon family (that is, UUU, UUC, UUA, and UUG). An unmodified U can read both purines, and G can read both pyrimidines. Tryptophan tRNA has a U in the wobble position, so it will read both the traditional UGG codon and the associated UGA stop codon as tryptophan. Similarly, the methionine codon reads both AUG and the associated AUA as methionine. Finally, human mtDNA has only a single arginine tRNA such that two of the six arginine codons (AGG and AGA) now function as stop codons.

14.2 Chloroplasts and their genomes

Chloroplasts (叶绿体) are sites of photosynthesis.

- **Found in plants and algae.**
- **40 –50 chloroplasts each leaf cell in corn.**



Chloroplasts in a moss

Fig. 14.6

- **Capture, conversion, and storage of solar energy in the bonds of carbohydrates.**

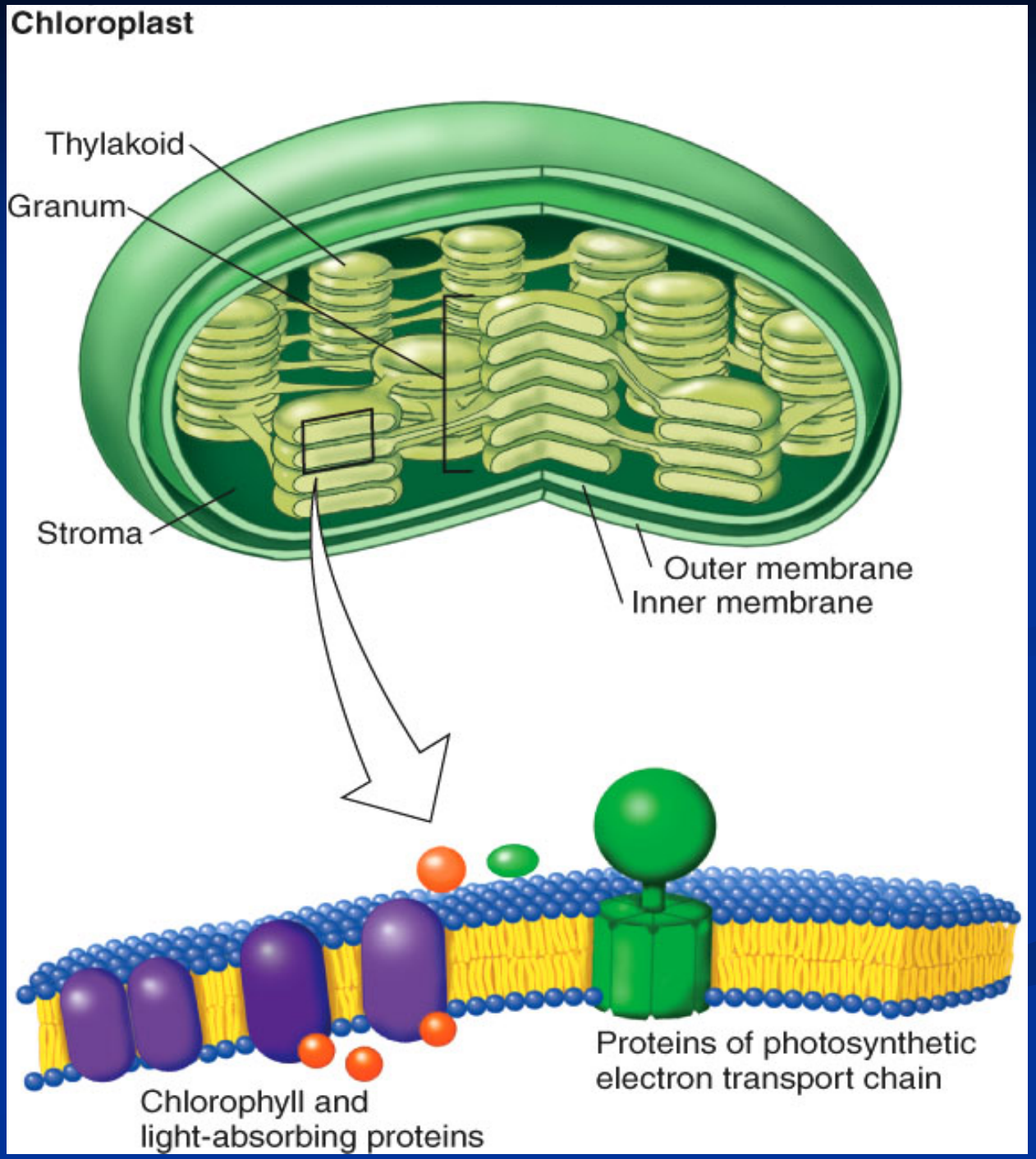
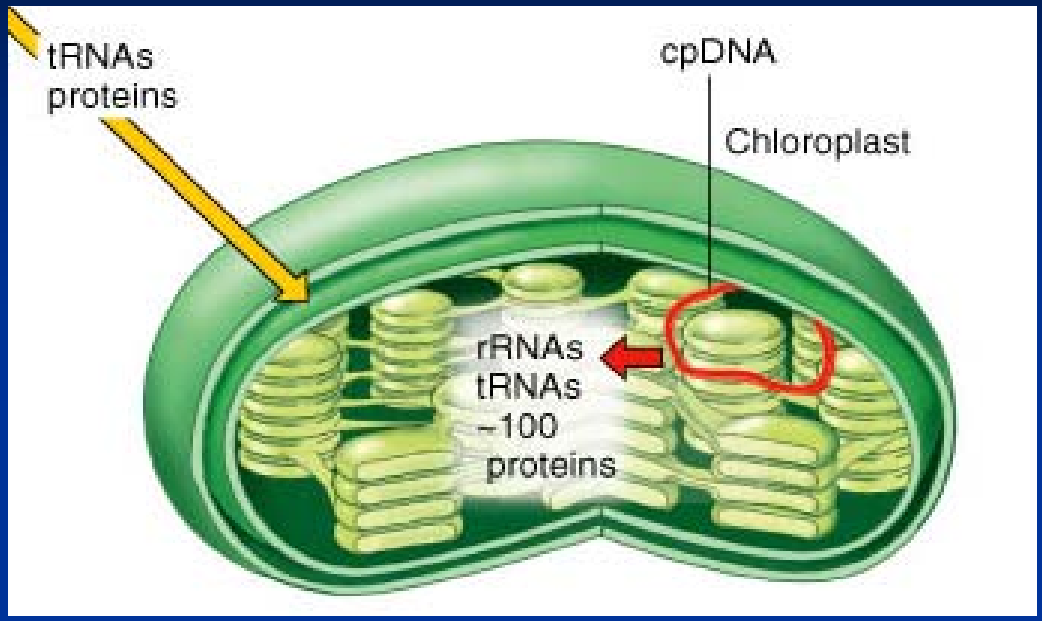


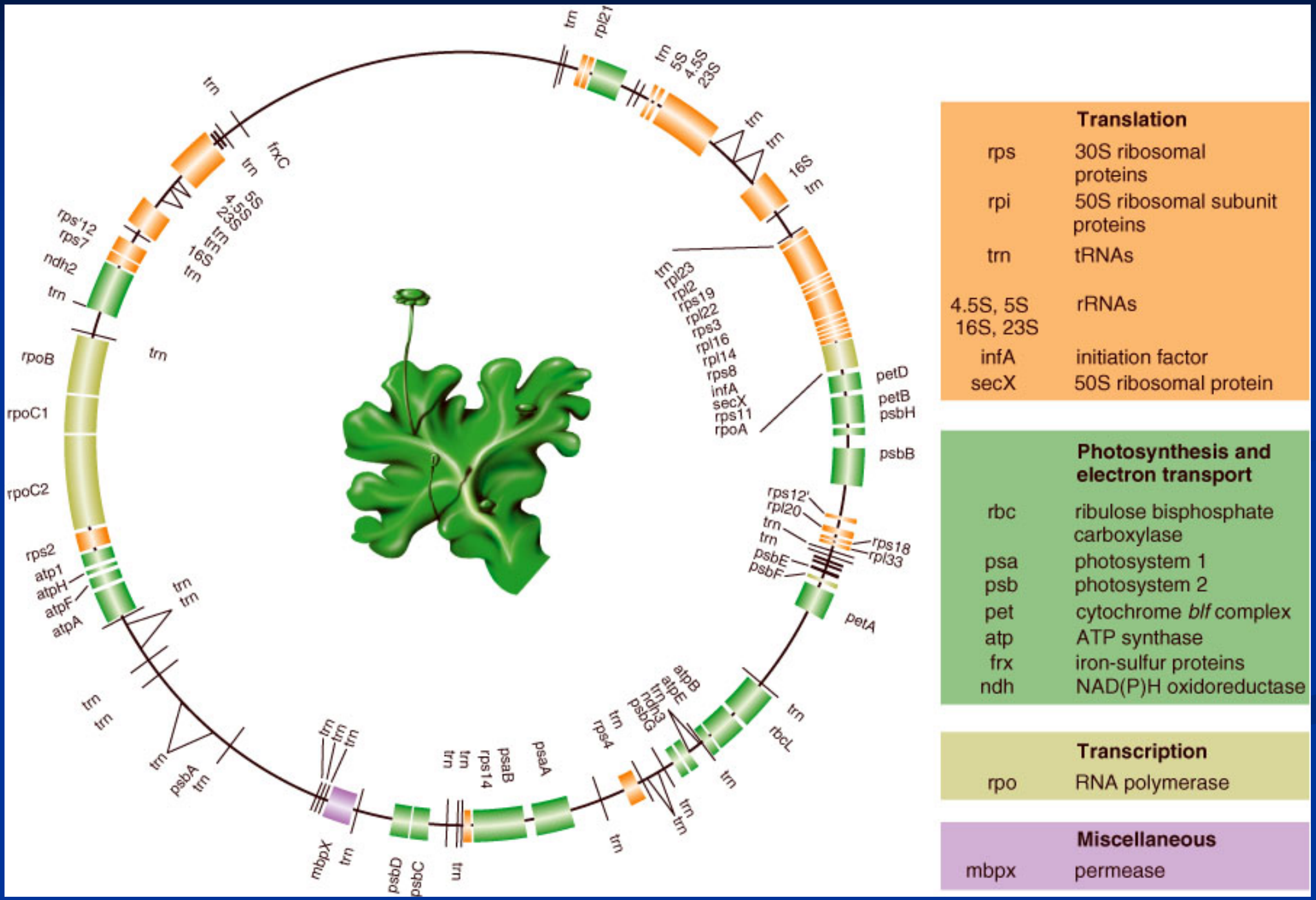
Fig. 14.6

The genomes of chloroplasts



- Size: 120-217 kb, mostly 120-160 kb.
- Genes are closely packed and separated by few nucleotides.
- Genes contain introns.
- Shape: most cpDNAs are linear.

Chloroplast DNA of the liverwort *M. polymorpha*



	Translation
rps	30S ribosomal proteins
rpi	50S ribosomal subunit proteins
trn	tRNAs
4.5S, 5S 16S, 23S	rRNAs
infA	initiation factor
secX	50S ribosomal protein

	Photosynthesis and electron transport
rbc	ribulose bisphosphate carboxylase
psa	photosystem 1
psb	photosystem 2
pet	cytochrome *b/f* complex
atp	ATP synthase
frx	iron-sulfur proteins
ndh	NAD(P)H oxidoreductase

	Transcription
rpo	RNA polymerase

	Miscellaneous
mbpx	permease

- **cpDNA-encoded proteins**
  - **Proteins or enzymes for photosynthesis.**
  - **RNA polymerase, ribosomal proteins, translation factors.**
  - **Translation initiation, elongation factors.**
- **Chloroplast transcription and translation resemble prokaryotic translation.**
  - **RNA polymerase is similar to bacterial counterparts.**
  - **Sensitive to bacterial translation inhibitors, such as chloramphenicol and erythromycin.**

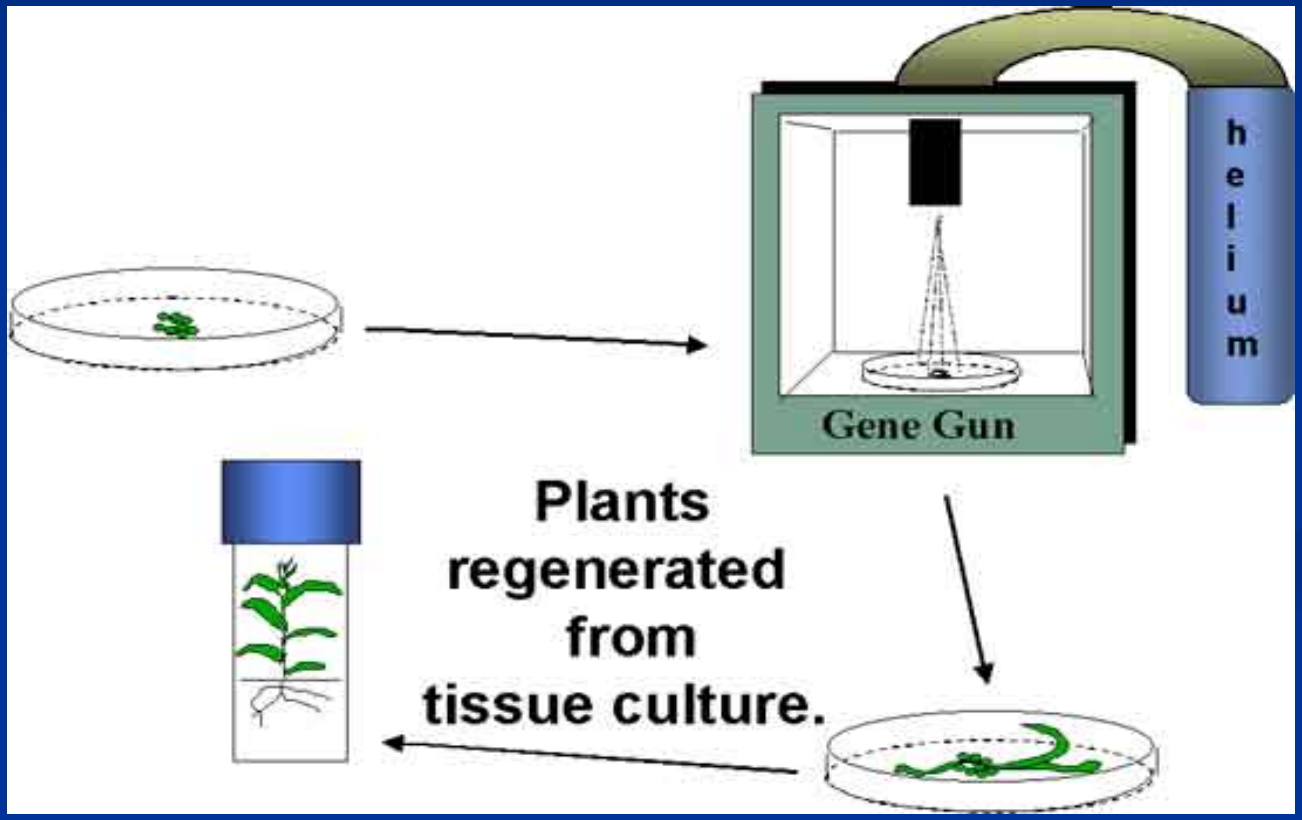
Organelle transformation

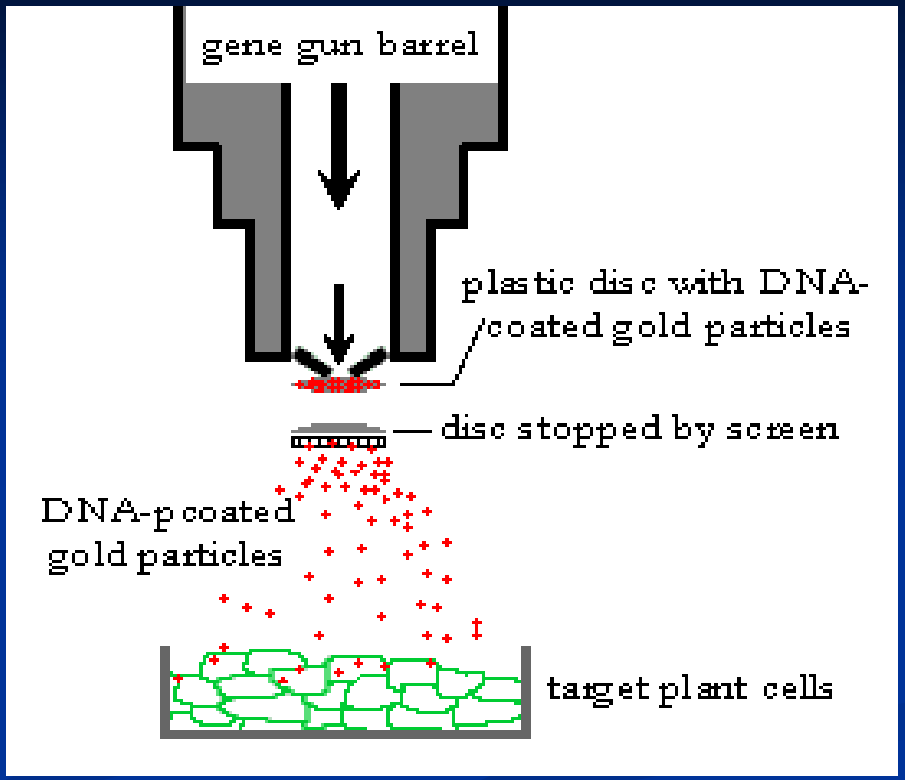
Biolistic transformation

- Developed in late 1980s.
- Coat small metal particles (1 μm) with DNA and shoot them at cells with the gene gun.
- Stable transformation of organelles is highly successful only for chloroplasts.

Helios gene gun (Bio-Rad)

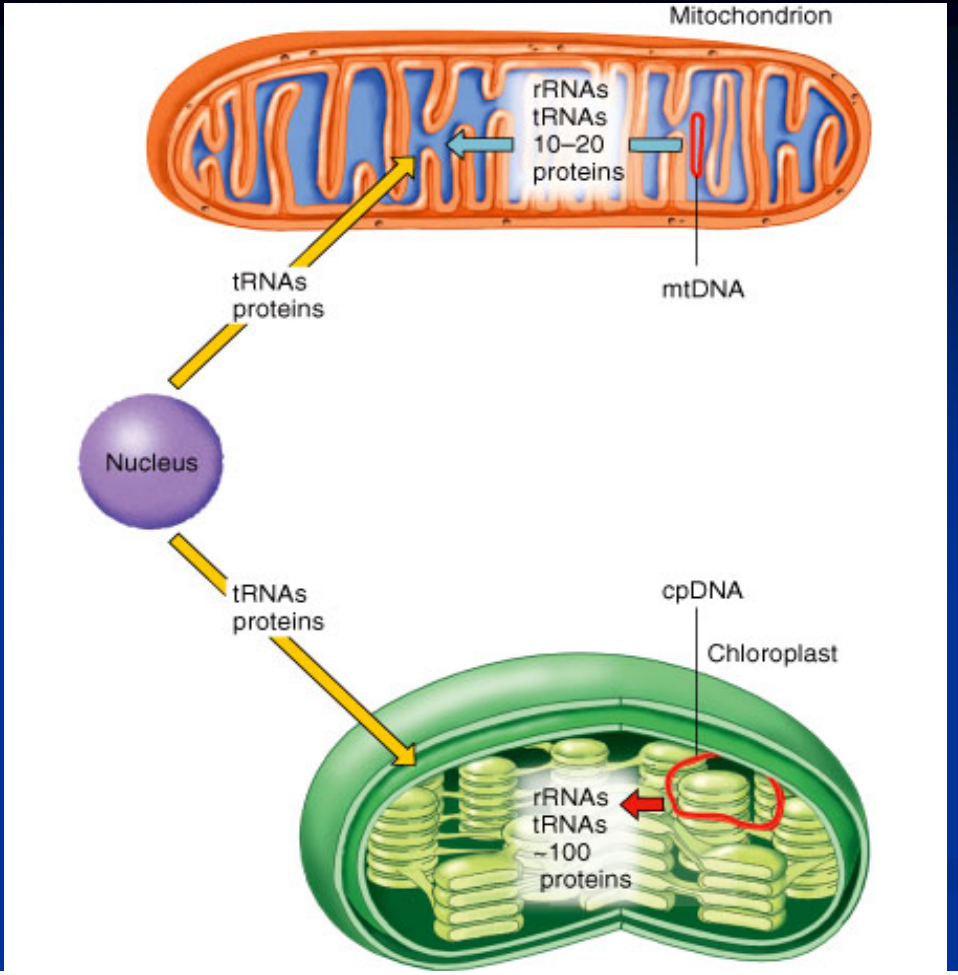
Fig 14.8


helium
Gene Gun
Plants
regenerated
from
tissue culture.


gene gun barrel
plastic disc with DNA-coated gold particles
disc stopped by screen
DNA-p coated gold particles
target plant cells

14.3 The relationship between organellar and nuclear genomes

Mitochondria and chloroplast functions require cooperation between organelle and nuclear genomes



Number and genomic location of oxidative phosphorylation genes

	Number of polypeptides					
	Electron transport chain				ATP synthase	
Genomic location	I	II	III	IV	V	Total
Mitochondrion	7	0	1	3	2	13
Nucleus	≥33	4	10	10	10	≥67
Total	≥40	4	11	13	12	≥80

Gene transfer occurs through an RNA intermediate or by movement of DNA pieces

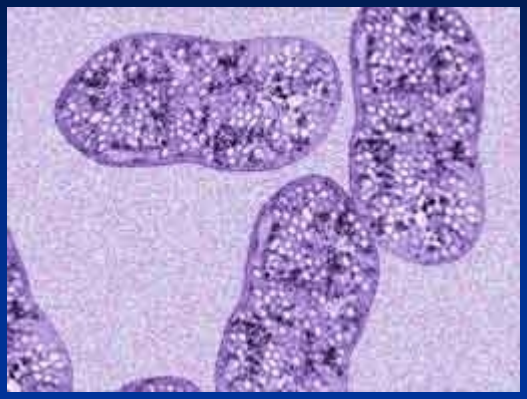
- Genes transfer between organelles and the nucleus.
  - *COXII* gene
    - Locates in mtDNA in many plants.
    - In nuclear genome in other plants.
    - In both nuclear genome (functional, intronless) and mtDNA (nonfunctional, intron) in several plant species.
- DNA movement between organelles.
  - Some plant mtDNAs carry fragments of cpDNA.
  - Nonfunctional copies of organelle DNA are found in the nuclear genomes of eukaryotes.

Origin of mitochondria and chloroplasts

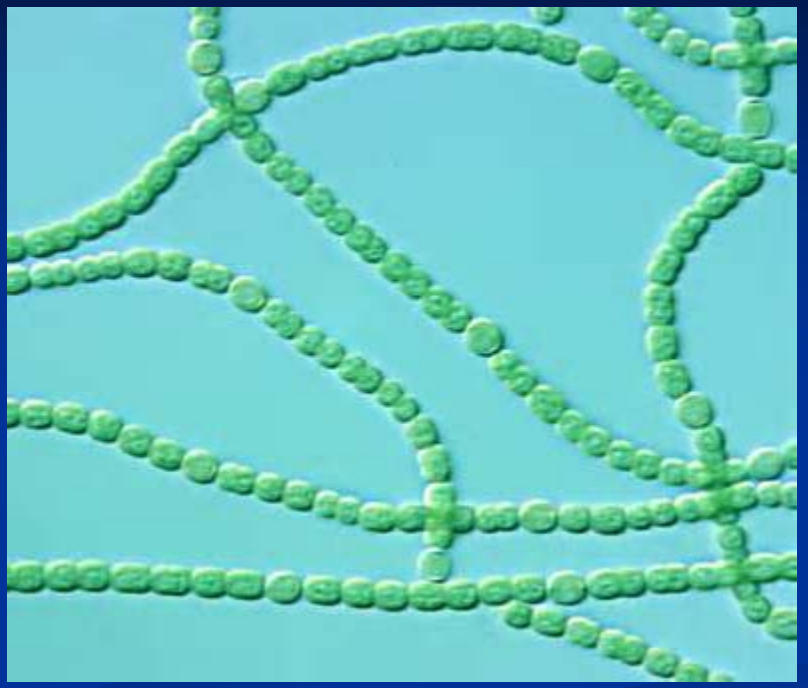
Endosymbiont theory:

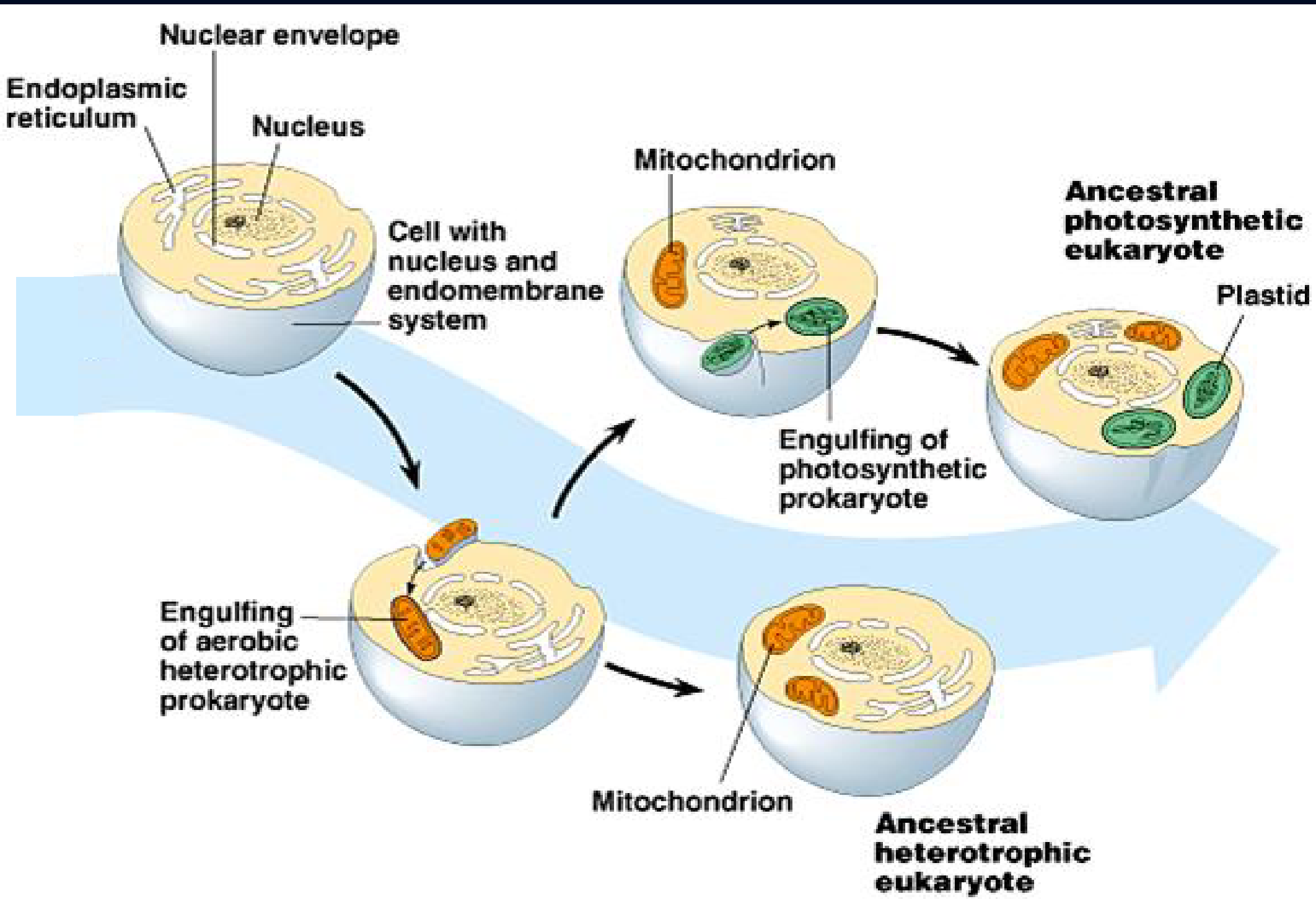
- 1970s, Lynn Margulis
- Mitochondria and chloroplasts originated more than a billion years ago.
- Ancient precursors of eukaryotic cells engulfed bacteria and established symbiotic relationship.
  - Mitochondrial genomes derives from a common ancestor of present-day **nonsulfur purple bacteria**.
  - Chloroplast genomes derives from **cyanobacteria**.

Nonsulfur purple bacteria



Cyanobacteria




Nuclear envelope
Endoplasmic reticulum
Nucleus
Cell with nucleus and endomembrane system
Mitochondrion
Ancestral photosynthetic eukaryote
Plastid
Engulfing of photosynthetic prokaryote
Engulfing of aerobic heterotrophic prokaryote
Mitochondrion
Ancestral heterotrophic eukaryote

- **Molecular evidence**
  - **Both chloroplasts and mitochondria have their own DNA and ribosomes.**
  - **mtDNA and cpDNA are not organized into nucleosomes by histones, similar to bacteria.**
  - **Mitochondria uses *N*-formyl methionine and $tRNA^{fmet}$ in translation.**
  - **Inhibitors of bacterial translation have same effect on mitochondrial translation, but not on eukaryotic cytoplasmic protein synthesis.**

14.4 Non-Mendelian inheritance of mitochondria and chloroplasts

Non-Mendelian inheritance: An inheritance pattern that does not follow Mendel's laws and does not produce Mendelian ratios among the progeny of various crosses.

- **Organelle DNA**
  - **Mitochondrial DNA**
  - **Chloroplast DNA**
- **Plasmid**

Inheritance of variegated leaves in four-o'clocks (*Mirabilis jalapa*)

- In 1909, Carl Correns.
- Reciprocal crosses showed different patterns of inheritance.
- Maternal inheritance pattern.

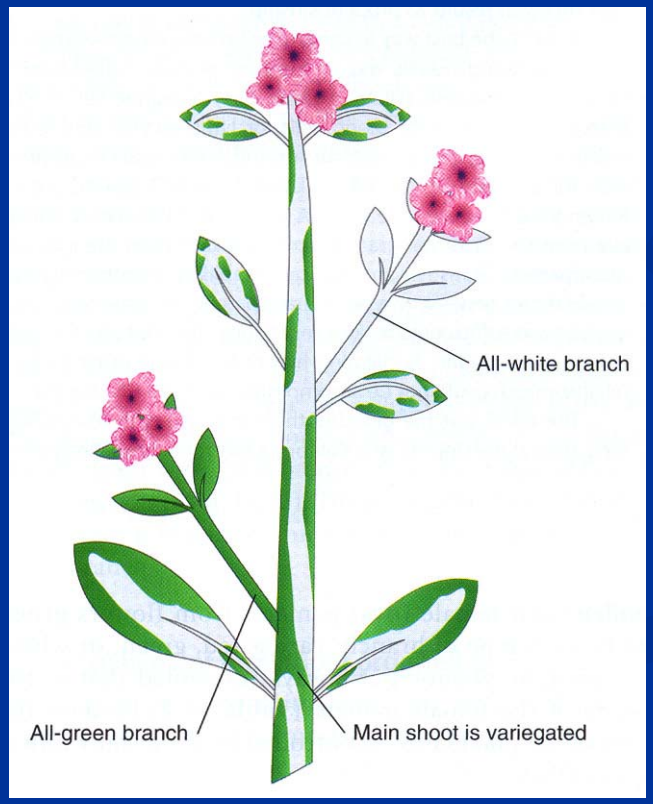


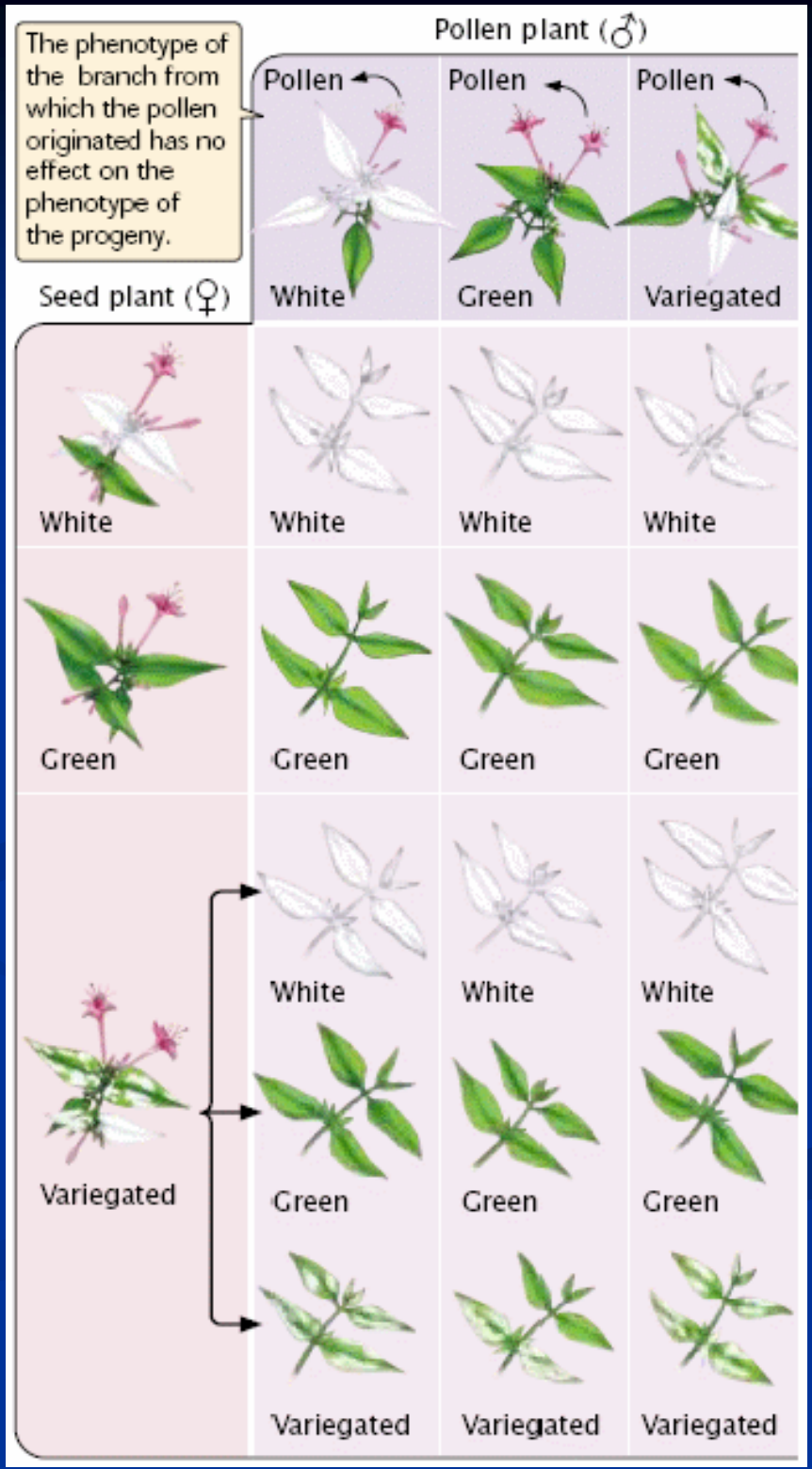
Fig 14.1, 14-10

Variegated leaf

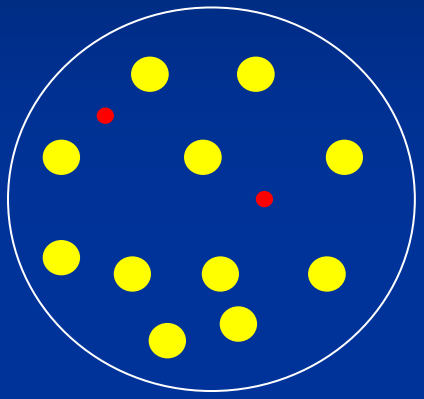
♀ White × ♂ Variegated → White

♀ Green × ♂ Variegated → Green

♀ Variegated × ♂ Green → White
or Green
or Variegated



Colony-size inheritance in yeast *Saccharomyces cerevisiae* found by French researchers in 1949



Yeast on glucose ⟶ ***grande*** **(95%),** ***petite*** **(5%)**

grande ⟶ ***grande, petite*** **colonies**

petite ⟶ ***petite***

grande × ***grande*** ⟶ ***grande*** (diploid) $\xrightarrow{\text{Sporulation}}$ **4** ***grande*** (haploid)

petite × ***petite*** ⟶ ***petite*** (diploid) ⟶ **No sporulation**

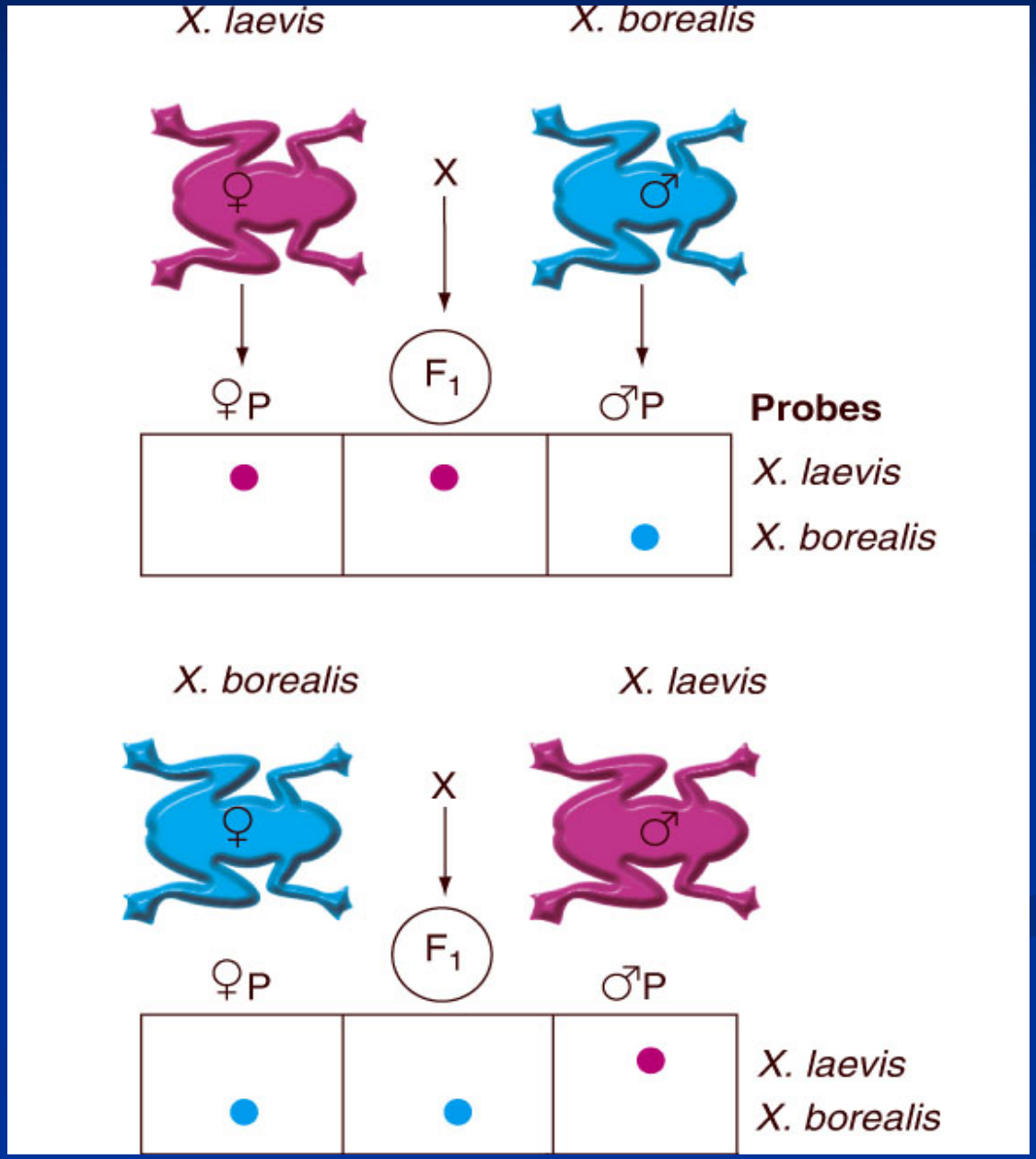
grande × ***petite*** ⟶ ***grande*** (diploid) $\xrightarrow{\text{Sporulation}}$ **4** ***grande*****: 0** ***petite*** (haploid)

Non-Mendelian inheritance of organelle DNA-encoded traits

- ***Uniparental inheritance*: Inheritance of organelle genes from only one parent.**
  - **Mitochondrial DNA-encoded traits**
  - **Chloroplast DNA-encoded traits**
- ***Biparental inheritance*: Inheritance of organelle genes from both parents.**
  - **Yeast mitochondrial DNA**
  - **Chloroplast DNA-encoded leaf variegation in geranium *Pelargonium zonale*.**

In most species, progeny inherit organelle DNA from only one parent, usually their mother

- Examination of mtDNA inheritance in the *Xenopus* frog.
  - Isolate mtDNA from two species.
  - DNA hybridization.
  - F1 hybrids retain only mtDNA from mother.



LHON, a maternally inherited human neurodegenerative disease

- **Leber's hereditary optic neuropathy (LHON), defective mitochondrial electron transport chain leads to optic nerve degeneration and blindness.**
- **Mutation in a gene encoding NADH dehydrogenase subunit 4: G-to-A substitution at nucleotide 11,778 in mtDNA.**

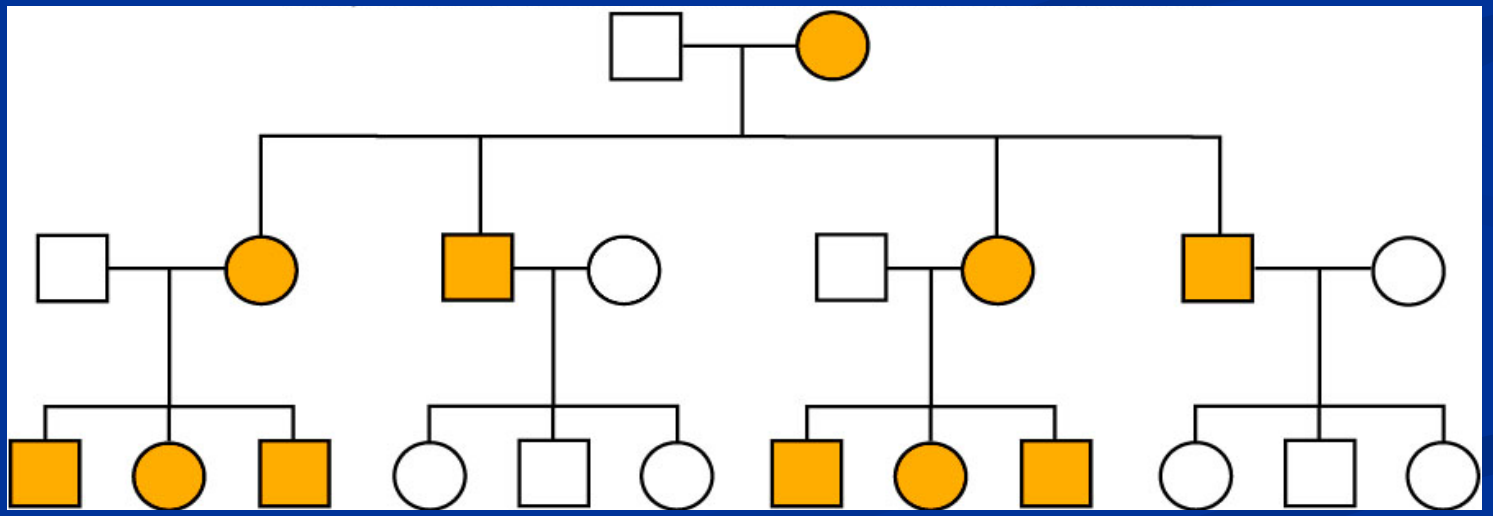


Fig 14.14

Segregation of organelles during mitosis

Cells can carry one type or a mixture of organelle genomes.

- ***Homoplasmy*** **– cells contain one type of organelle DNA.**
  - **Mitotic products contain same type, except for rare mutation.**
- ***Heteroplasmy*** **– cells contain a mixture of organelle genomes.**
  - **Mitotic progeny could be heteroplasmic, homoplasmic wild type, or homoplasmic mutant.**

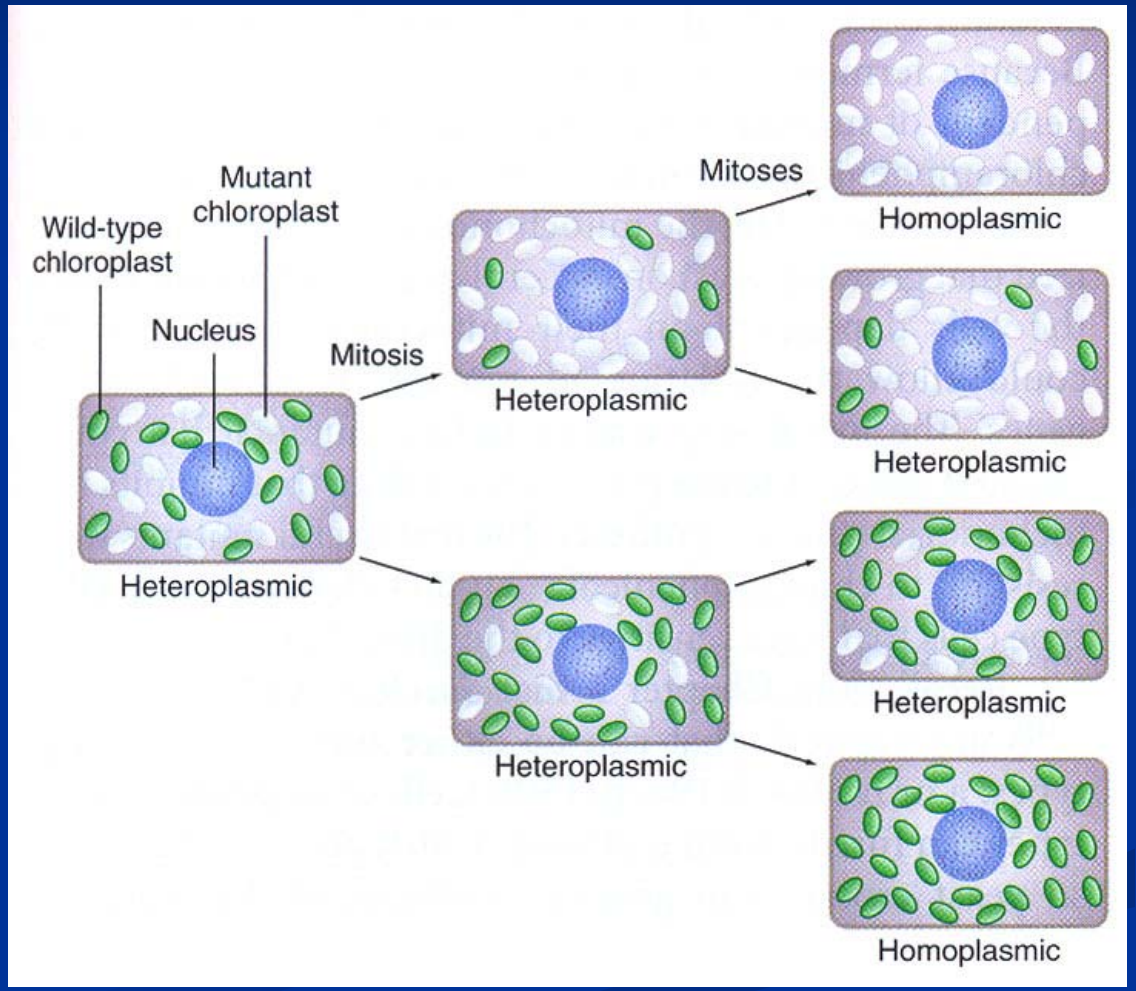


Fig 14.11

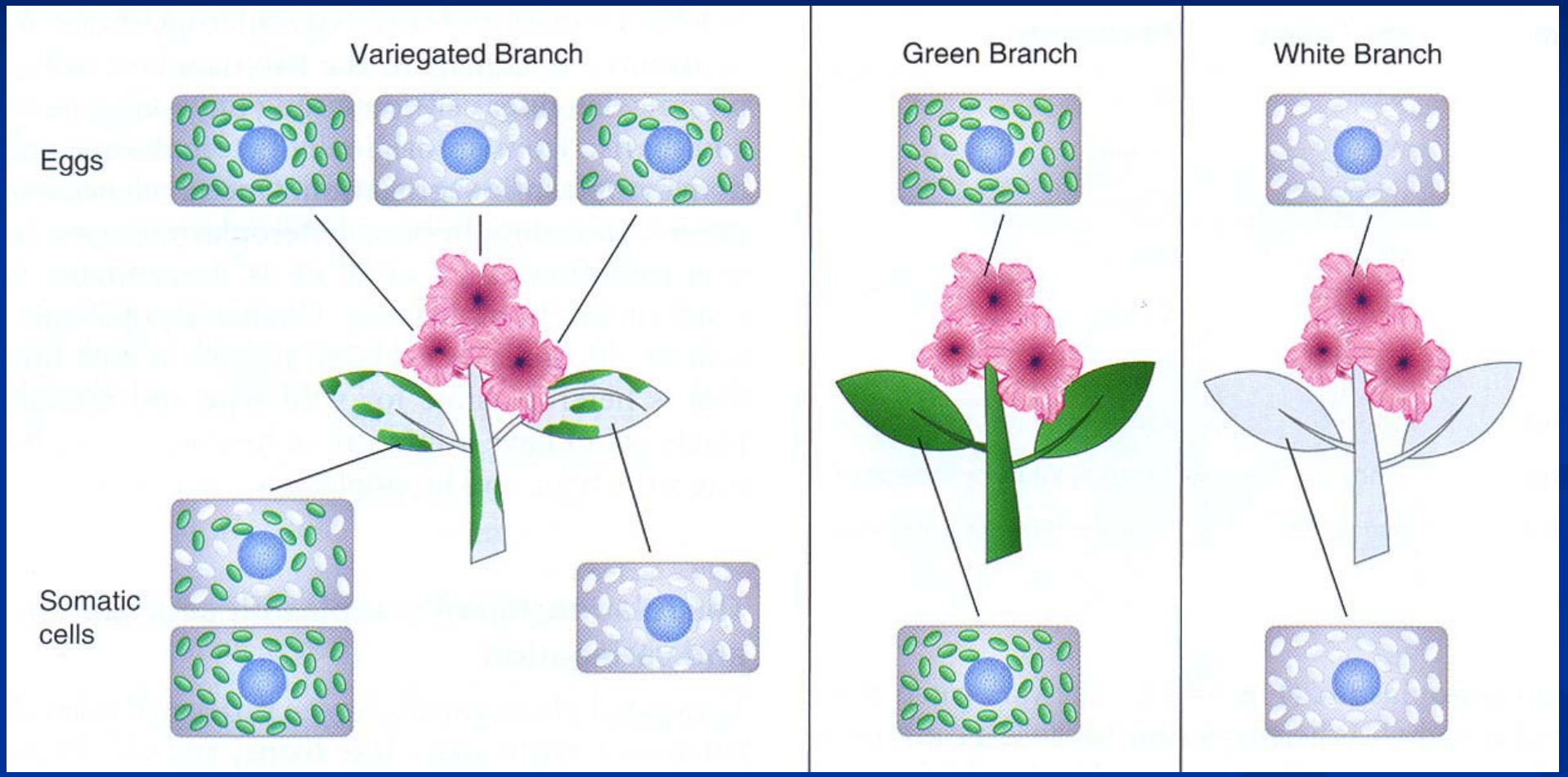

Variegated Branch
Green Branch
White Branch
Eggs
Somatic cells

Fig 14.12

Mitotic segregation produces an uneven distribution of organelle genes in heteroplasmic cells

Women with heteroplasmic LHON mutation:

- **Some ova may carry large number of wild-type mitochondria and few mitochondria with LHON mutation.**
- **Other ova may carry mainly mitochondria with LHON mutation and few wild-type.**
- **Consequence of heteroplasmy after fertilization**
  - **Some cells produce tissues with normal ATP production and others with low production.**
  - **If low production cells are in optic nerve, LHON results.**

Mechanisms that contribute to uniparental inheritance

- **Differences in gamete size** (big egg, small sperm).
- **Organelles or organelle DNA of male gametes are degraded in some organisms.**
- **Paternal organelle genomes are distributed to cells that are not destined to become part of the embryo during early development in some plants.**
- **Paternal organelles are not allowed to enter the egg during fertilization in some animals.**
- **Paternal organelles in the zygote are destroyed after fertilization in some organisms.**

Biparental inheritance and mitotic segregation of mtDNA-encoded traits in yeast

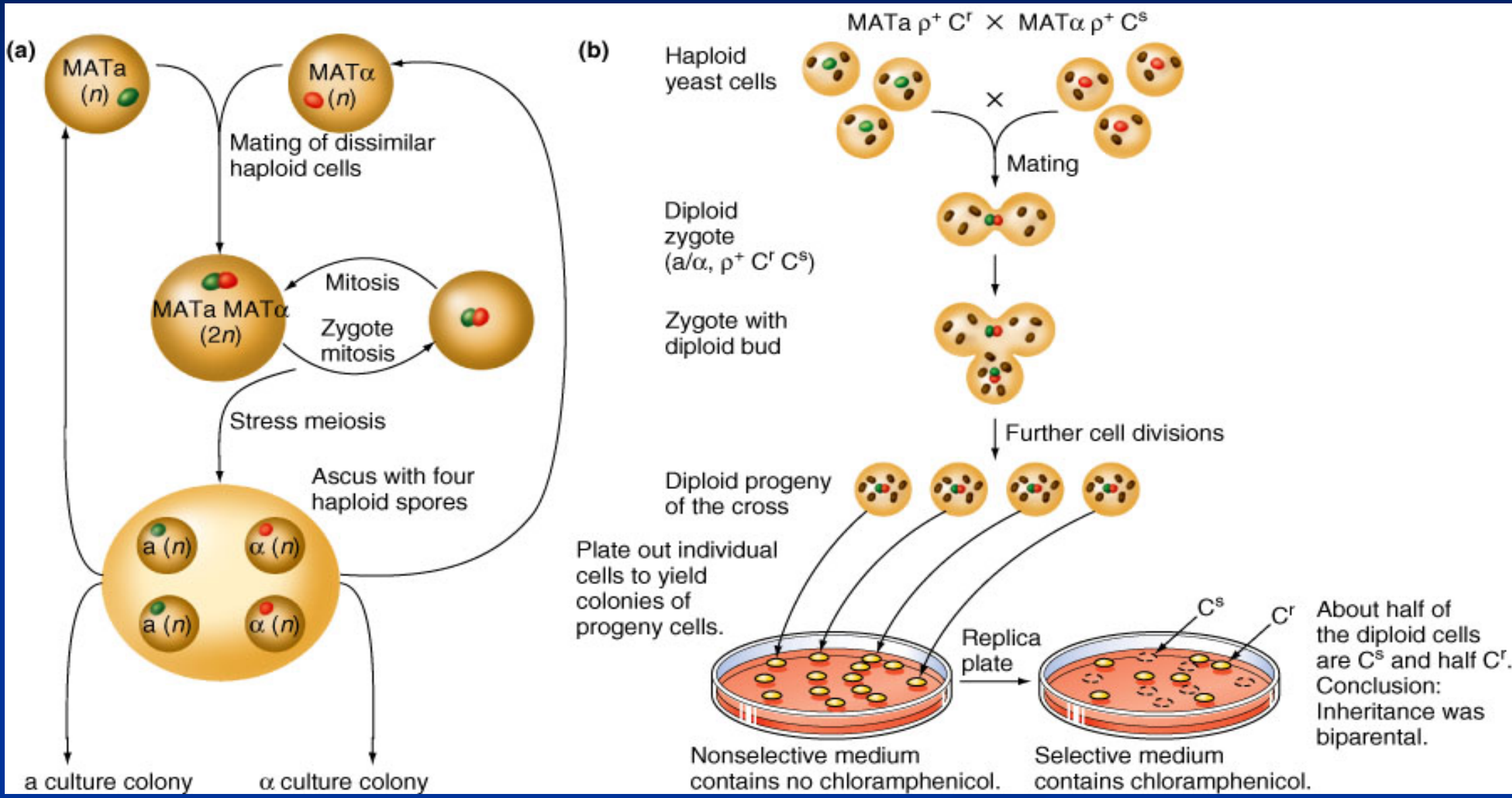
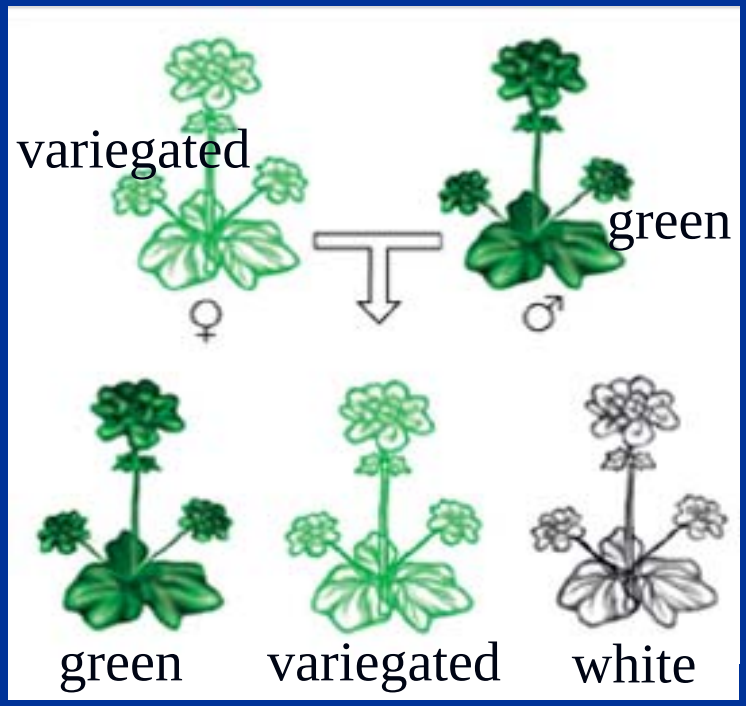
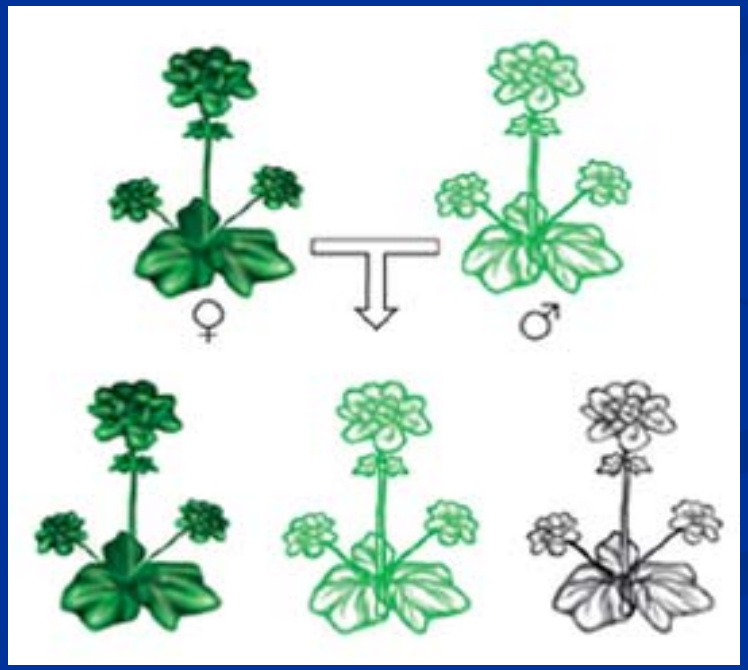


Fig 14.13

Biparental inheritance of leaf variegation in geranium *Pelargonium zonale*





Variegated leaves are a chimeric condition. Chloroplasts are from both parents.

Fig. 14.36

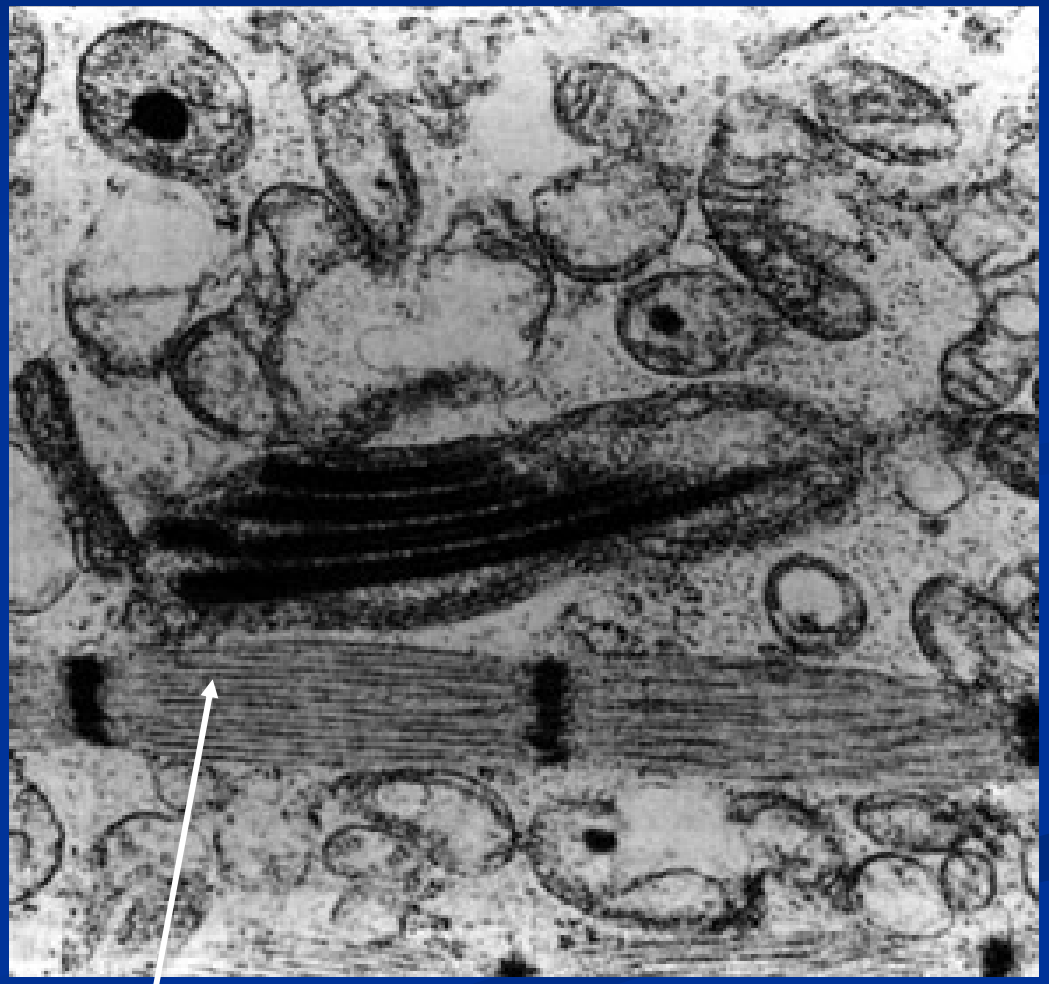
Summary of the genetic principles of non-Mendelian inheritance

- Parental alleles segregate at the ratio of 4:0 instead of 2:2.
- In most organisms, inheritance of organelle-encoded traits is uniparental, mainly maternal. Biparental inheritance is rare.
- When cells carrying a mixture of organelles with different genotypes divide, organelle genotype segregates due to uneven partitioning of organelles.

14.5 Mutant mitochondria and human disease

MERRF: Myoclonic epilepsy and ragged red fiber disease

- Symptoms include uncontrolled jerking, muscle weakness, deafness, heart problems, kidney problems, progressive dementia.
- Caused by a mutation in the $tRNA^{Lys}$ gene, or other tRNA genes, which disrupts the production of proteins involved in ATP production.



Abnormal mitochondria in muscle

Maternal inheritance of MERRF

- Individuals with MERRF disease are heteroplasmic.
- The percentage of mutant mitochondria in an individual varies and corresponds with the severity of the disease.

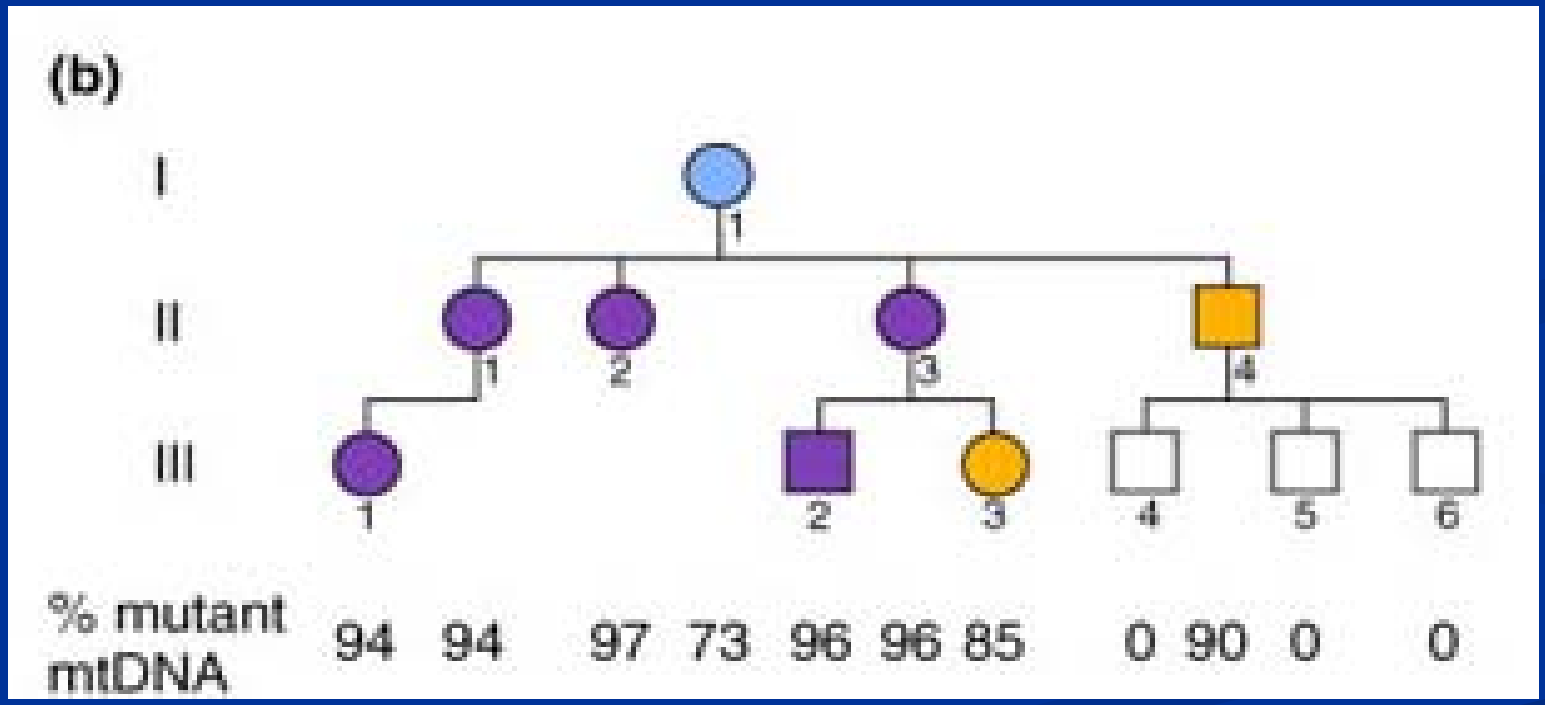
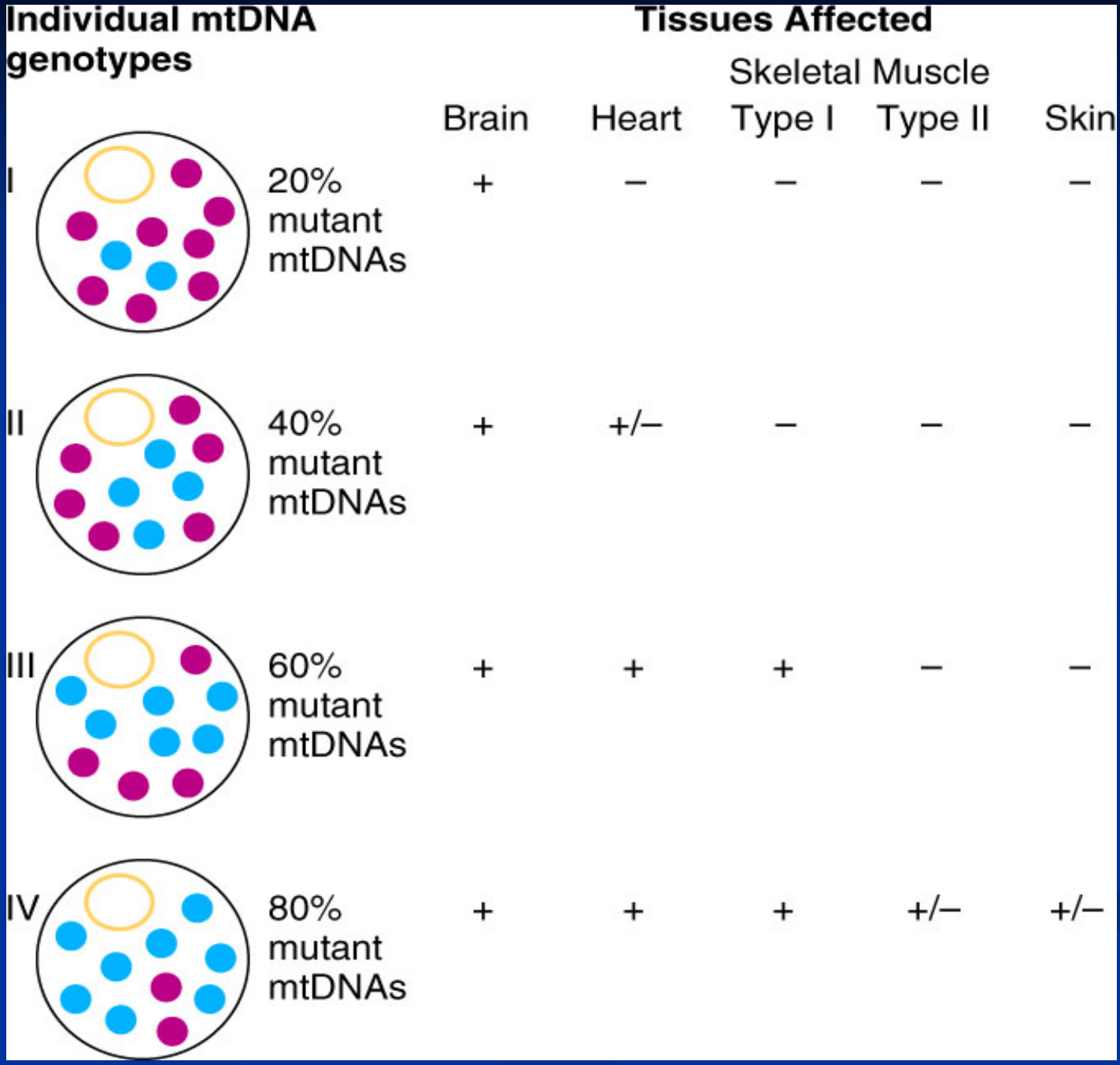


Fig. 14.16

Proportion of mutant mtDNA and tissue in which they reside influence phenotype



Individual mtDNA genotypes		Tissues Affected				
				Skeletal Muscle		
		Brain	Heart	Type I	Type II	Skin
I	20% mutant mtDNAs	+	–	–	–	–
II	40% mutant mtDNAs	+	+/–	–	–	–
III	60% mutant mtDNAs	+	+	+	–	–
IV	80% mutant mtDNAs	+	+	+	+/–	+/–

Fig. 14.17

Mitochondrial inheritance in identical twins

- Symptoms of neurodegenerative diseases or other mutations may manifest in one twin, but not in the other.
- Nuclear genomes are identical, but mitochondrial genomes could be different.
  - In a fertilized egg carrying a mixture of normal and mutant mtDNA, two populations of mtDNA may unevenly partition into two twins.
  - During embryonic development of each twin, mutant mtDNA may partition into cells that will die, or into cells that will develop into heart and optic nerves.

mtDNA has high rate of mutation

- 10 times higher rate of mutation than nuclear DNA.
  - Oxidative phosphorylation system generates a high concentration of DNA-damaging free radicals.
- Provides a tool for studying evolutionary relationships among closely related organisms.
  - Mitochondrial DNA test as evidence of kinship.
  - The hypothesis of a "mitochondrial Eve". Maternal lineage of humans trace back to a few women who lived in Africa about 200,000 years ago.

mtDNA mutations and aging

- ***Hypothesis*: Accumulation of mutations in mtDNA over lifetime and biased replication of mtDNA with deletion result in age-related decline in oxidative phosphorylation system.**
- ***Evidence*:**
  - **mtDNA accumulated 16 times more oxidative damage than nuclear DNA.**
  - **Significant decreases of cytochrome c oxidase in hearts from autopsies.**
  - **Rate of DNA deletions in mtDNA increases with age.**
  - **Alzheimer's patients have abnormally low energy metabolism.**

Oocyte nuclear transplantation can sidestep transmission of mitochondrial disease

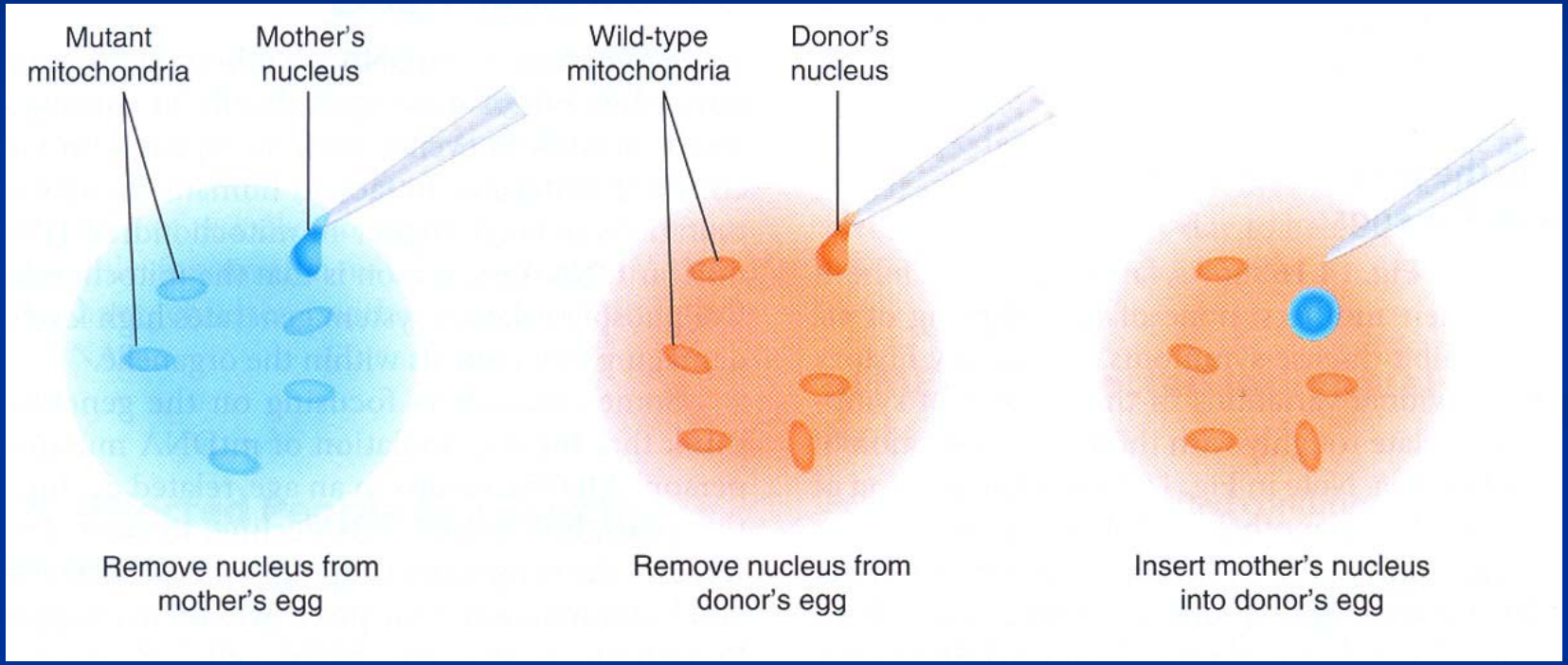


Fig. 14.18, 14.19