Mitochondria

A cellular organelle probably of endosymbiotic origin that resides in the cytosol of most nucleated (eurkaryotic) cells.

This organelle produces energy by oxidising organic acids and fats with oxygen by the process of **oxidative phosphorylation** and generates oxygen radicals (ROS)as a toxic by-product

Modern Views of Mitochondrial Functions in Mammalian Cells

1. Act as the power plant of the cell to sustain life of the human and animals

2. Take a center stage in the studies of mechanisms of electron transport and oxidative phosporylation

3. Major regulator of energy metabolism (via energy charge or phosphorylation potential).

4. Serve as an evolutionary link for higher organisms (conservation in the electron transport system and divergence in respiratory enzymes and mtDNA sequences).

5. A good model for the study of organelle biogenesis and inter-genomic communications between mitochondria and the nucleus

6. Play an important role in calcium homeostasis and calcium signaling of the cell

7. Permeability transition pore opening/closure in the mitochondrial membranes during the early phase of execution and regulation of cell death

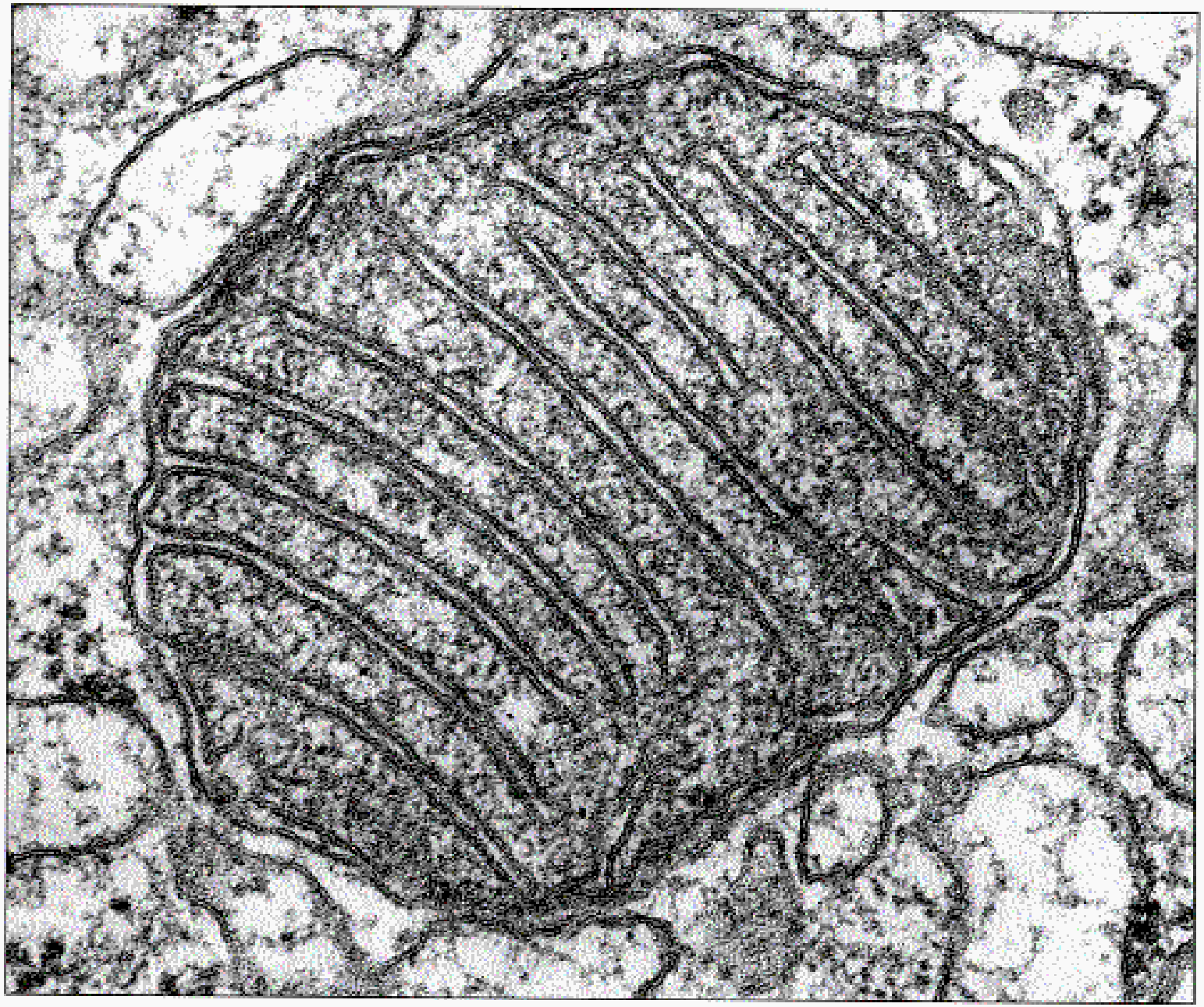
8. Mitochondrial genetics and mtDNA mutations in neuromuscular diseases, aging and age-related diseases

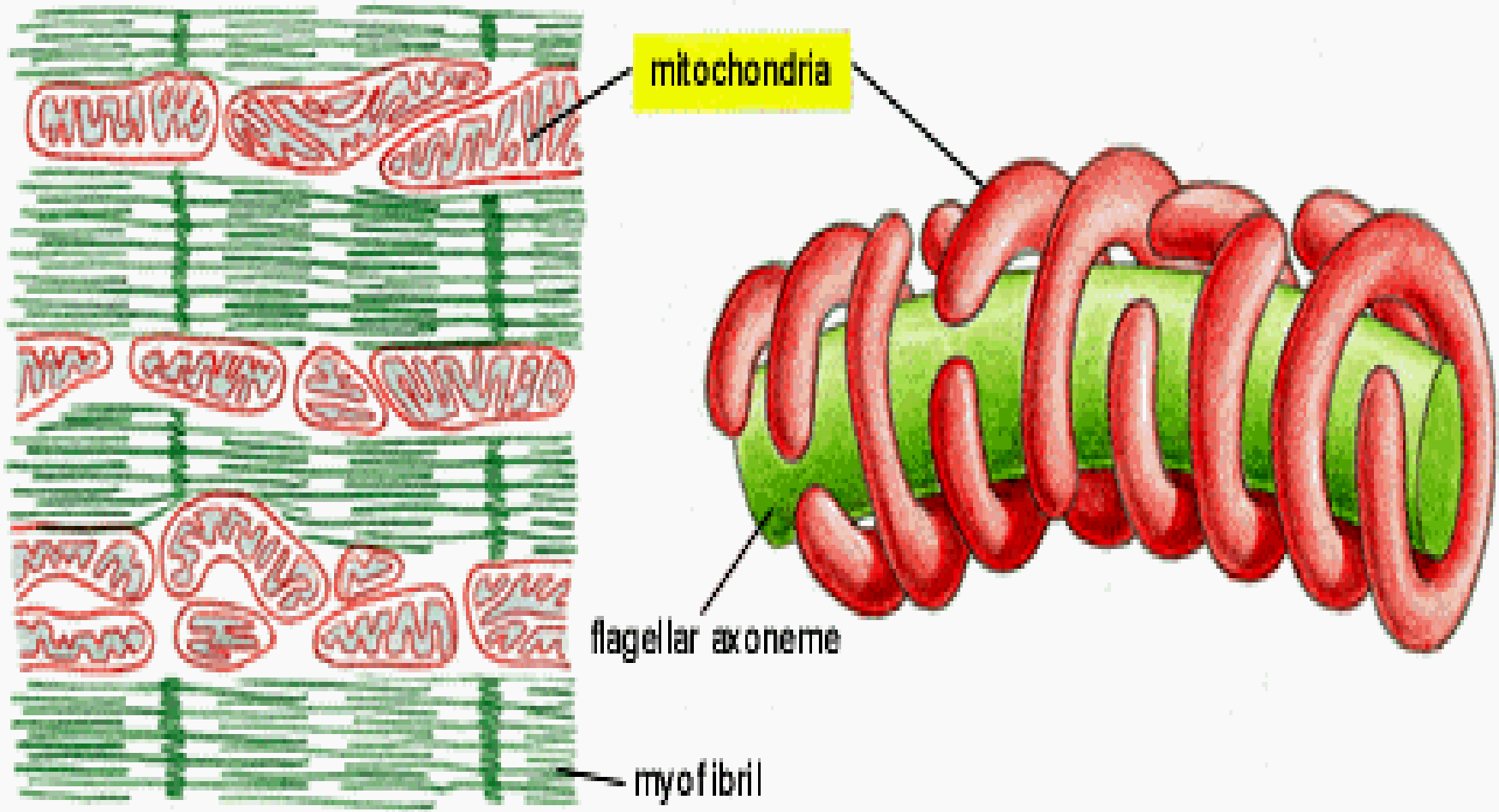
9. Proteins involved in the initiation and propagation of apoptosis

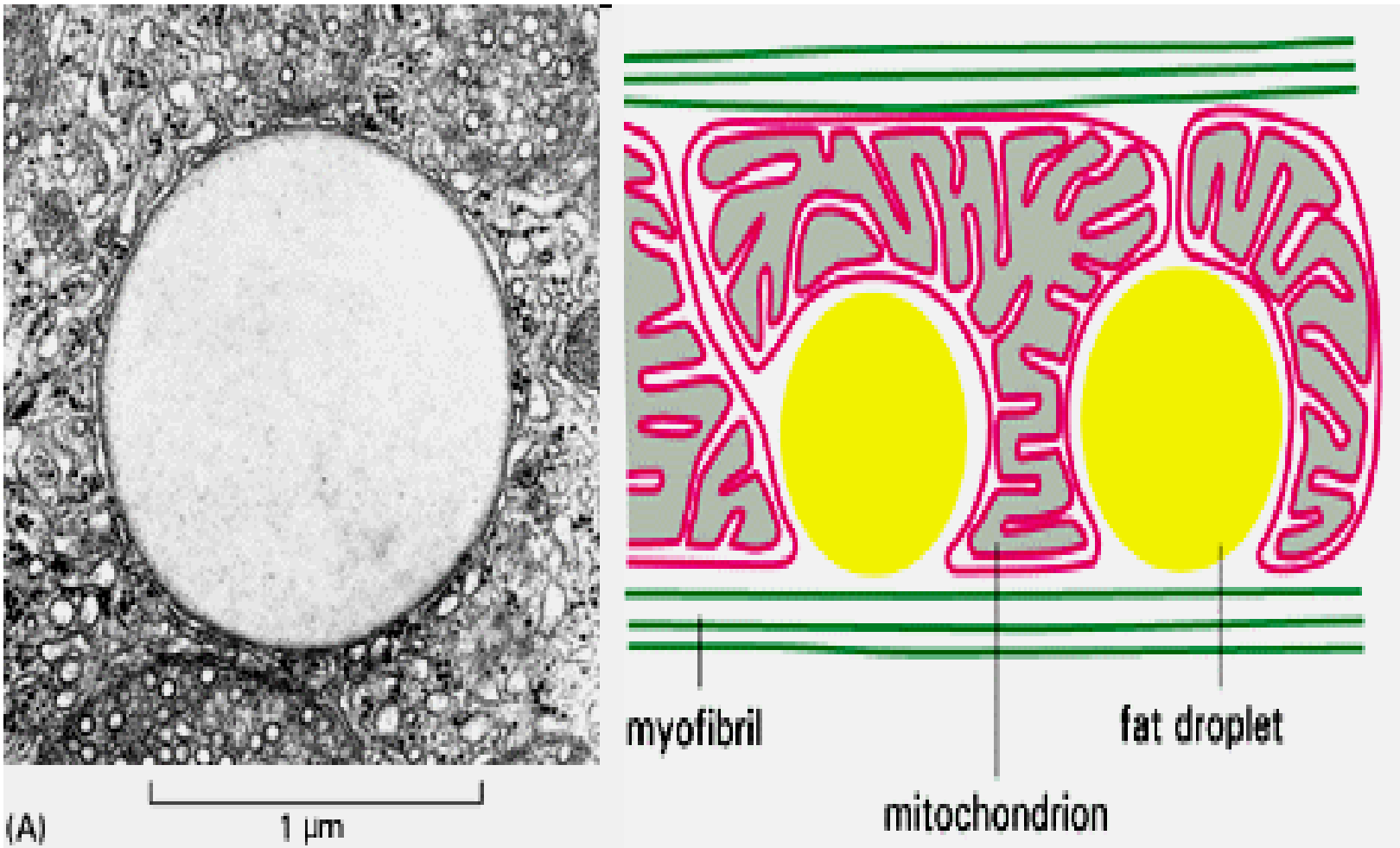
10. Act as an arbitrator, executioner, and regulator of life and death of the cell

11. The most sensitive intracellular organelles in response to physiological and pathological signals and oxidative stress

12. It provides a unique system for the study of intracellular dynamics during different physiological and pathological conditions


100 nm


mitochondria
flagellar axoneme
myofibril
CARDIAC MUSCLE
SPERM TAIL


(A)
1 µm
myofibril
mitochondrion
fat droplet

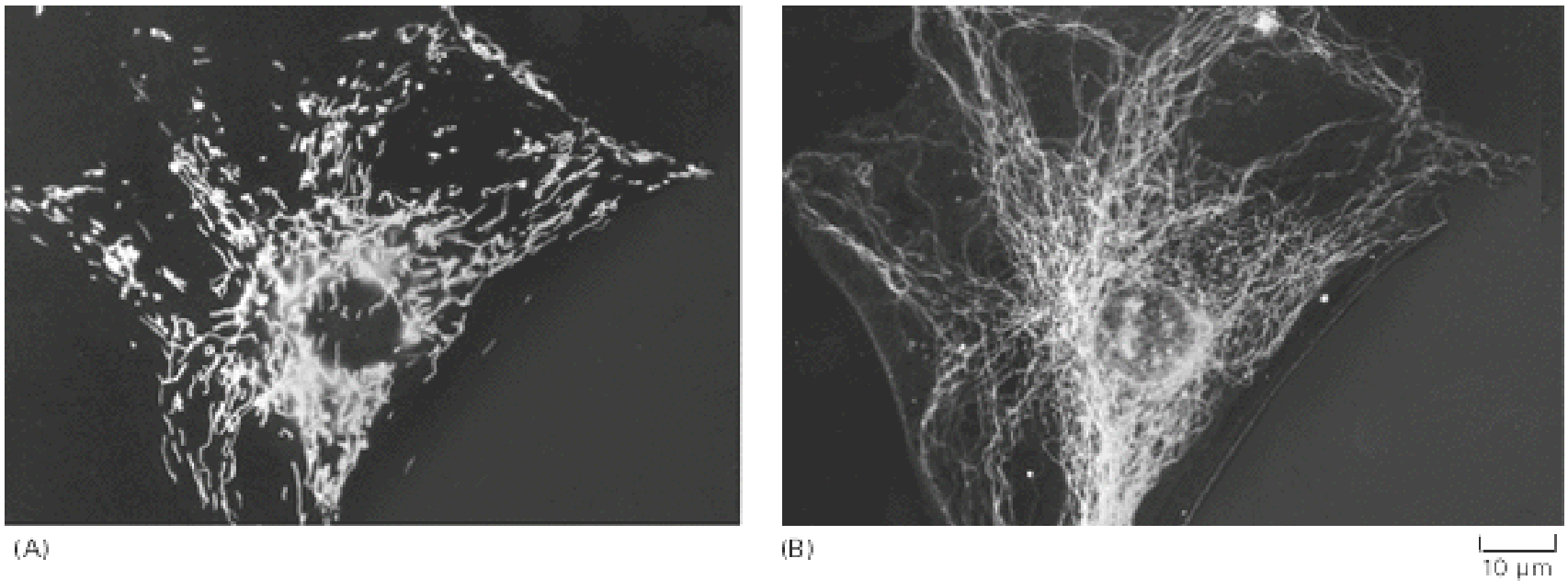
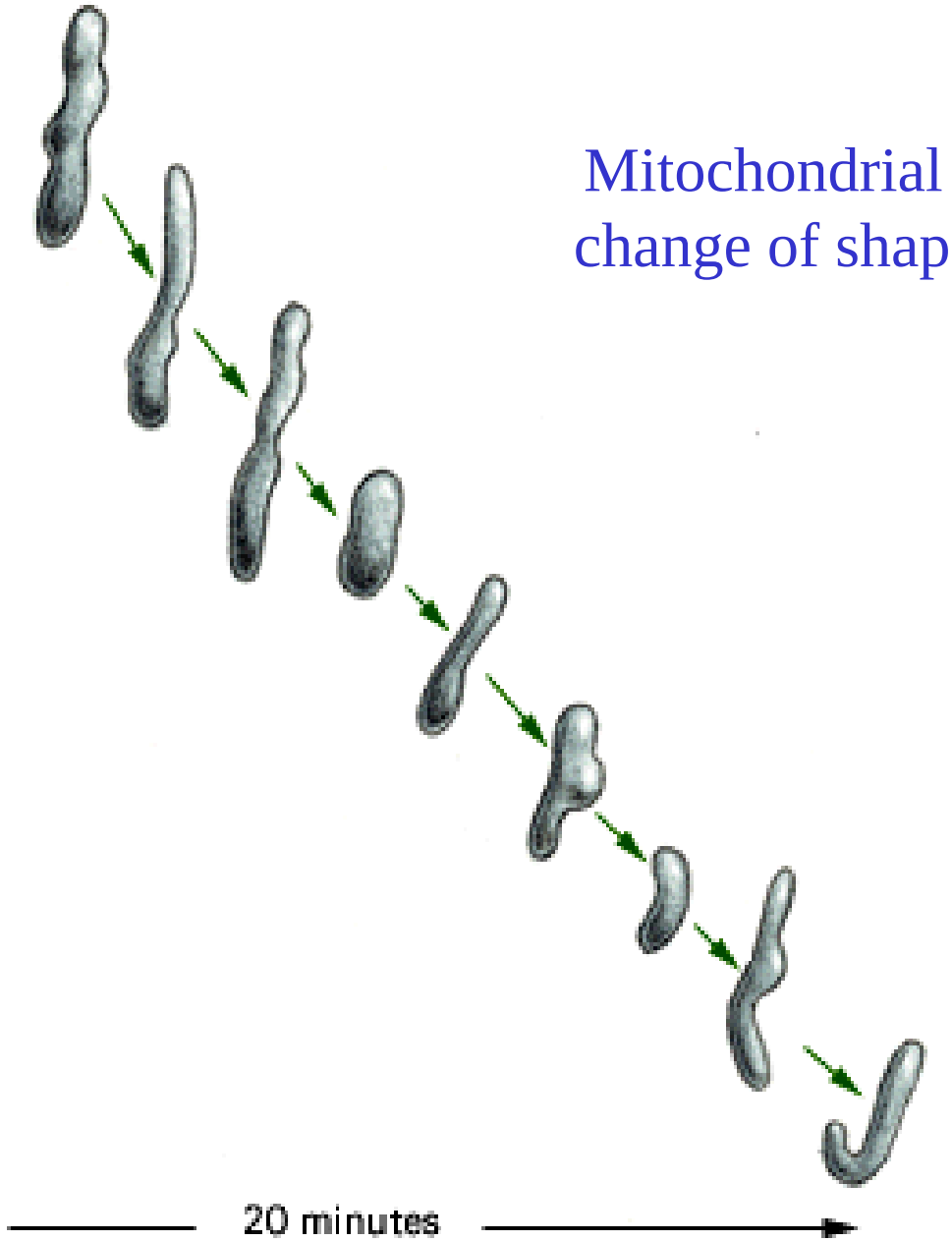


Figure 14-4. Relationship between mitochondria and microtubules. (A) Light micrograph of chains of elongated mitochondria in a living mammalian cell in culture. The cell was stained with a vital fluorescent dye (rhodamine 123) that specifically labels mitochondria. (B) Immuno-fluorescence micrograph of the same cell stained (after fixation) with fluorescent antibodies that bind to microtubules. Note that the mitochondria tend to be aligned along microtubules. (Courtesy of Lan Bo Chen.)

Mitochondrial plasticity: rapid change of shapes in living cells

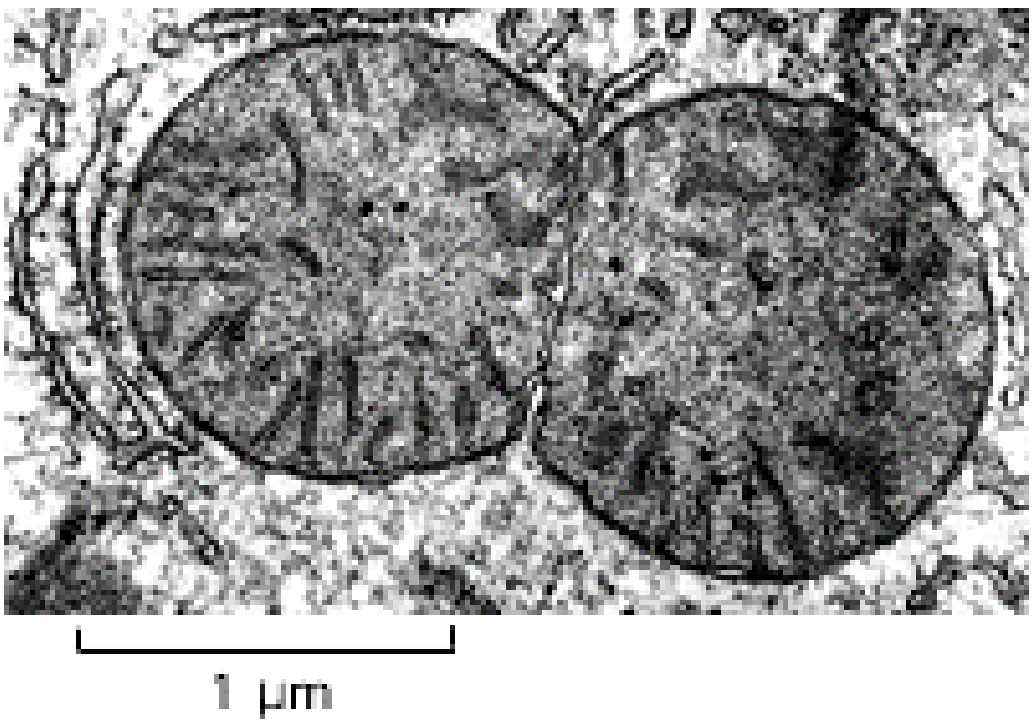


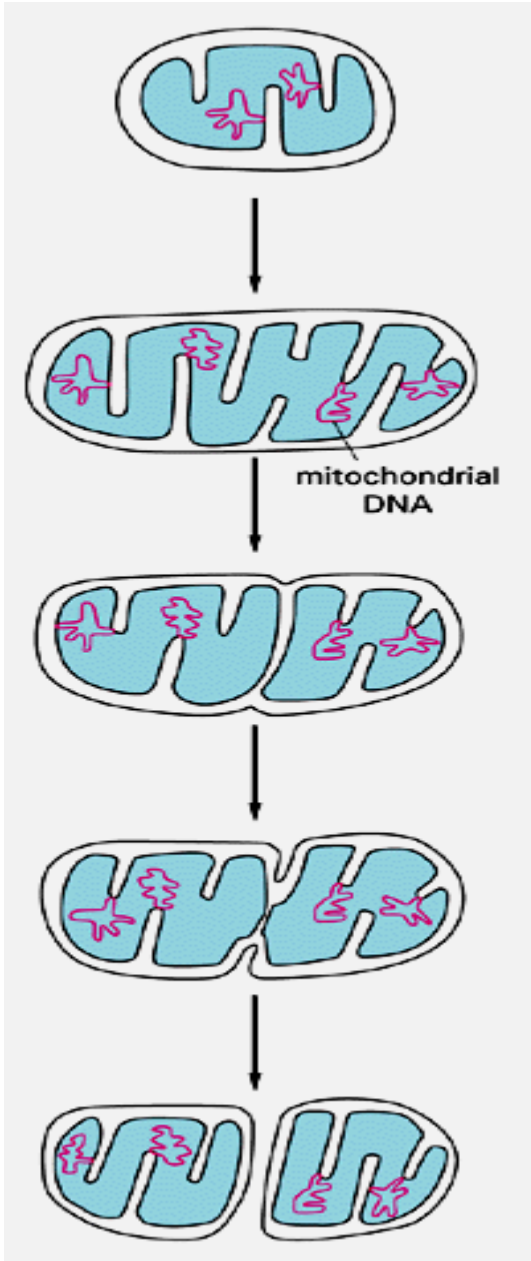
Number of Mitochondria per cell

•Most somatic cells	100-10,000
•Lymphocyte	1000
•Oocytes	100,000
•Sperm	few hundred

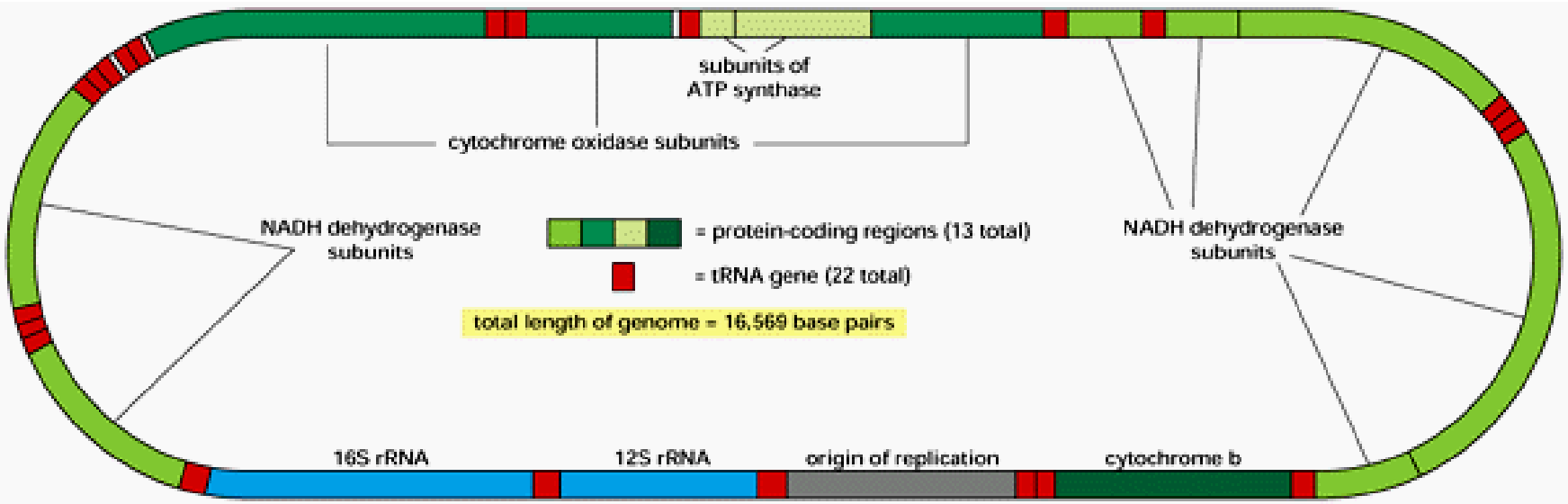
•No mitochondria in red cells and some terminally differentiated skin cells

Mitochondrial biogenesis





Mitochondrial genome



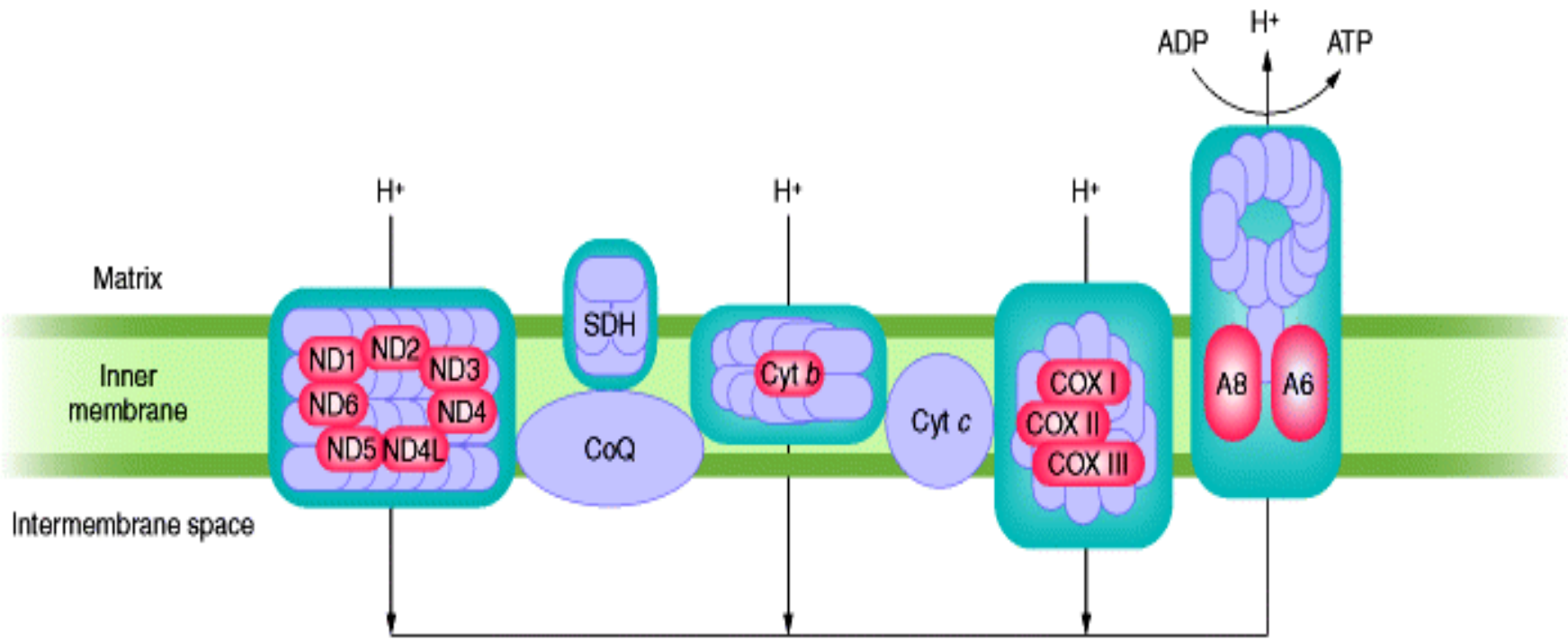
–22 tRNA genes

–13 polypeptide-encoding gene (for respiratory chain)

–2 ribosomal RNA genes

Two-way Communication and Coordination between Two Genomes

Coordinated Expression of Proteins by Genes in Both Nuclear and the Mitochondrial Genome



Subunits	Complex I	Complex II	Complex III	Complex IV	Complex V
nDNA-encoded	35	4	10	10	12
mtDNA-encoded	7	0	1	3	2

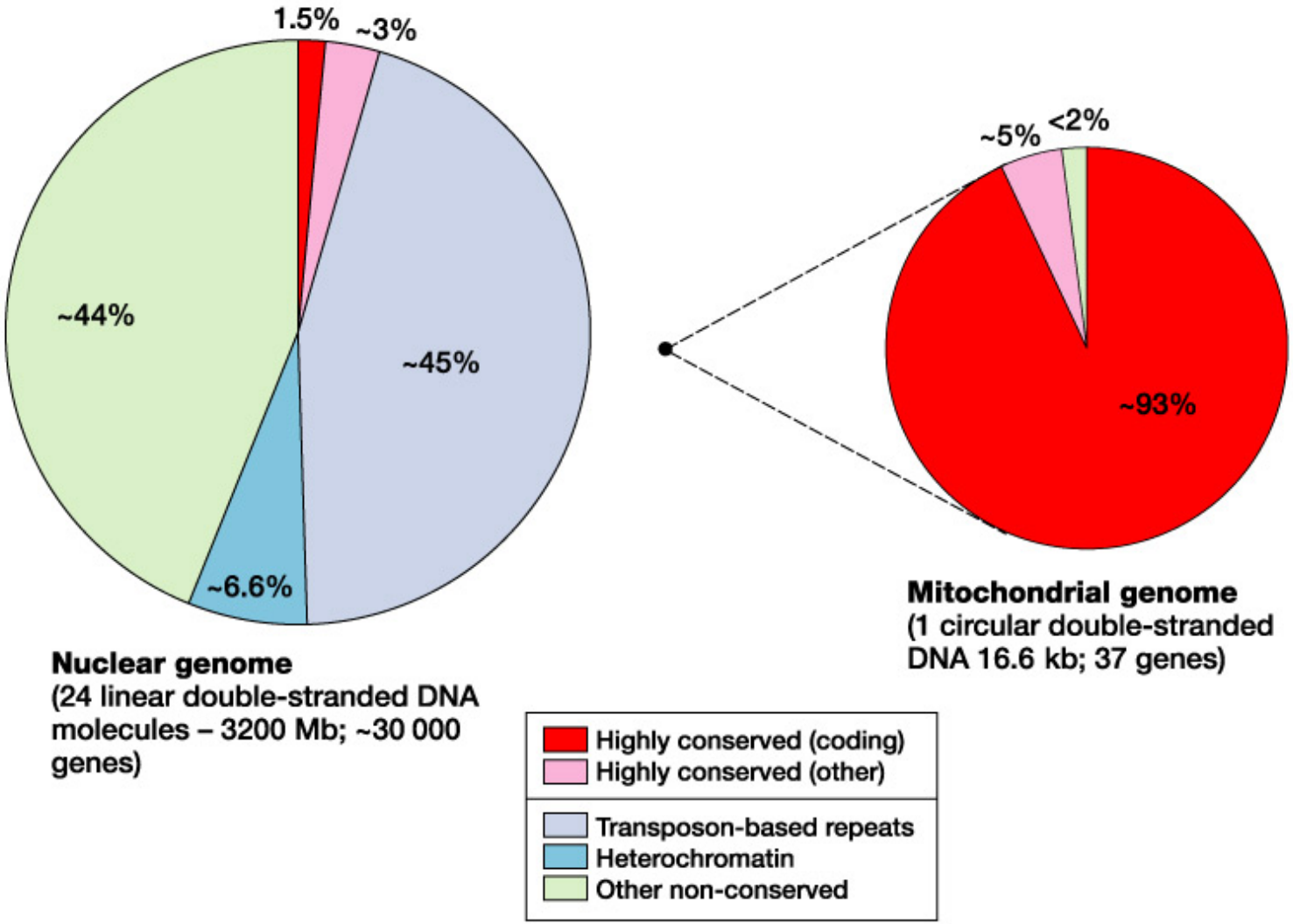
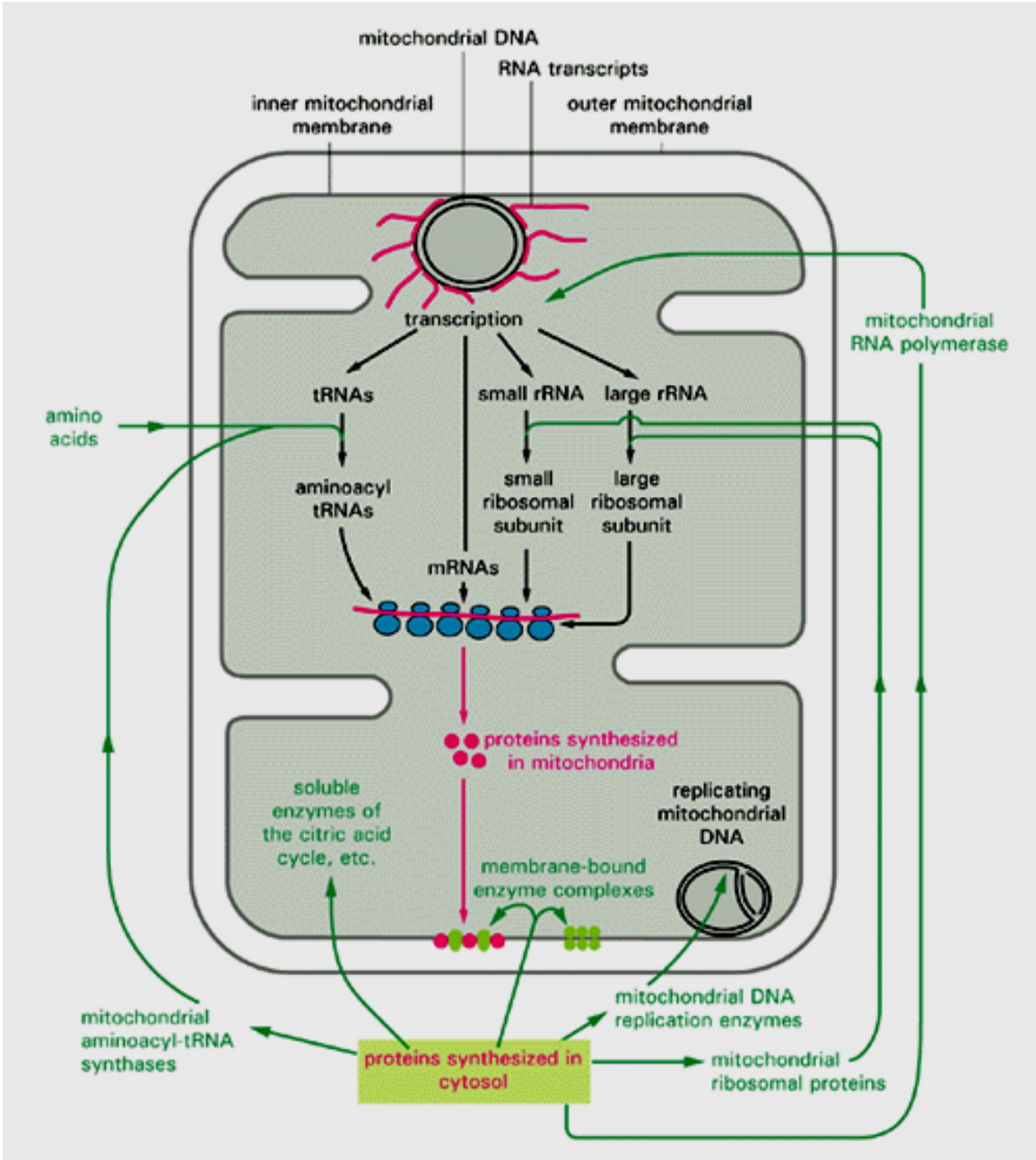


Figure 9-1 Human Molecular Genetics, 3/e. (© Garland Science 2004)

The human nuclear and mitochondrial genomes

	Nuclear Genome	Mitochondrial Genome
Size	3200 Mb	16.6 kb
No. of different DNA molecules	23 (in XX cells) or 24 (in XY cells); all linear	One circular DNA molecule
Total no. of DNA molecules per cell	46 in diploid cells, but varies according to ploidy	Often several thousands (but variable
Associated protein	Several classes of histone & nonhistone protein	Largely free of protein
No. of genes	~ 30 000 ~35-000	37
Gene density	~ 1/100 kb	1/0.45 kb


mitochondrial DNA
RNA transcripts
inner mitochondrial membrane
outer mitochondrial membrane
transcription
mitochondrial RNA polymerase
tRNAs
small rRNA
large rRNA
amino acids
aminoacyl tRNAs
small ribosomal subunit
large ribosomal subunit
mRNAs
proteins synthesized in mitochondria
soluble enzymes of the citric acid cycle, etc.
replicating mitochondrial DNA
membrane-bound enzyme complexes
mitochondrial DNA replication enzymes
mitochondrial aminoacyl-tRNA synthases
proteins synthesized in cytosol
mitochondrial ribosomal proteins

“Mitochondrial Diseases” = Respiratory Chain Disorders

- More than 40 known types
- Mitochondrial disease is a difficult disorder to identify because it can take many forms, and range from mild to severe
- The problems it causes may begin at birth or not occur until later in adult life.
- It is estimated that mitochondrial disease affects between 40,000 and 70,000 Americans, occurring in one in 2,500 to 4,000 births.

Modes of inheritance of RC defects

- Maternal - mtDNA point mutations & duplications
- Sporadic - mtDNA single deletions
- Autosomal Recessive - subunit genes, SURF1, etc
- Autosomal Dominant - multiple mtDNA deletions
- X-linked - DDP1, ABC7, (Barth syndrome)
- Complex - LHON

- In most diagnoses, we don’t know!

The clinical features of mitochondrial disease 1: "Classical" presentation

- MELAS (Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes)
- MERRF (Myoclonic Epilepsy with Ragged Red Fibres)
- Leber Hereditary Optic Neuropathy (LHON)
- External Ophthalmoplegia
    - Kearns-Sayre syndrome
    - Chronic progressive external ophthalmoplegia
- NARP (Neurogenic weakness Ataxia with Retinitis Pigmentosa)

Clinical Features of Mitochondrial disease 2: Features suggestive of mitochondrial illness but not classical syndrome

Neuromuscular

- External opthalmoplegia
- Ptosis
- Deafness
- Neuropathy
- Migraine
- Seizures
- Encephalomyopathy
- Dementia
- Ataxia
- Stroke-Like episodes
- Spastic paraparesis
- Neuropathy
- Pigmentary retinopathy
- Optic atrophy
- Dystonia

Cardiac
HCOM
Heart Block

Endocrine
Diabetes Mellitus
Hypogonadism
Infertility

GIT
Dysphagia
Vomiting
Pseudo-obstruction
Pancreatic failure
Liver failure

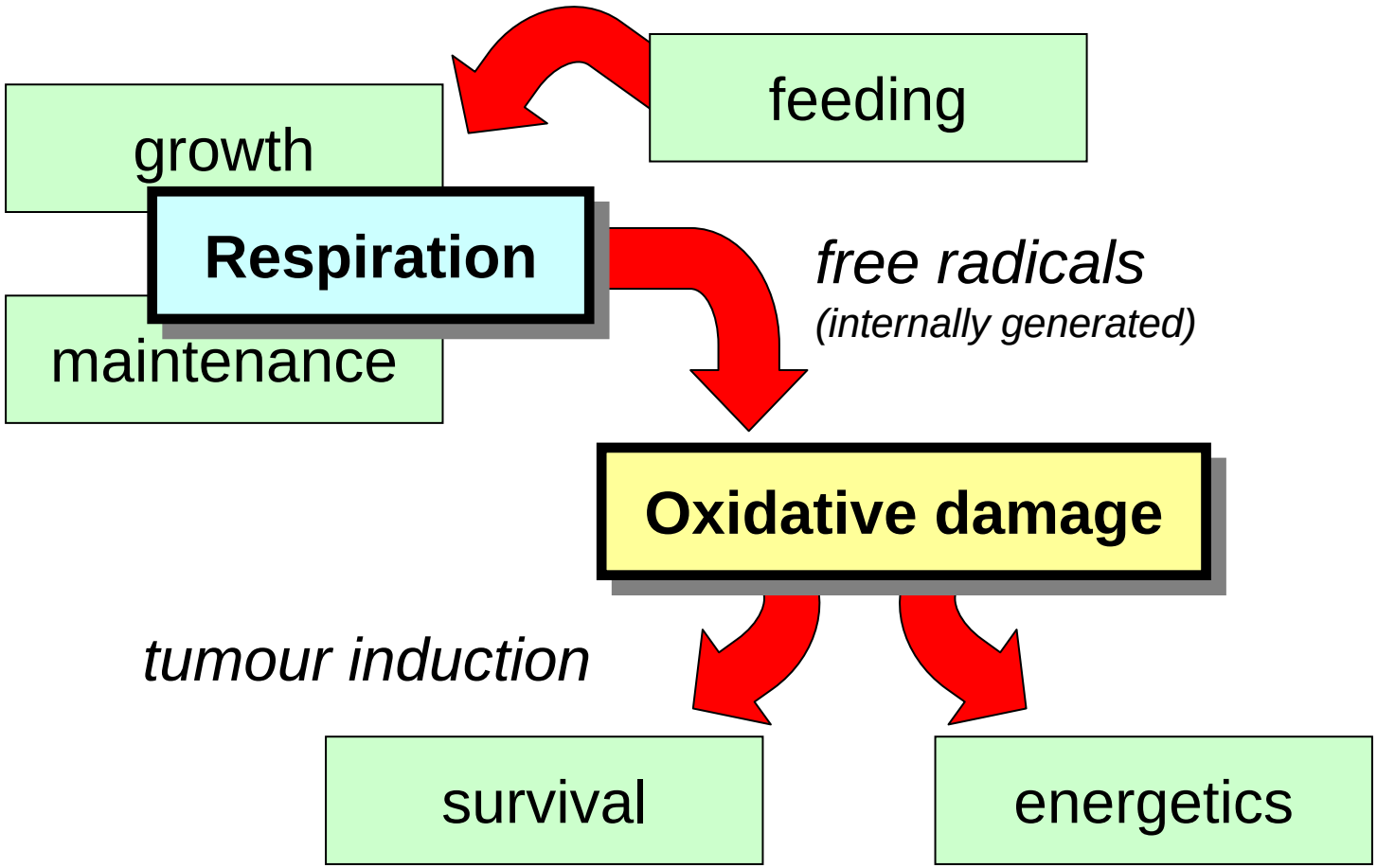
Haematological
Sideroblastic anaemia
Pancytopenia

Psychiatric
Depression
Psychosis

Dermatological
Lipomatosis

Renal
Aminoaciduria
Tubule dysfunction
Fanconi syndrome

Intracellular reactive oxygen species



Major sources of Reactive Oxygen Species (ROS)

- Mitochondria (Electron Transport Chain)
- Peroxisomal β–oxidation system
- Xanthine and glucose oxidases
- Cyclooxygenases
- Plasmamembrane-localized NAD(P)H oxidoreductase complex

Mitochondria and ROS

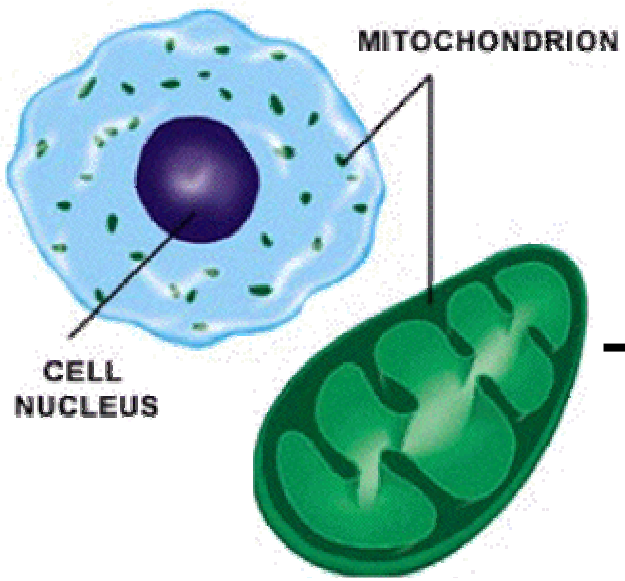
Reactive oxygen species (ROS): hydroxyl radical, superoxide, hydrogen peroxide
Reactive nitrogen species (RNS)

Reactive Oxygen Species (ROS)

$^1O_2$ $^{\bullet}OH$ $O_2^{\bullet -}$ H_2O_2

Reactive Nitrogen Species (RNS)

NO ONOO N_2O_3



ROS (H_2O_2, $^{\cdot}O_2^{\cdot}$, $^{\cdot}OH$)

↓

Oxidative damage

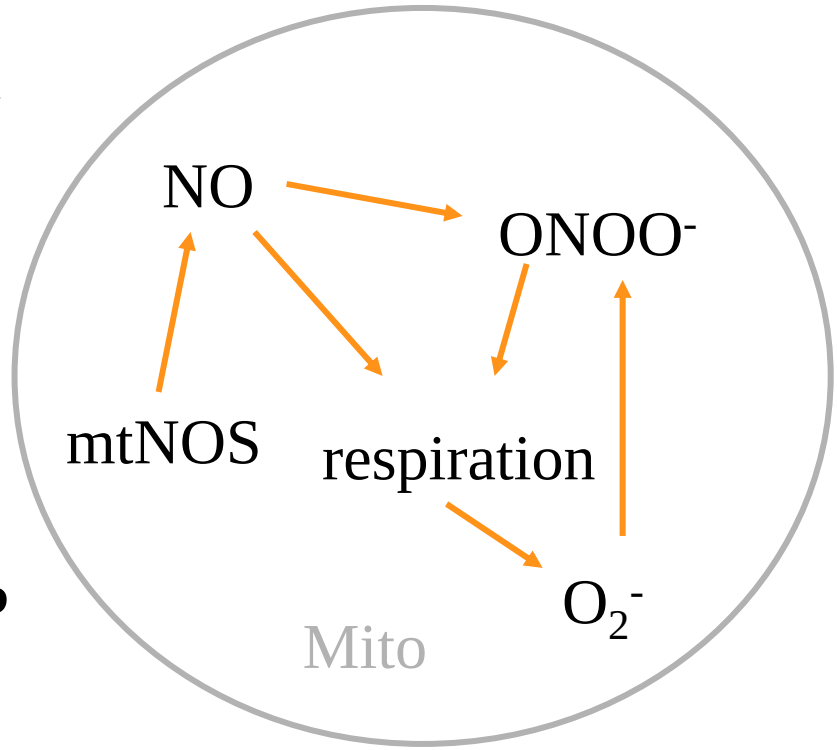
↓

Heart disease and aging

Source: http://onlinetc.its.brooklyn.cuny.edu/ Core81/chap3.html

NO in mitochondria?

- Reversibly inhibits respiration (complex IV)
- Reacts with superoxide to form $ONOO^-$
- Irreversibly inhibits respiration (complex II-III)
- Is there a mitochondrial NOS?



"Radical" Reactions

$O_2 + e \Leftrightarrow O_2^{\bullet-}$ Equation 1

$O_2 + 2e + 2H^+ \rightarrow H_2O_2$ Equation 2

$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow SOD \rightarrow H_2O_2 + O_2$ Equation 3

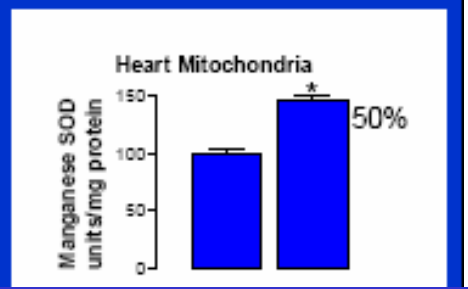
$H_2O_2 + 2GSH \rightarrow GPX \rightarrow H_2O + GSSG + ROH$ Equation 4

$2H_2O_2 + \rightarrow Catalase \rightarrow 2H_2O + O_2$ Equation 5

$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + HO^- + Fe^{3+}$ Equation 6

$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$ Equation 7

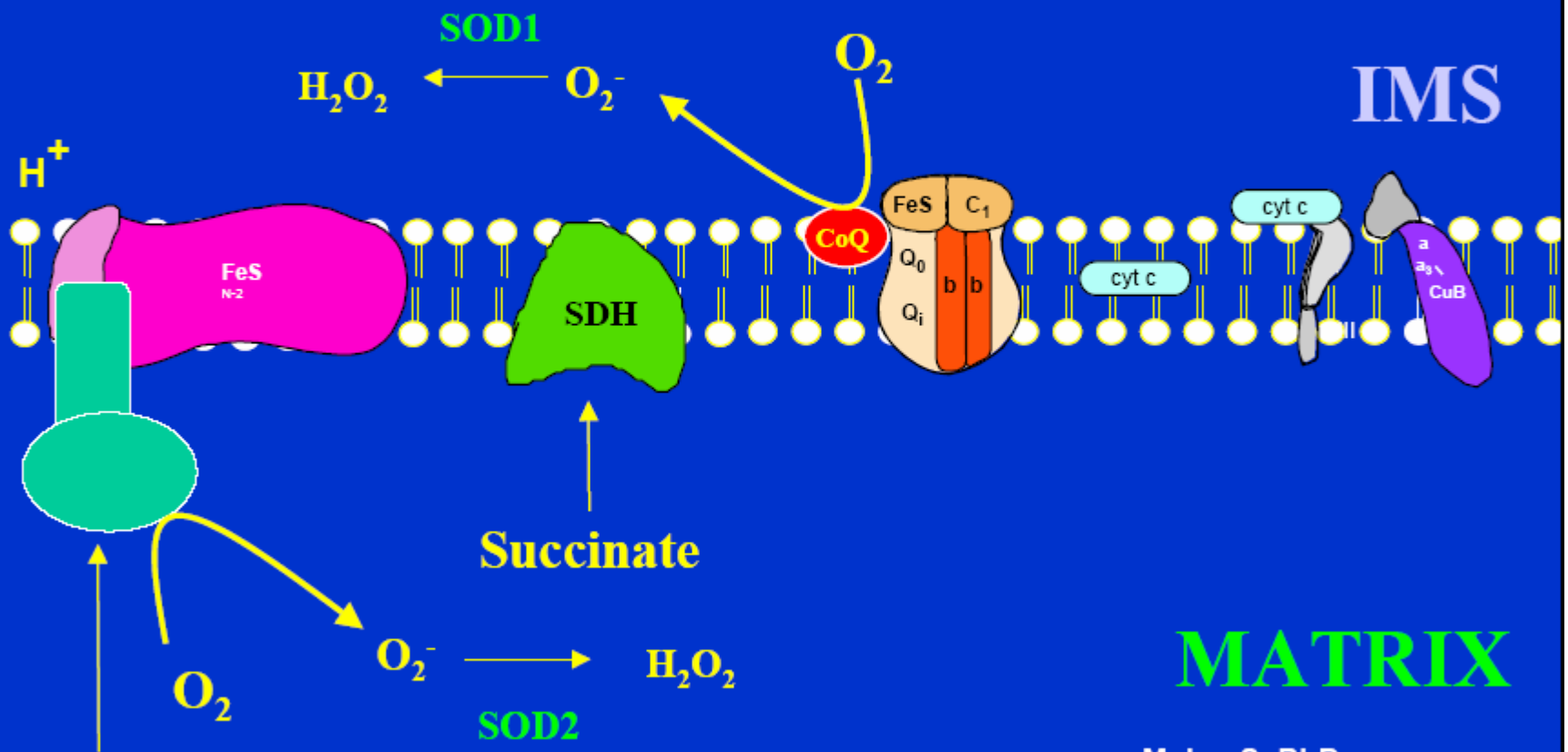
$O_2^{\bullet-} + M^{n+} \rightarrow O_2 + M^{(n-1)+}$ Equation 8

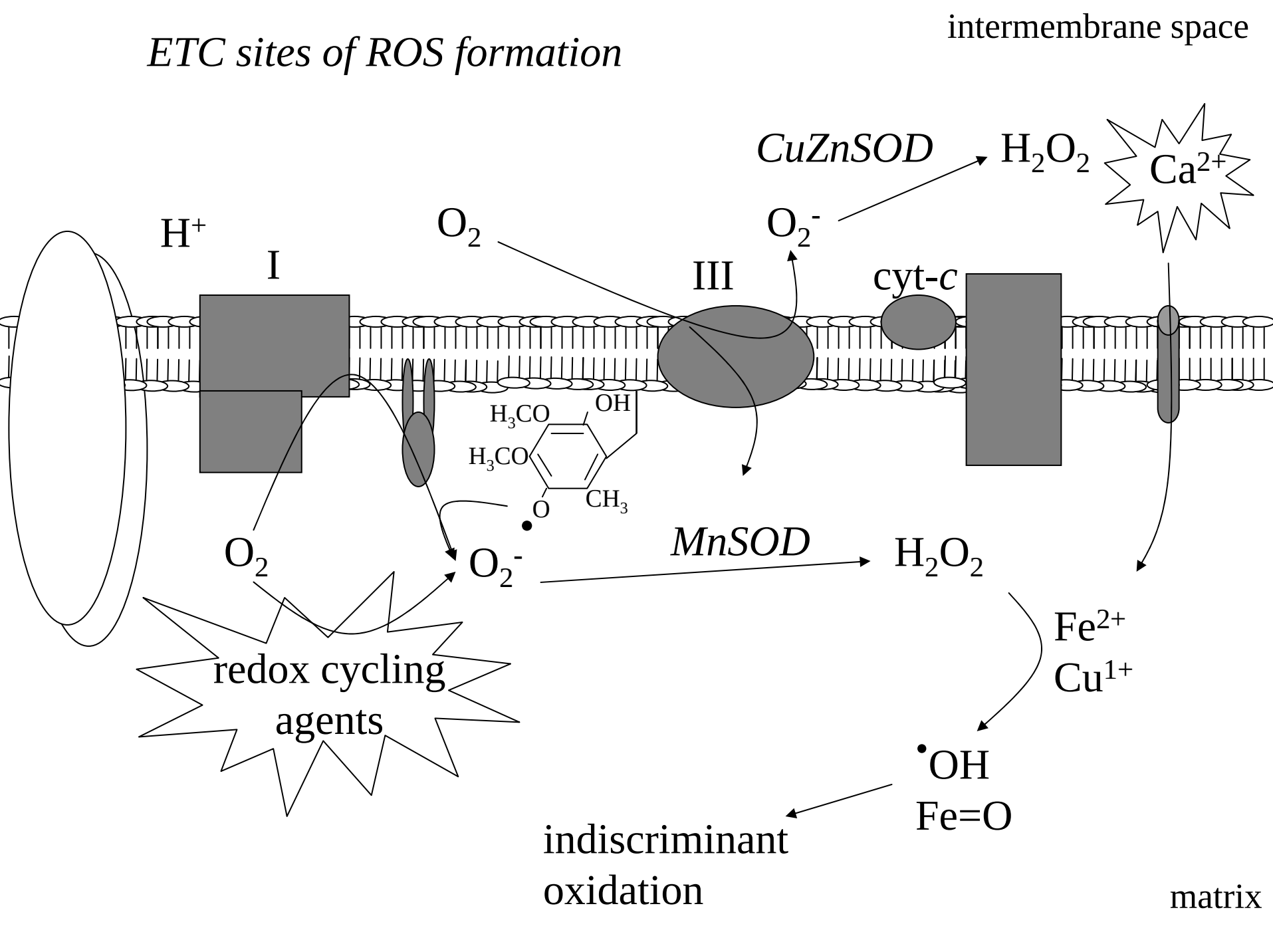


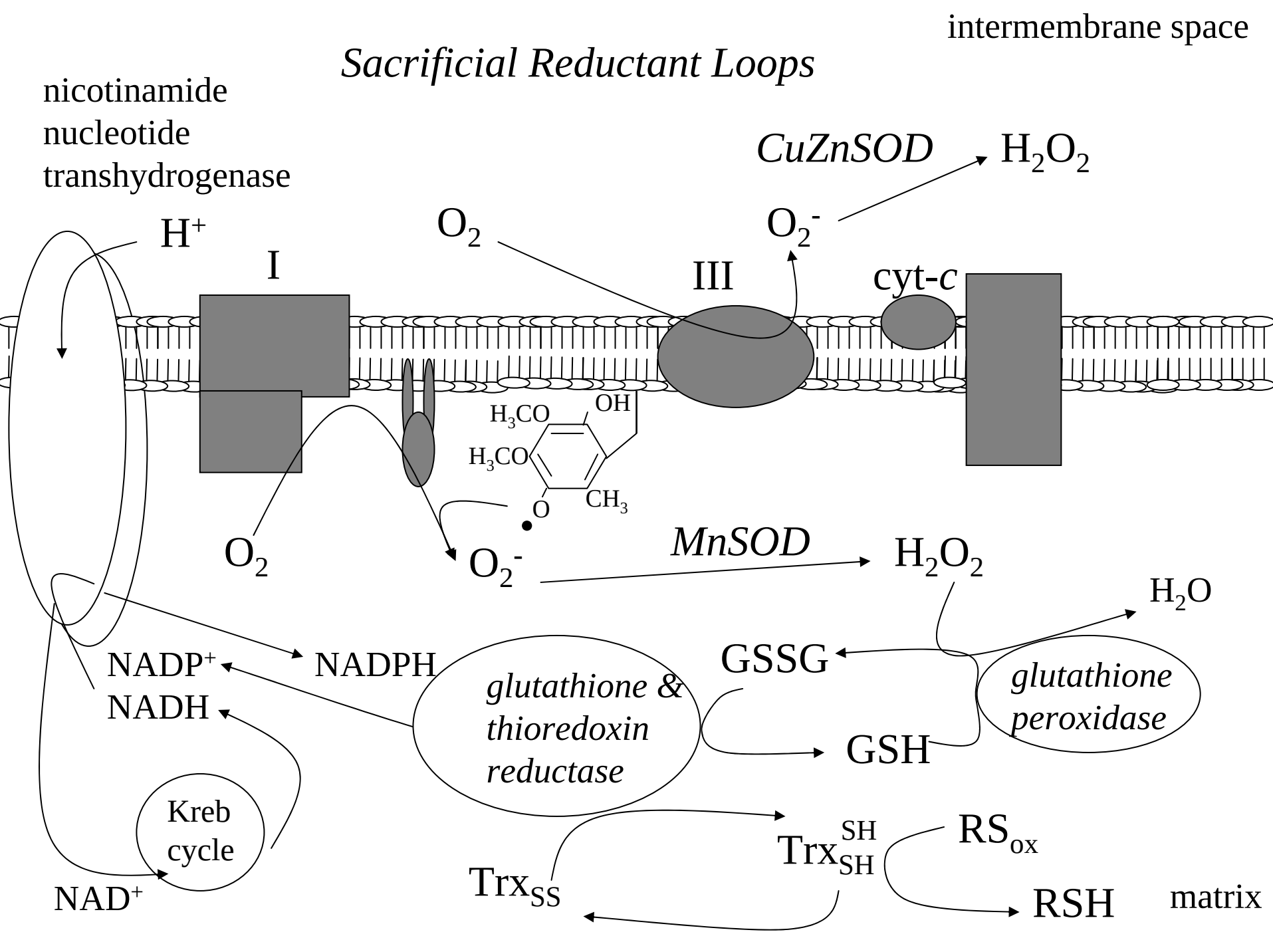
Primary antioxidants metabolizing ROS

- Glutathione (GSH) and related enzymes (peroxidases, reductases, S-transferases, and efflux pumps)
- Thioredoxin and Peroxiredoxin families
- Catalase
- Superoxide dismutases (SODs)

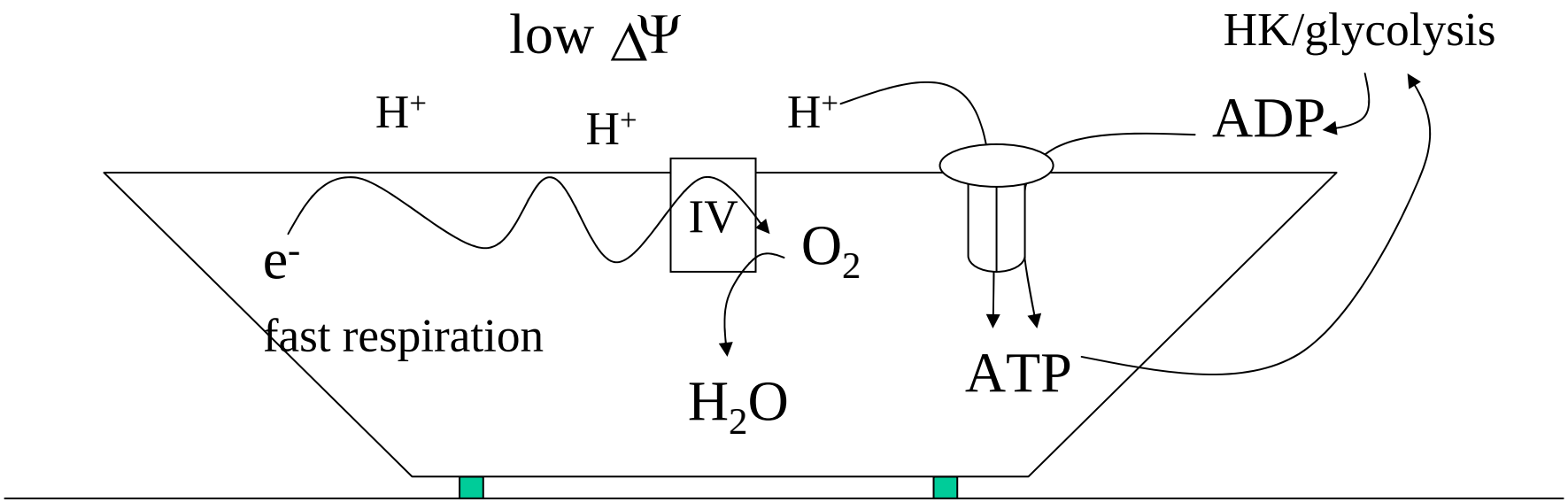
Reactive Oxygen species and the Respiratory Chain



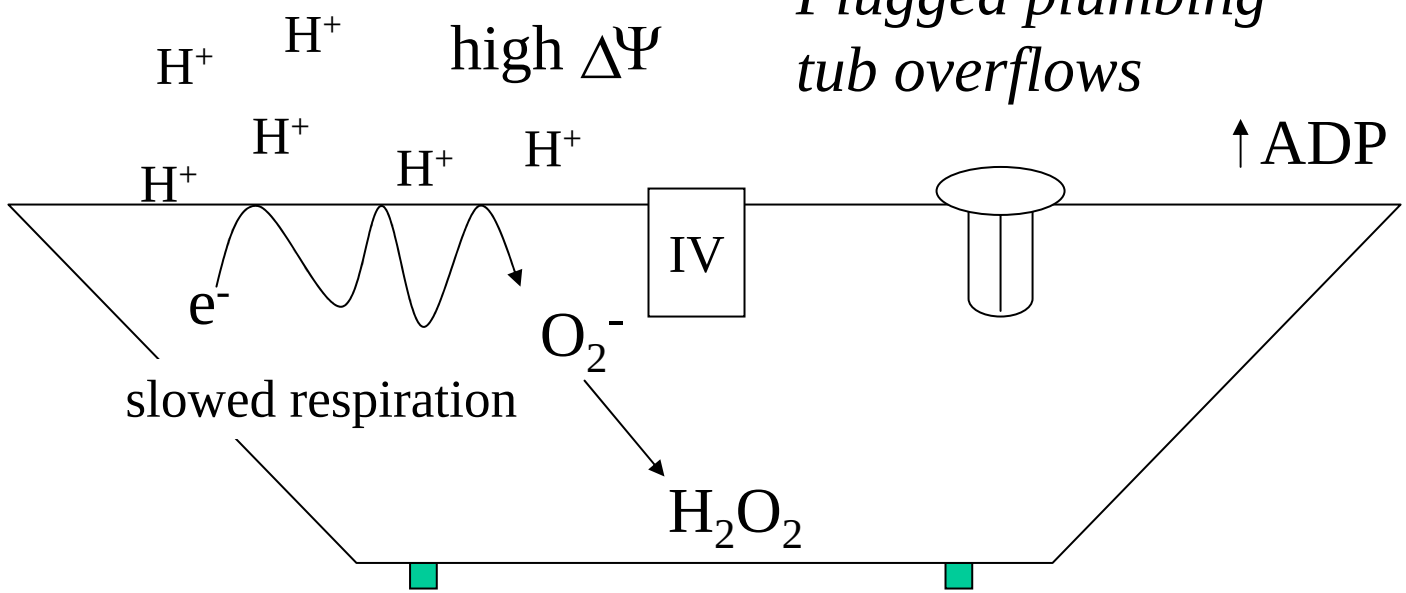

ETC sites of ROS formation
intermembrane space
CuZnSOD
H2O2
Ca2+
H+
O2
O2-
I
III
cyt-c
H3CO
OH
H3CO
CH3
O
O2
O2-
MnSOD
H2O2
Fe2+
Cu1+
redox cycling
agents
•OH
Fe=O
indiscriminant
oxidation
matrix


intermembrane space
Sacrificial Reductant Loops
nicotinamide nucleotide transhydrogenase
CuZnSOD
H2O2
O2-
H+
O2
I
III
cyt-c
H3CO
OH
H3CO
CH3
O
O2
O2-
MnSOD
H2O2
H2O
NADP+
NADPH
NADH
glutathione & thioredoxin reductase
GSSG
GSH
glutathione peroxidase
Kreb cycle
NAD+
TrxSS
TrxSH SH
RSox
RSH
matrix

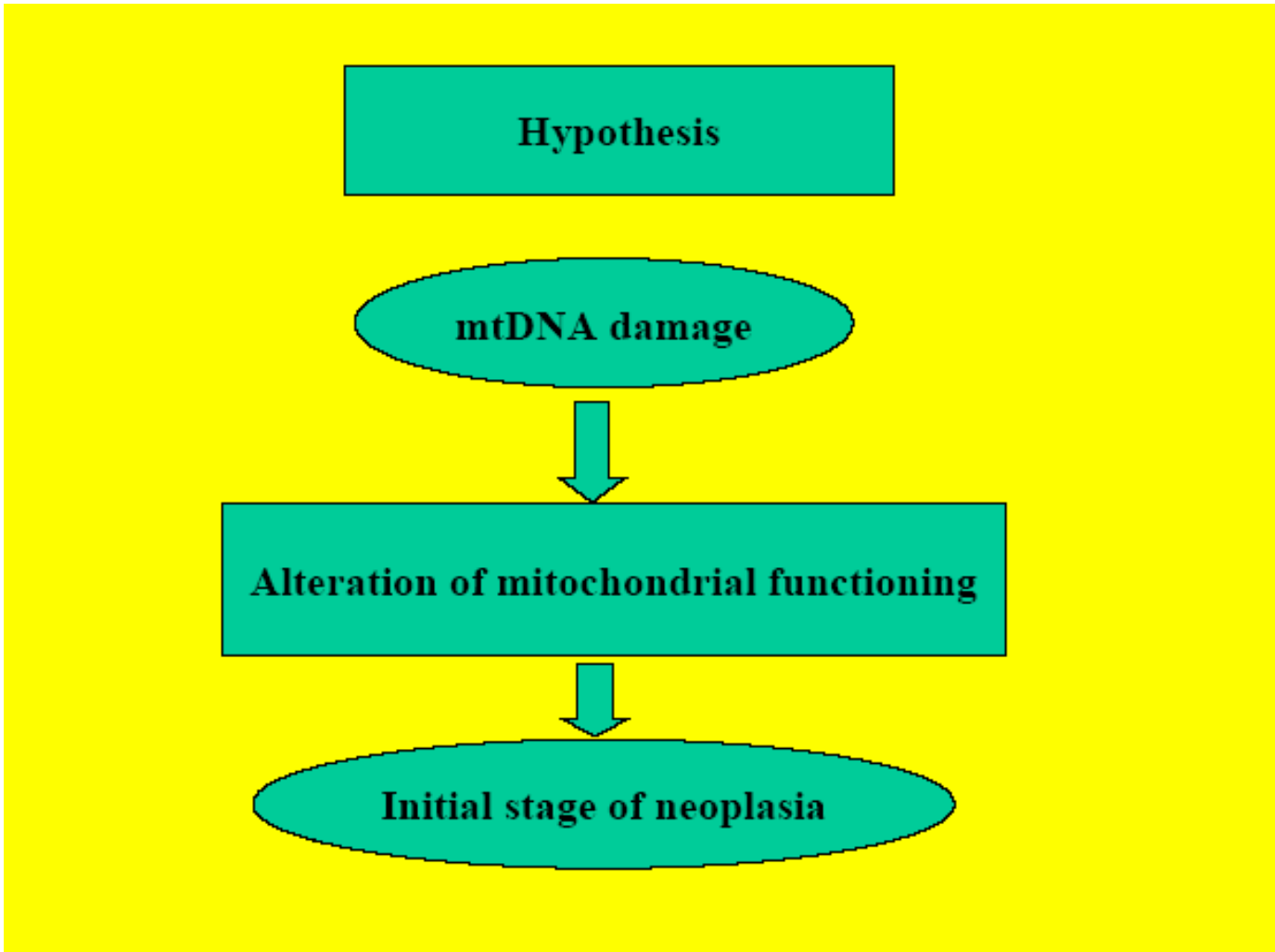
ETC ROS formation and the bathtub



Plugged plumbing tub overflows



mtDNA damage



mtDNA

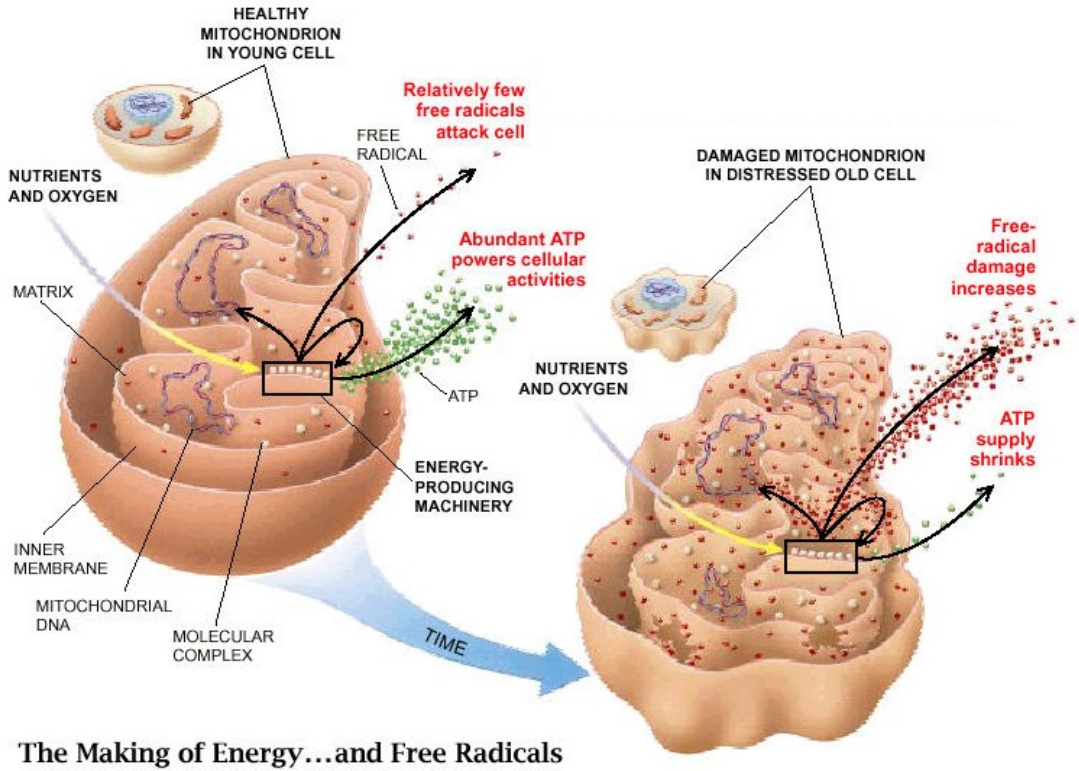
- Lack of protective histones
- Limited capacity for repair (DNA polymerase γ)
- Lack of introns (>> probability that a mutation will be detrimental)

mtDNA: an attractive target for pathological damage

- Proximity of mtDNA to the site of ROS generation
- Mitochondrial genome is highly sensitive to genotoxic agents

Amplification mechanisms

1) Affected mitochondria produce more ROS



Weindruch R 1996 Caloric restriction and aging. *Scientific American* **231**, 46-52**.**

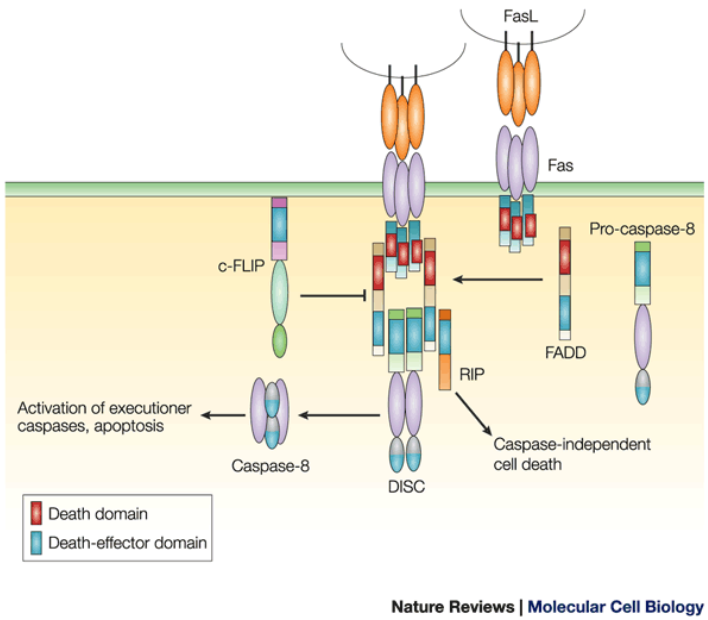
2) Affected mitochondria grow and degrade at different rates

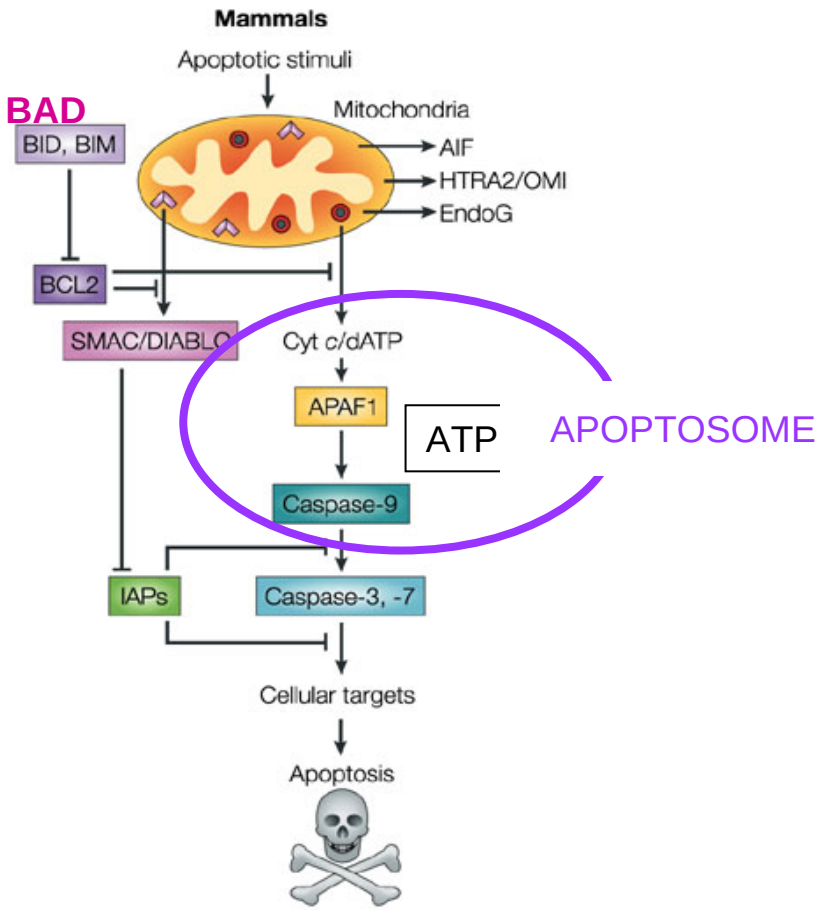
- **Kowald A 2001** The mitochondrial theory of aging, *Biological Signals & Receptors* **10,** 162-175.

- **Kowald A & Kirkwood TBL 2000** Accumulation of defective mitochondria through delayed degradation of damaged organelles and its possible role in the aging of post-mitotic cells. *Journal of Theoretical Biology* **202**, 145-160.

Cancer: evade apoptosis

- Fas/TNFa extrinsic pathway for apoptosis
- Mitochondrial intrinsic pathway
- Both pathways have caspases in common
- Ironically, oncogenes can also induce apoptosis





Growth & death signal