Membrane Biochemistry

Lectures by

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Lectures in Membrane Biochemistry

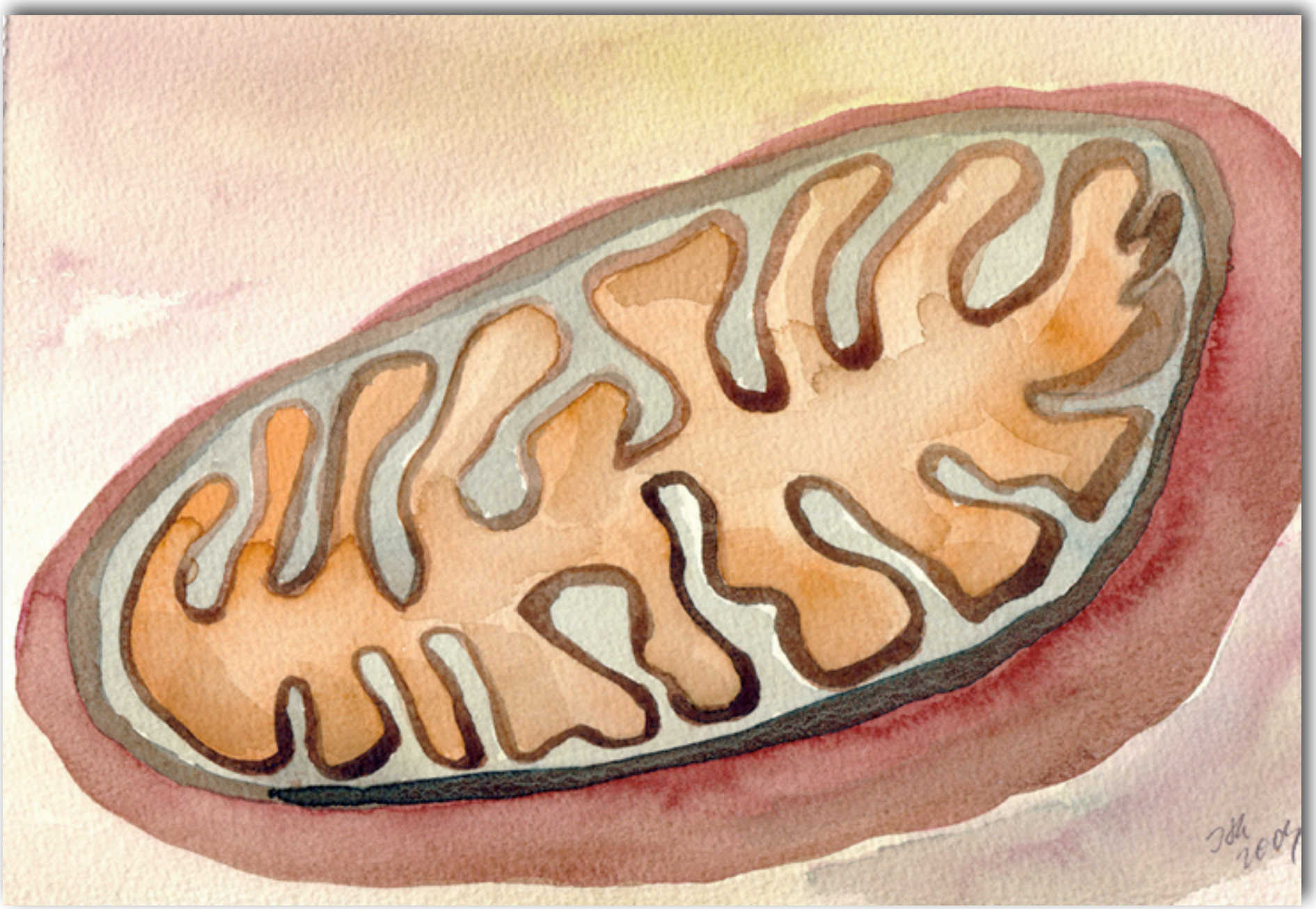
- The endomembrane system - endocytosis and exocytosis (Acrobat, .pdf file)
- The endomembrane system - vesicular transport and protein trafficking (Acrobat, .pdf file)
- Transport across membranes 1 - Proteins (Acrobat, .pdf file)
- Transport across membranes 2 - Small molecules and ions (Acrobat, .pdf file)

Course web pages

Membrane Biochemistry web pages

General reference

Cell and Molecular Biology: Concepts and Experiments
Gerald Karp. Fifth Edition 2008. John Wiley & Sons Inc.



Membrane Biochemistry

Bioenergetics

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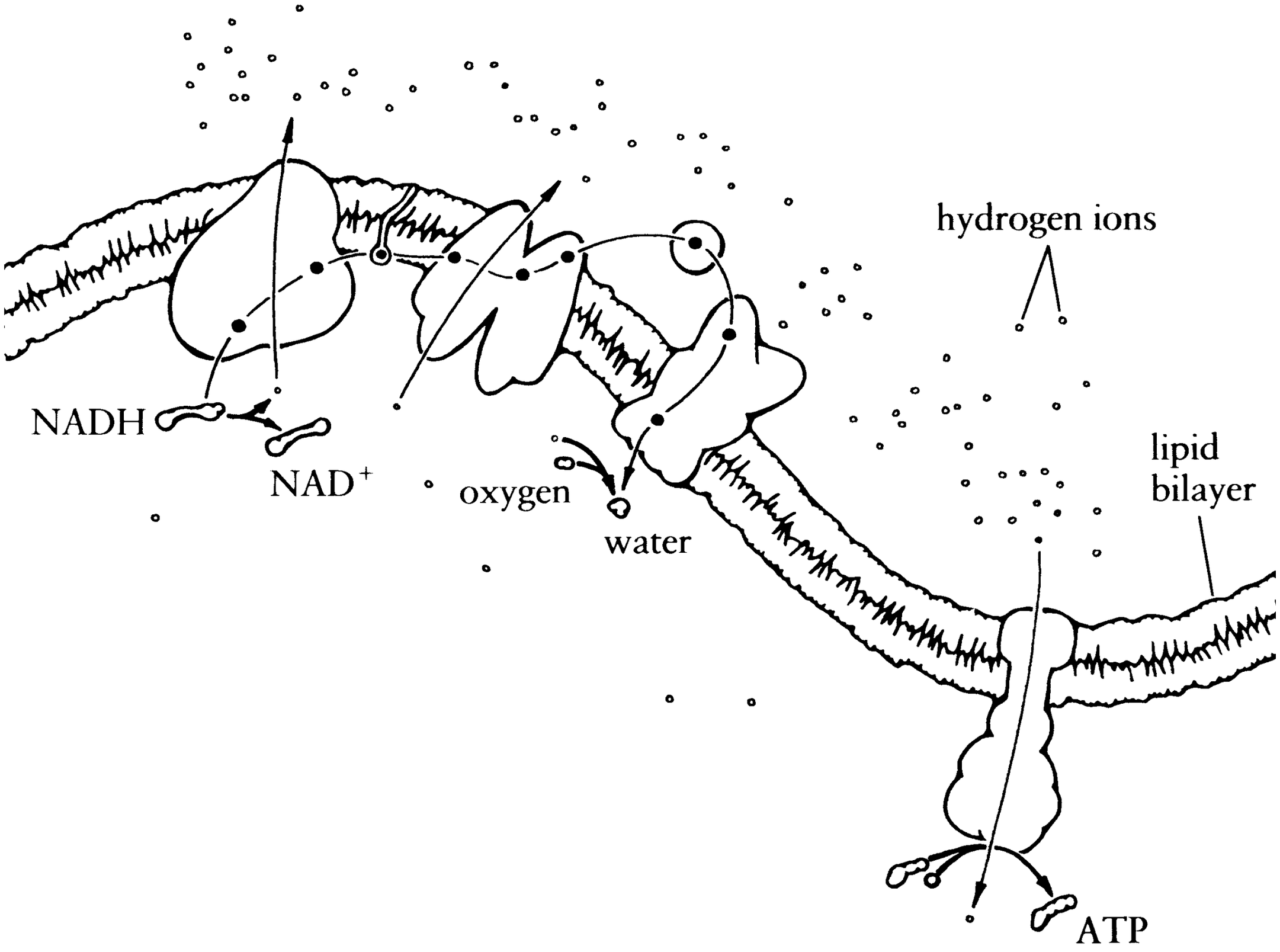
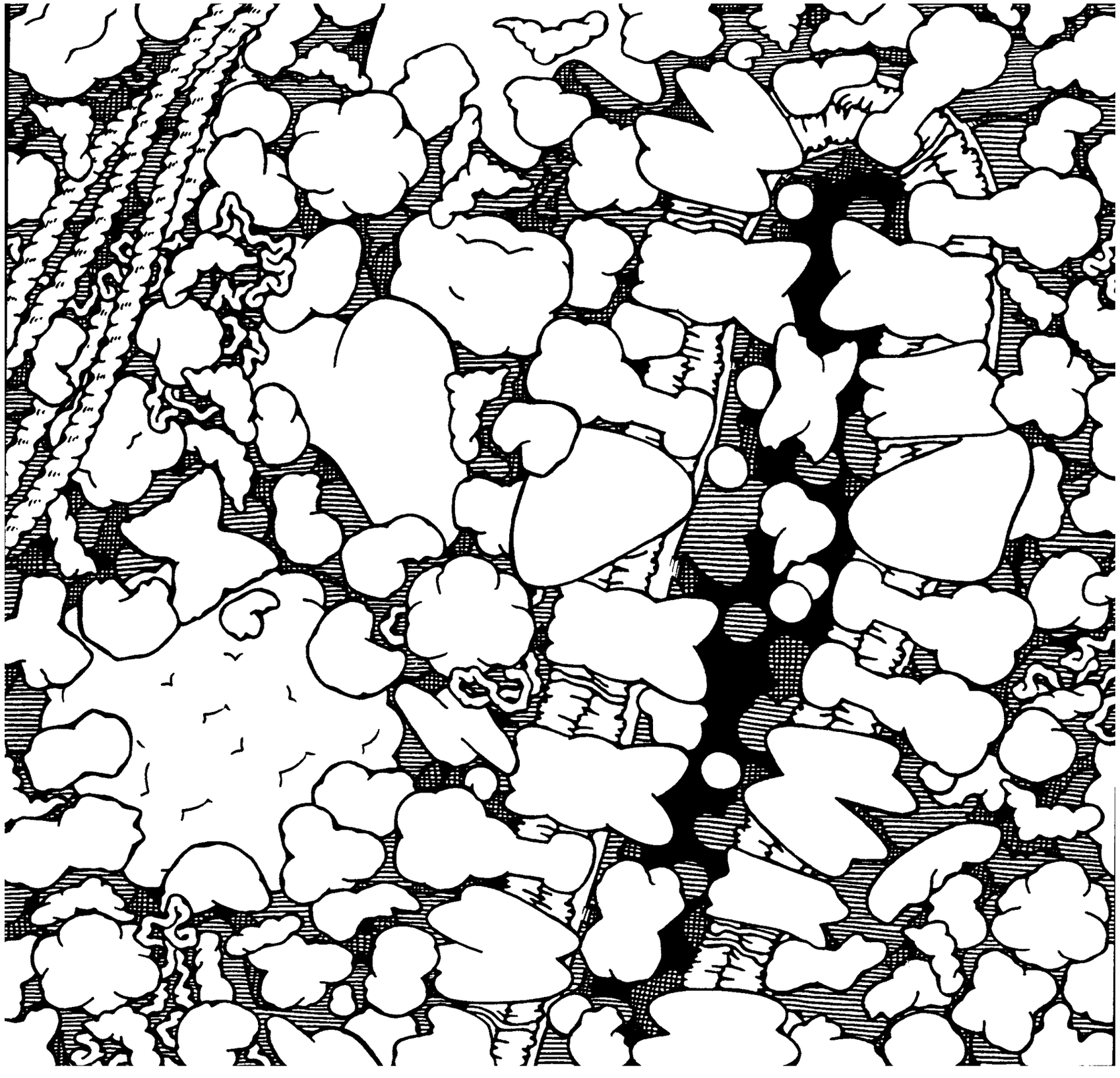

hydrogen ions
NADH
NAD+
oxygen
water
lipid
bilayer
ATP



Allen, J. F. (2003) Phil. Trans. R. Soc. B458, 19-38

Inter-membrane space

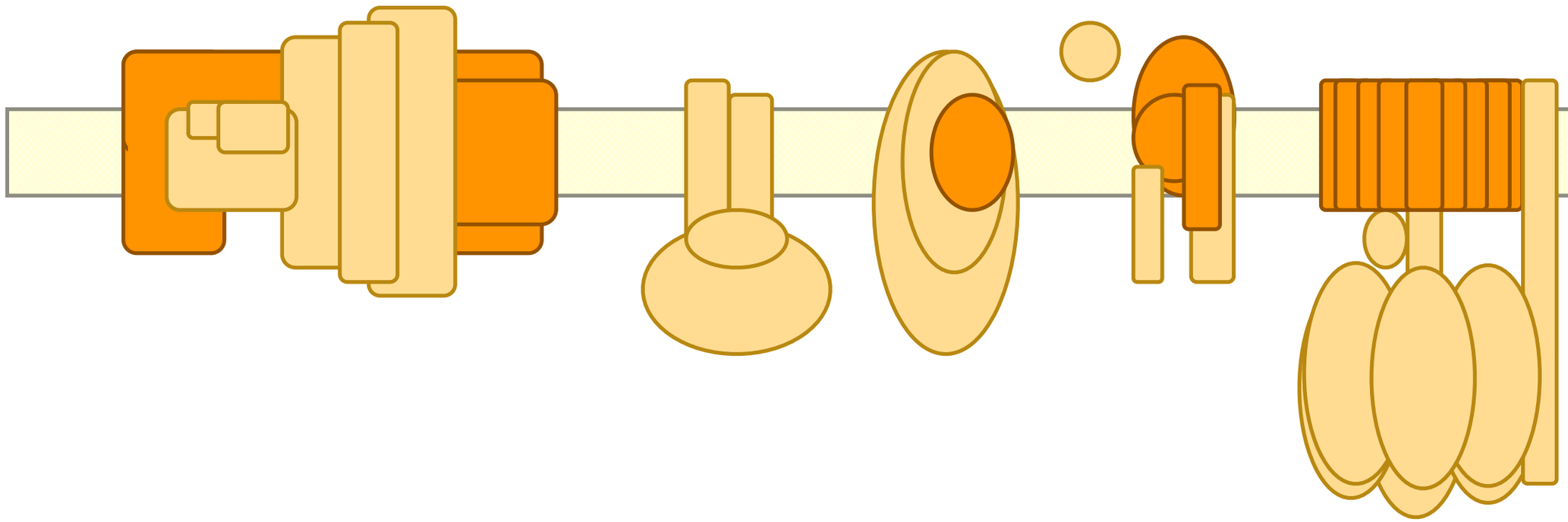
I

II

III

IV

ATPase



Mitochondrial matrix

Allen, J. F. (2003) Phil. Trans. R. Soc. B458, 19-38

Protein subunit encoded in mitochondrial DNA

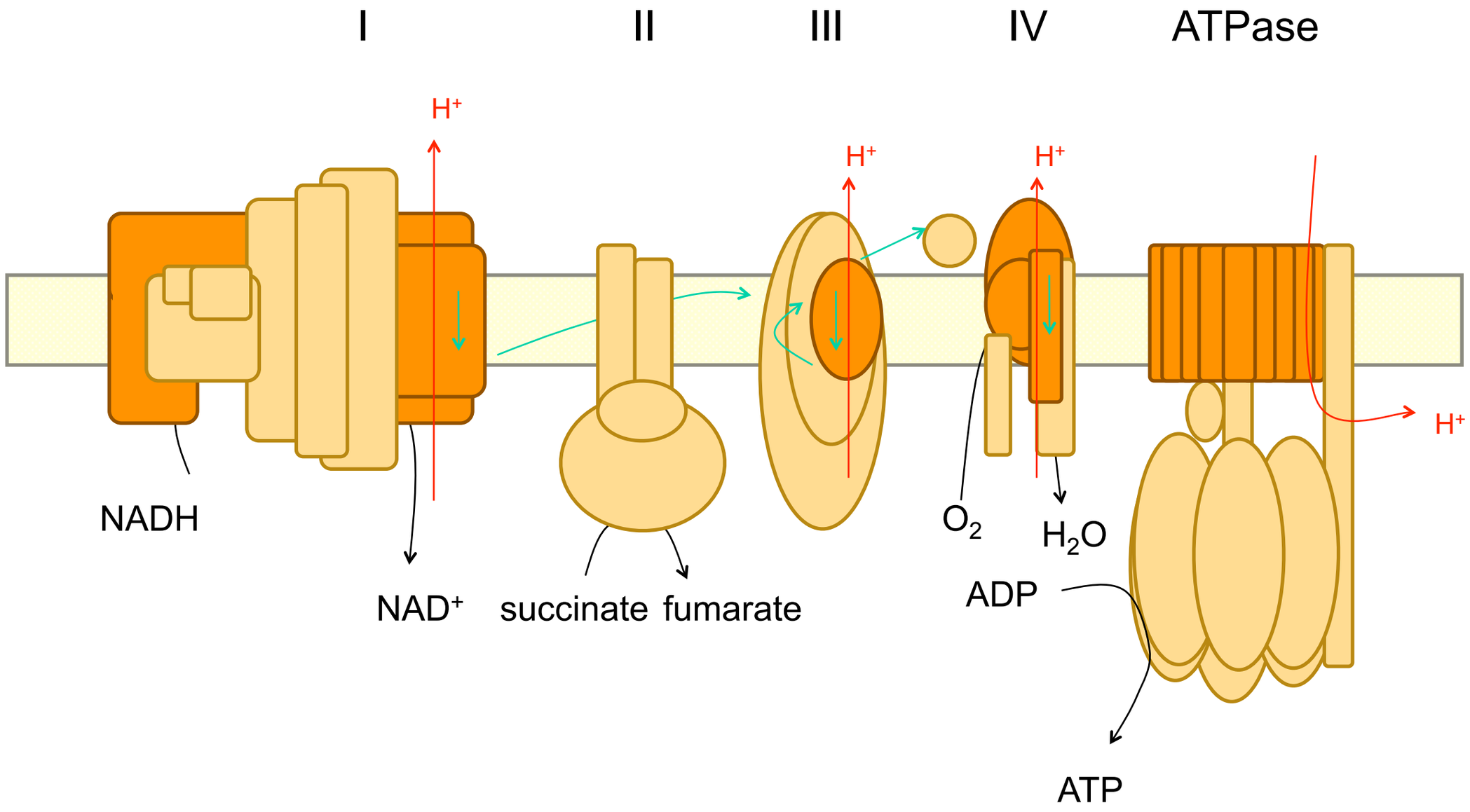
Protein subunit encoded in nuclear DNA

Mitochondrial inner membrane

Direction of vectorial proton translocation

Direction of electron transfer

Inter-membrane space



Mitochondrial matrix

MEMBRANE BIOCHEMISTRY

BIOENERGETICS

One definition of a living organisms is one that defies entropy i.e increases its order / energy rather than energy decreasing.

Living organisms need this energy for biosynthesis, reproduction and movement.

The ultimate source of energy for nearly all living organisms is sunlight, which is turned into chemical energy by photosynthesis in phototrophs.

This chemical energy is then utilised by heterotrophic organisms such as ourselves as a source of energy.

Eukaryotic phototrophs capture light energy in sub-cellular organelles called **chloroplasts**

Eukaryotic heterotrophs capture chemical energy in sub-cellular organelles called **mitochondria**

Prokaryotes carry out these processes in their **plasma membrane.**

In all three cases electron transfer within a membrane (driven either by light or chemical / redox energy) is linked to the storage of energy in the form of adenosine triphosphate (ATP)

So theses systems use a membrane to couple electron transfer within a membrane to the synthesis of ATP, and are called COUPLING MEMBRANES

So there are three types of coupling membrane

a) That found in mitochondria, the inner mitochondria membrane, and you will study this in the practicals

b) That found in chloroplasts, the thylakoid membrane

c) That found in prokaryotes, their plasma membrane

In these lectures we are going to describe the experiments that lead to the formulation of the chemiosmotic hypothesis, that suggested that electron transfer within a membrane was linked to the synthesis of ATP and other energy-requiring processes (such as active transport) by the creation of an electrochemical gradient of protons across the coupling membrane.

For this Peter Mitchell was awarded the Nobel prize in Chemistry in 1978.

In the practicals we will study

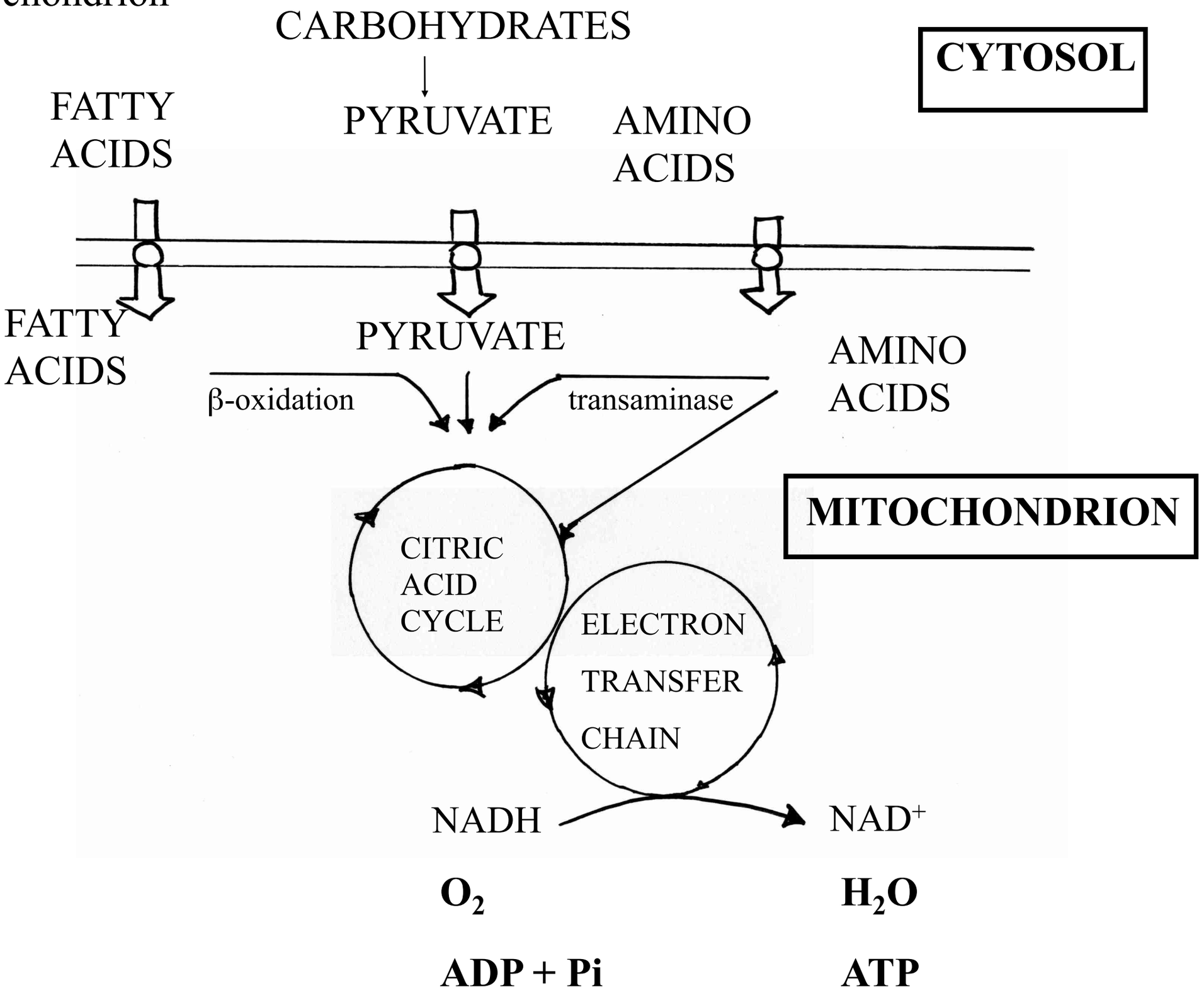
a) The electron transfer chain in animal mitochondria using the oxygen electrode

b) the linkage between electron transfer and ATP synthesis using the oxygen electrode

Mitochondria major catabolic sub-cellular organelle, catalysing breakdown (oxidation) of all three major types of biological macromolecule i.e. fatty acids (lipids), pyruvate (carbohydrates) and amino-acids (protein).

Energy released is stored as chemical energy in form of ATP, and mitochondria also provide precursors for biosynthesis in cytosol.

Relationship between the major oxidative pathways of the mitochondrion



Described relationship amongst major oxidative pathways in mitochondria. major functions can be conveniently listed as

1) the oxidation of pyruvate to acetyl coA by pyruvate dehydrogenase

2) the oxidation of fatty acids to acetyl coA (animals only) in β-oxidation

3) the oxidation of acetyl coA to CO_2 and reduced cofactors (i.e. NADH and succinate) in citric acid cycle

4) the oxidation of reduced cofactors by oxygen forming water and releasing energy (respiratory electron transfer)

5) the synthesis of ATP from ADP and phosphate using energy released during electron transfer (oxidative phosphorylation) There is also transamination of amino-acids to produce acetyl coA or intermediates of TCA cycle.

Humans require a continuous supply of energy to survive.

Typically, we need a power supply of around 100 watts.

The majority of the energy we use is supplied by breakdown of ATP, which is continuously re-formed from ADP and phosphate.

Each day we use the equivalent of up to half a ton of ATP.

The majority of the ATP is regenerated in our mitochondria.

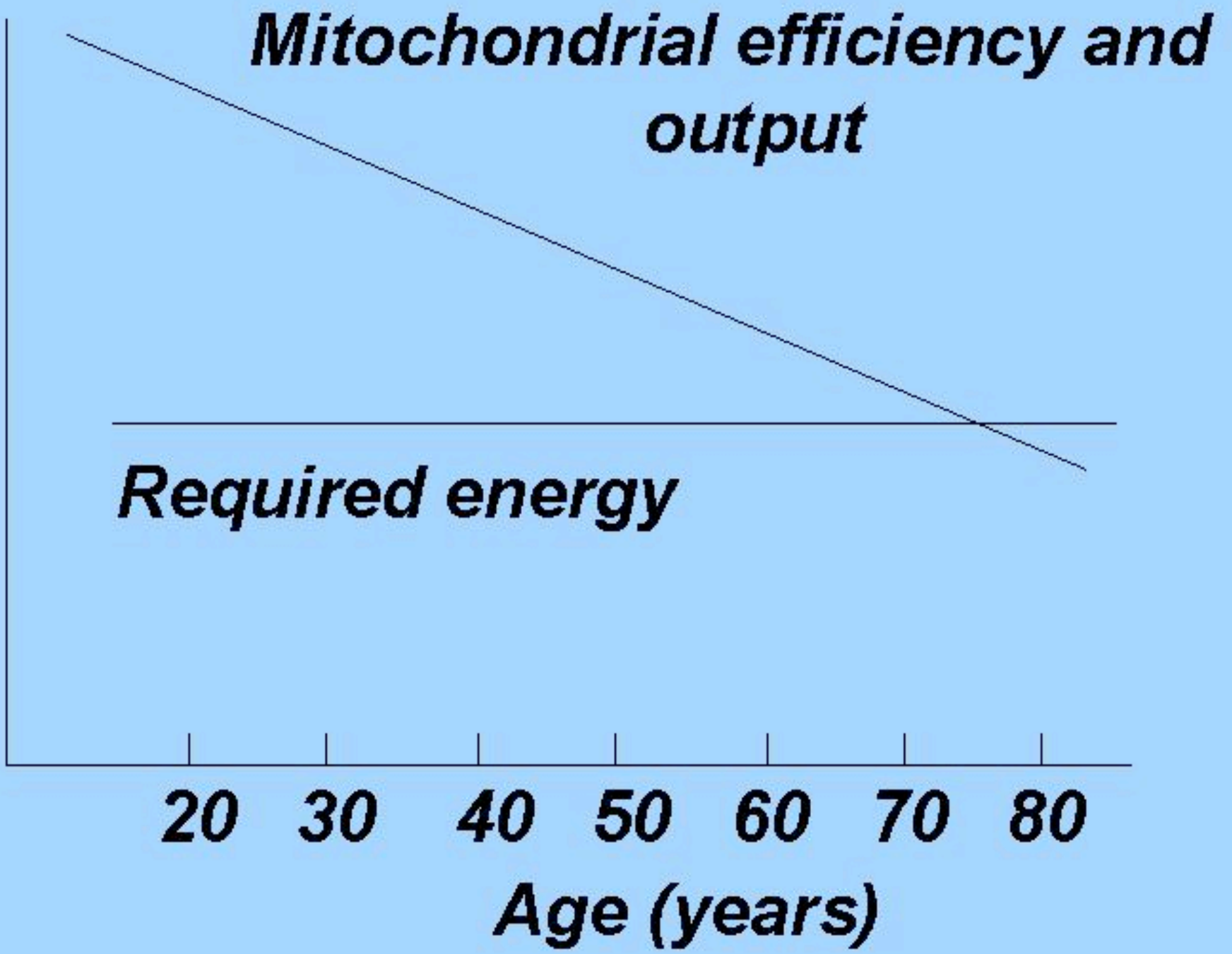
Energy yield from a molecule of glucose

Reaction	NADH	$FADH_2$	ATP
Glycolysis	+2	0	+2
Conversion of pyruvate to acetyl-CoA	+2	0	0
Citric acid cycle	+6	+2	+2
Oxidative phosphorylation (via glycerol-phosphate shuttle)	–10	–2	+26
Number of ATPs are rounded up to the nearest integer **Net ATP produced**	**0**	**0**	**30**

- **ATP synthases are generally operating at a potential gradient of roughly 250 mV**
- **If we assume that all of our 100 watt power requirement arises from these protons, our net transmembrane proton flux would have to be 400 amps, or roughly 2.5 x 10^{21} protons per second.**
- **3 ATP are formed for each 10 protons.**
- **ATP is reformed at a rate of around 10^{21} per second, equivalent to a turnover rate of ATP of 85 kg/day.**

(Rich, P.R. (2003) The cost of living. Nature, 421, 583)

Mitochondrial Efficiency and Ageing



The mitochondrial theory of ageing

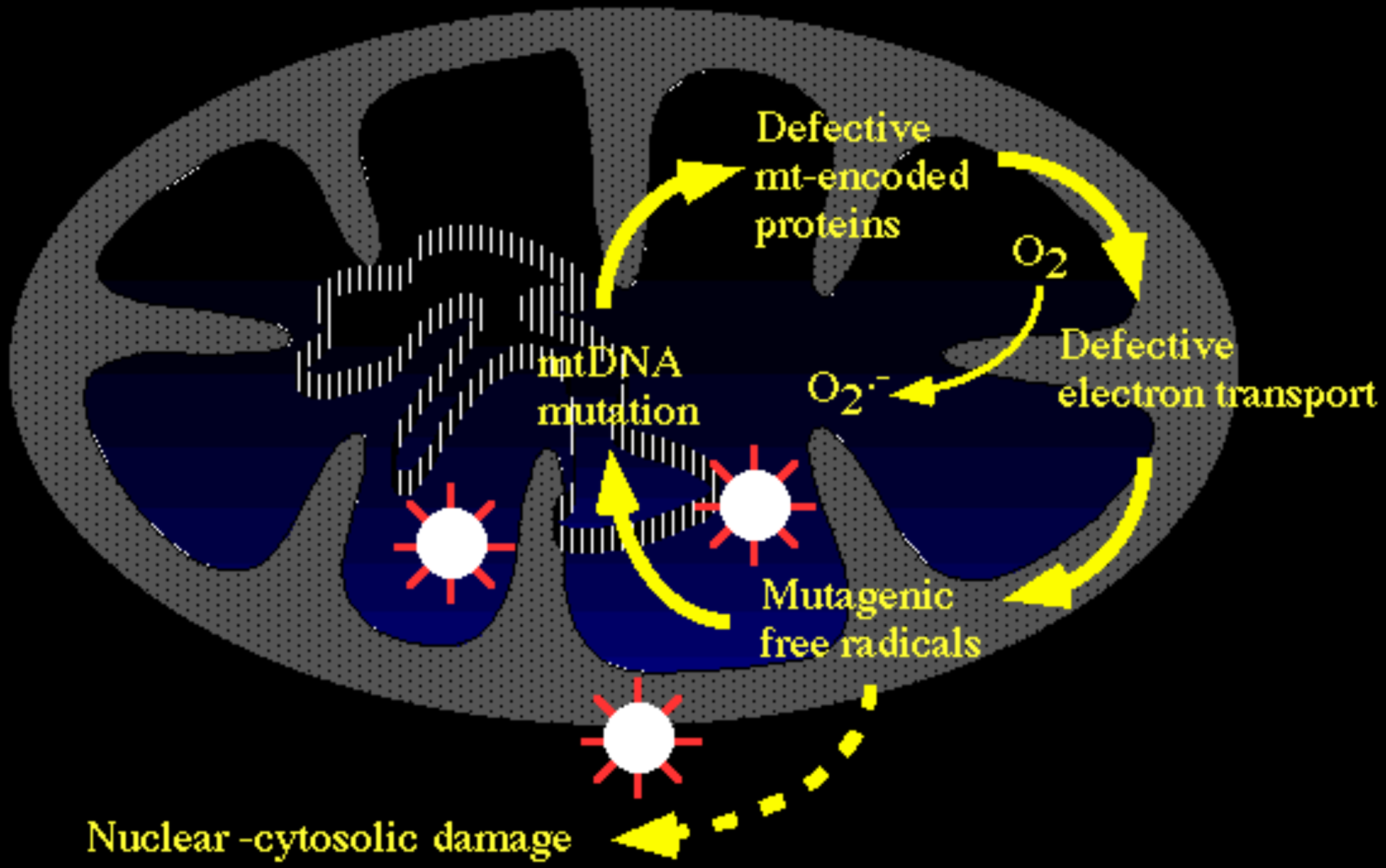
The mitochondrial theory of ageing

"Errors" in electron transfer - transfers to the "wrong" electron acceptor - occur at fixed frequency.

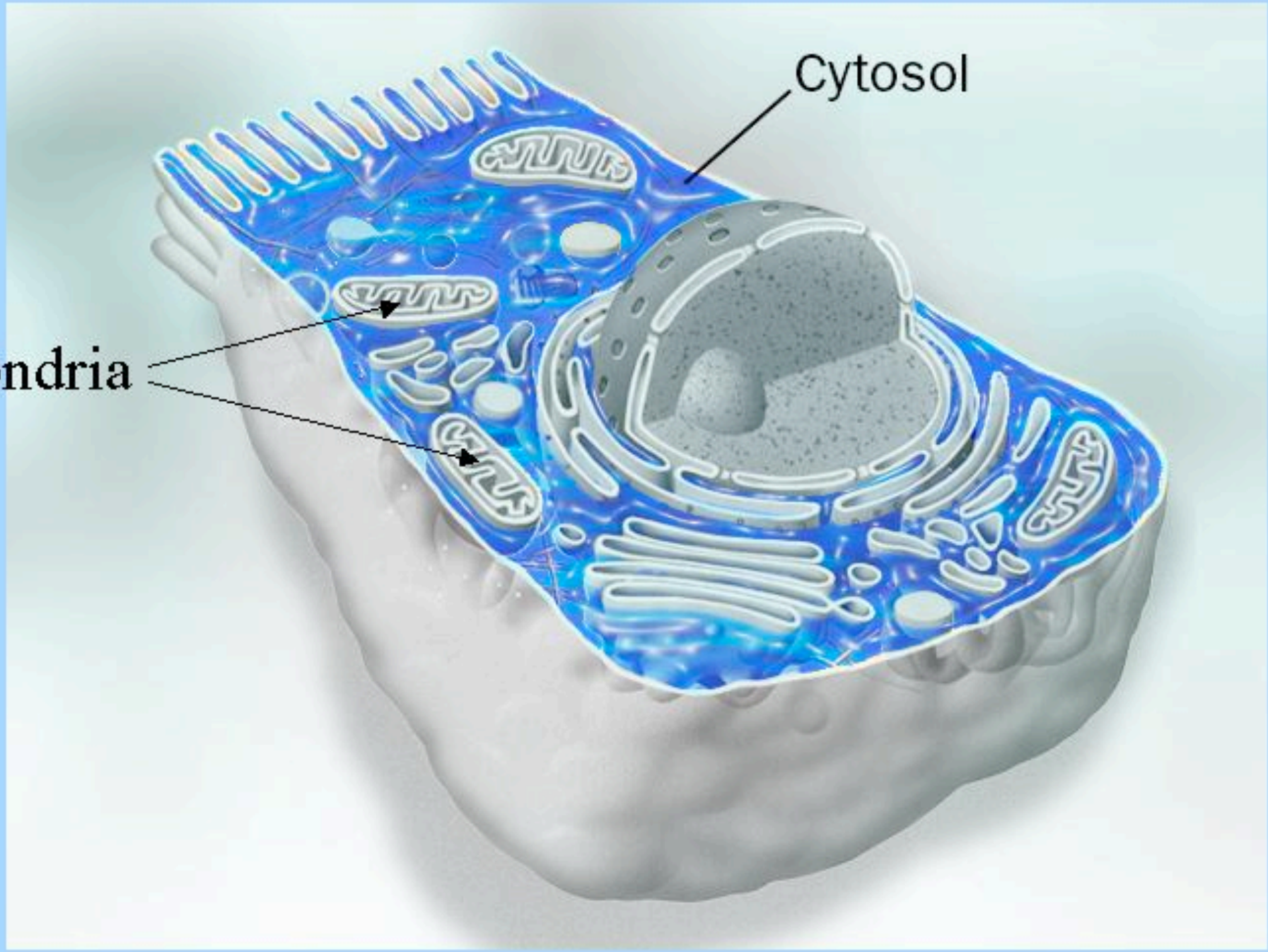
The mitochondrial theory of ageing

"Errors" in electron transfer - transfers to the "wrong" electron acceptor - occur at fixed frequency.

The products of these reactions damage mitochondrial genes, which then produce defective proteins, which then make more "errors" in electron transfer....damaging more genes, making more defective proteins....and so on.


Defective
mt-encoded
proteins
O_2
Defective
electron transport
mtDNA
mutation
$O_2^{\cdot -}$
Mutagenic
free radicals
Nuclear -cytosolic damage

A Typical Animal Cell



Origins of Mitochondria

- All the gaseous oxygen in the present atmosphere is believed to have had a biological origin, and was mostly formed sometime between 3,000,000,000 and 1,000,000,000 years ago, as a result of photosynthesis by cyanobacteria and the earliest green plants.

- Electron transport began in prokaryotes. The rising atmospheric concentration of highly toxic and reactive oxygen was a serious threat to early eukaryotes.

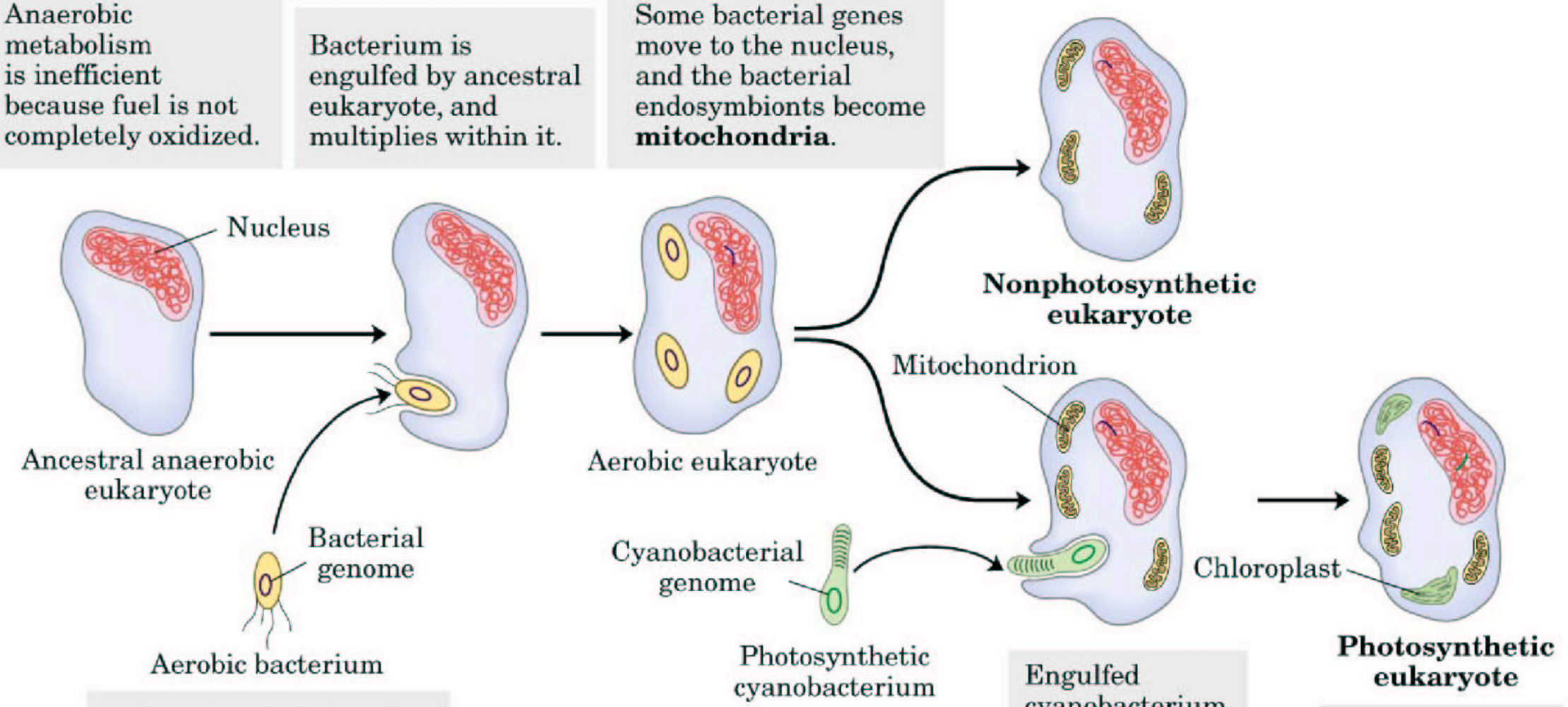
- Mitochondria are thought to have evolved at least 2000 million years ago from primitive bacteria which started with a symbiotic relationship with early eukaryotic cells.

- There followed a gradual transfer of mitochondrial genetic functions to the eukaryotic cell nucleus, where they were better integrated with the other cellular controls.

Anaerobic metabolism is inefficient because fuel is not completely oxidized.

Bacterium is engulfed by ancestral eukaryote, and multiplies within it.

Symbiotic system can now carry out aerobic catabolism. Some bacterial genes move to the nucleus, and the bacterial endosymbionts become **mitochondria**.



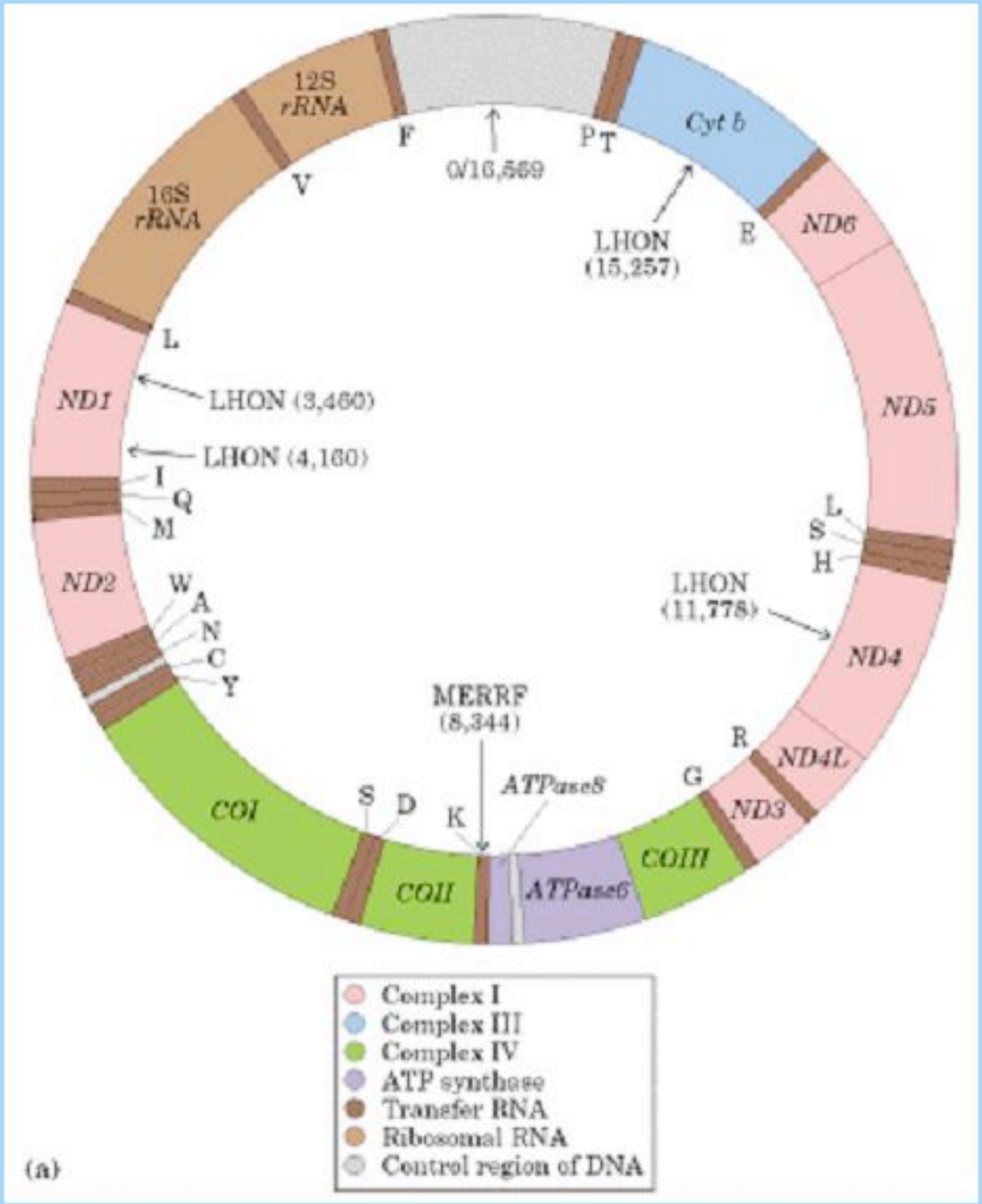
Aerobic metabolism is efficient because fuel is oxidized to CO_2.

Light energy is used to synthesize biomolecules from CO_2.

Engulfed cyanobacterium becomes an endosymbiont and multiplies; new cell can make ATP using energy from sunlight.

In time, some cyanobacterial genes move to the nucleus, and endosymbionts become **plastids (chloroplasts)**.

Mitochondria contain their own DNA



Circular

Maternally-inherited

Codes for 13 proteins, 2 rRNAs
And a full set of t RNAs

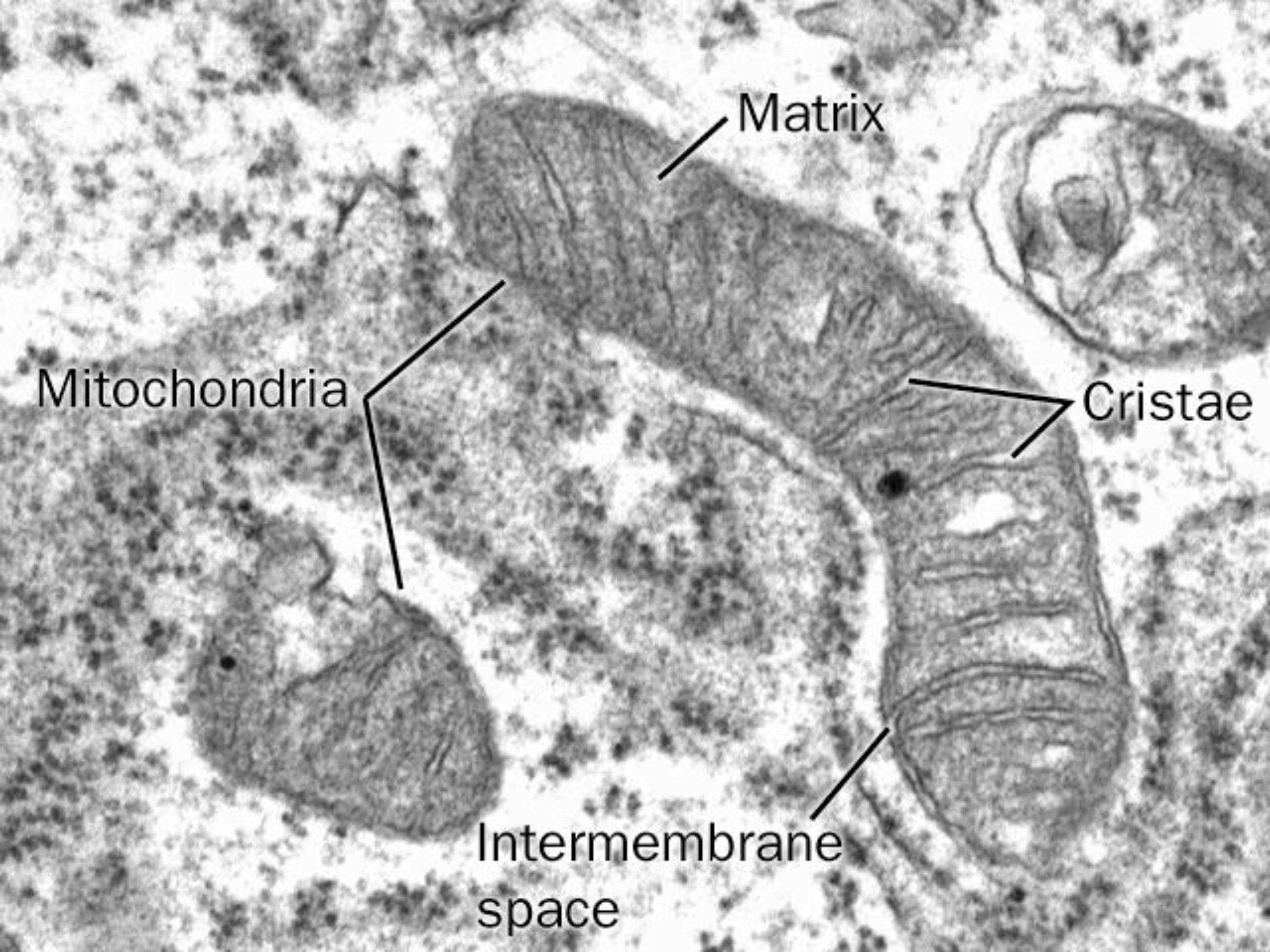
Many copies per cell

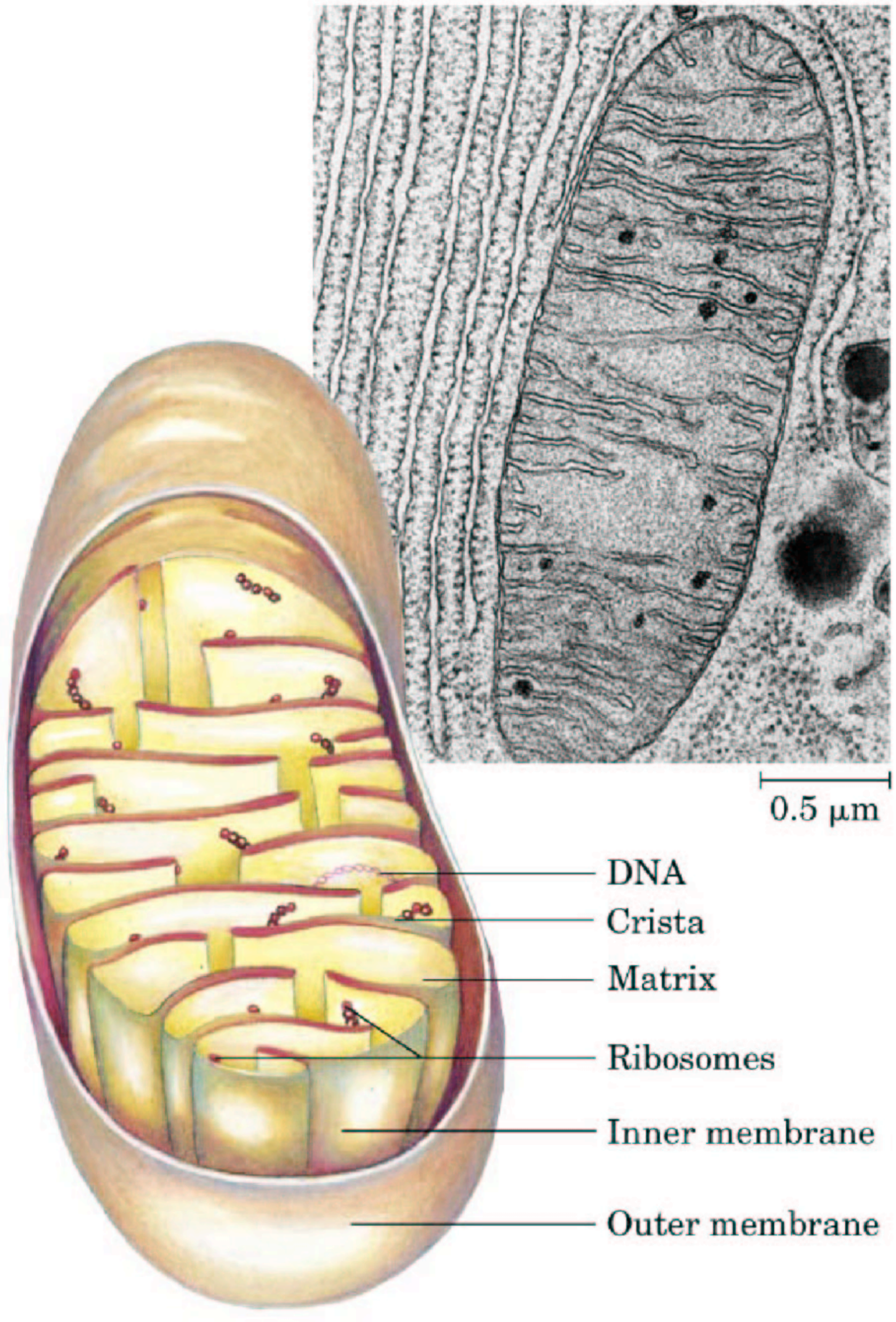
Bacterial-like replication

Structure of Mitochondria

Most cells contain several hundred –thousand mitochondria. Shape is very varied. Typically, they have a diameter of about 1 μm, but other shapes or even reticulate networks are found.

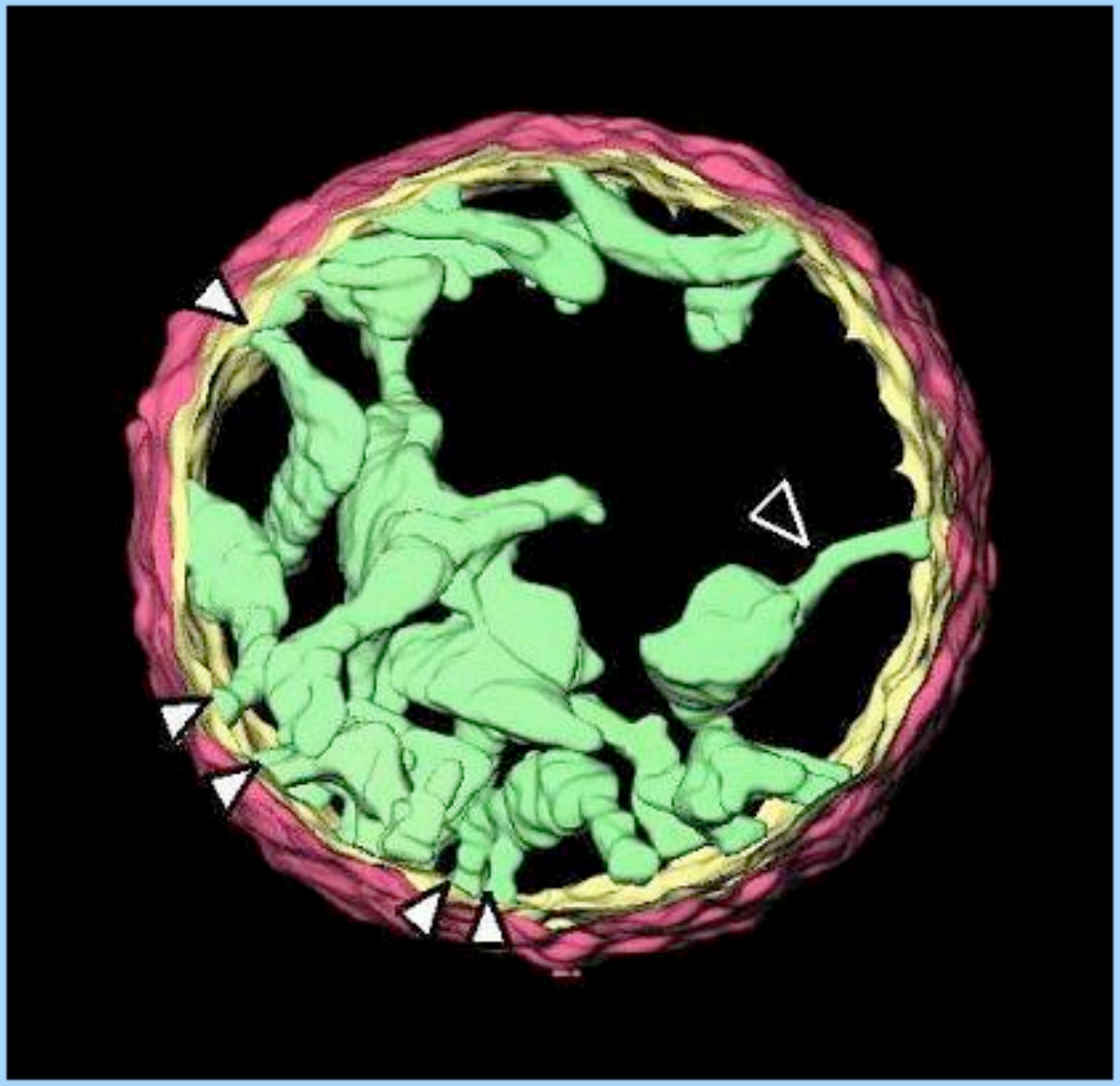
Typically oval-shaped sub-cellular organelles about 2 μM in length and 0.5 μM in diameter, although shape and size may vary depending on specialisation of the tissue they are found in (i.e. cup-shaped to increase surface area and thus exchange metabolites with cytosol).

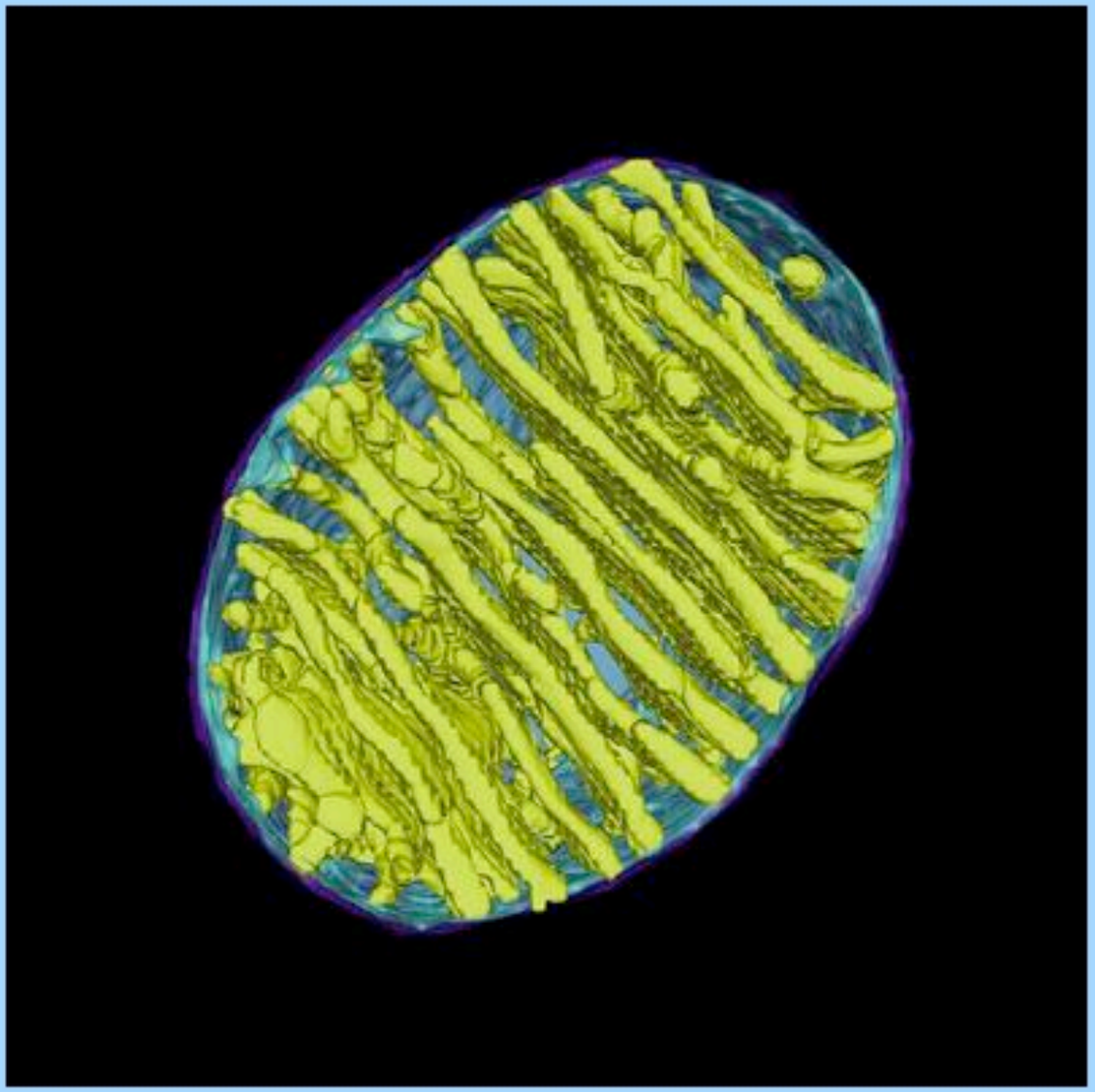

Matrix
Mitochondria
Cristae
Intermembrane
space

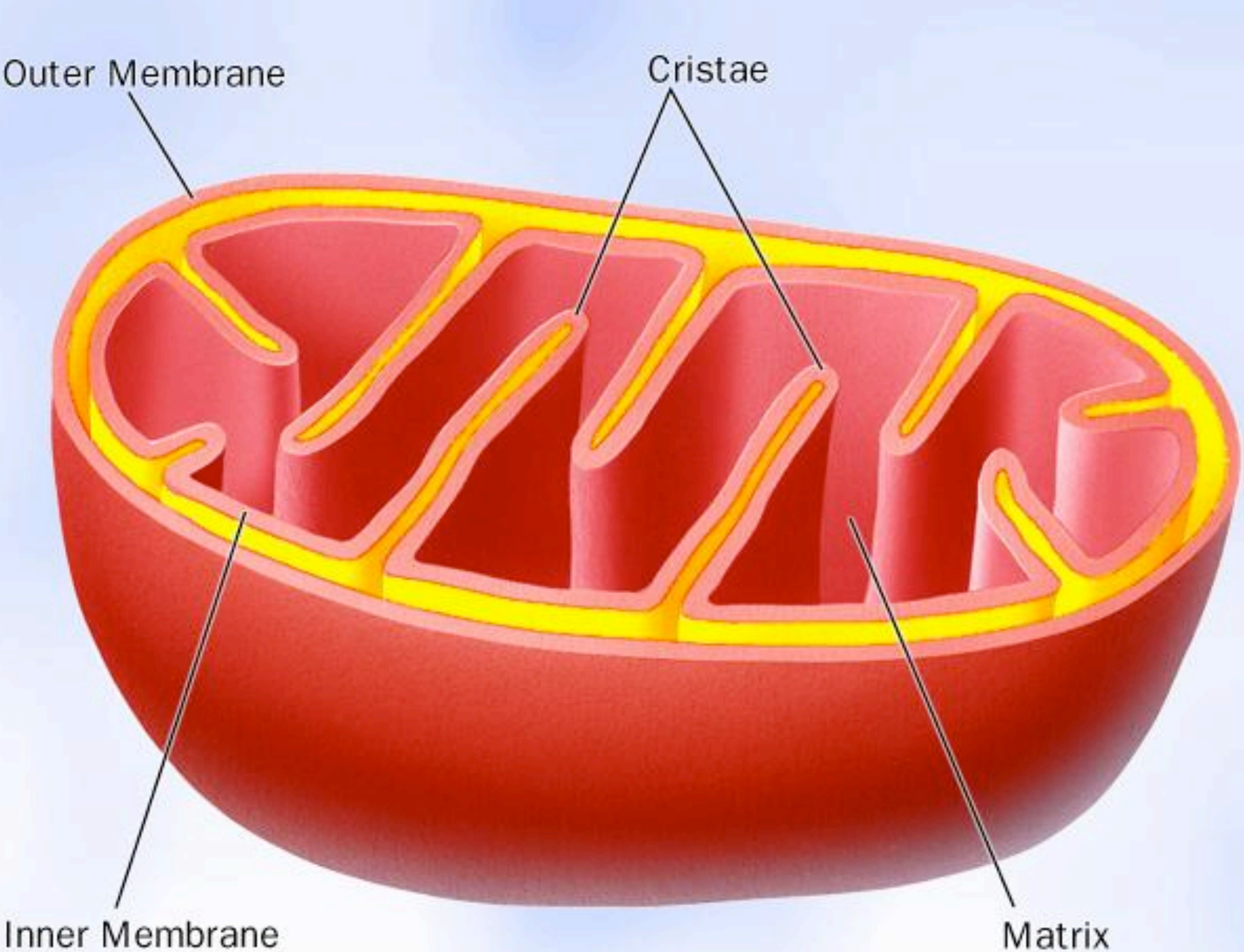

0.5 μm
DNA
Crista
Matrix
Ribosomes
Inner membrane
Outer membrane

Mitochondrial Cristae 3-D Structure

(courtesy of T. Frey and C. Manella)






Outer Membrane
Cristae
Inner Membrane
Matrix

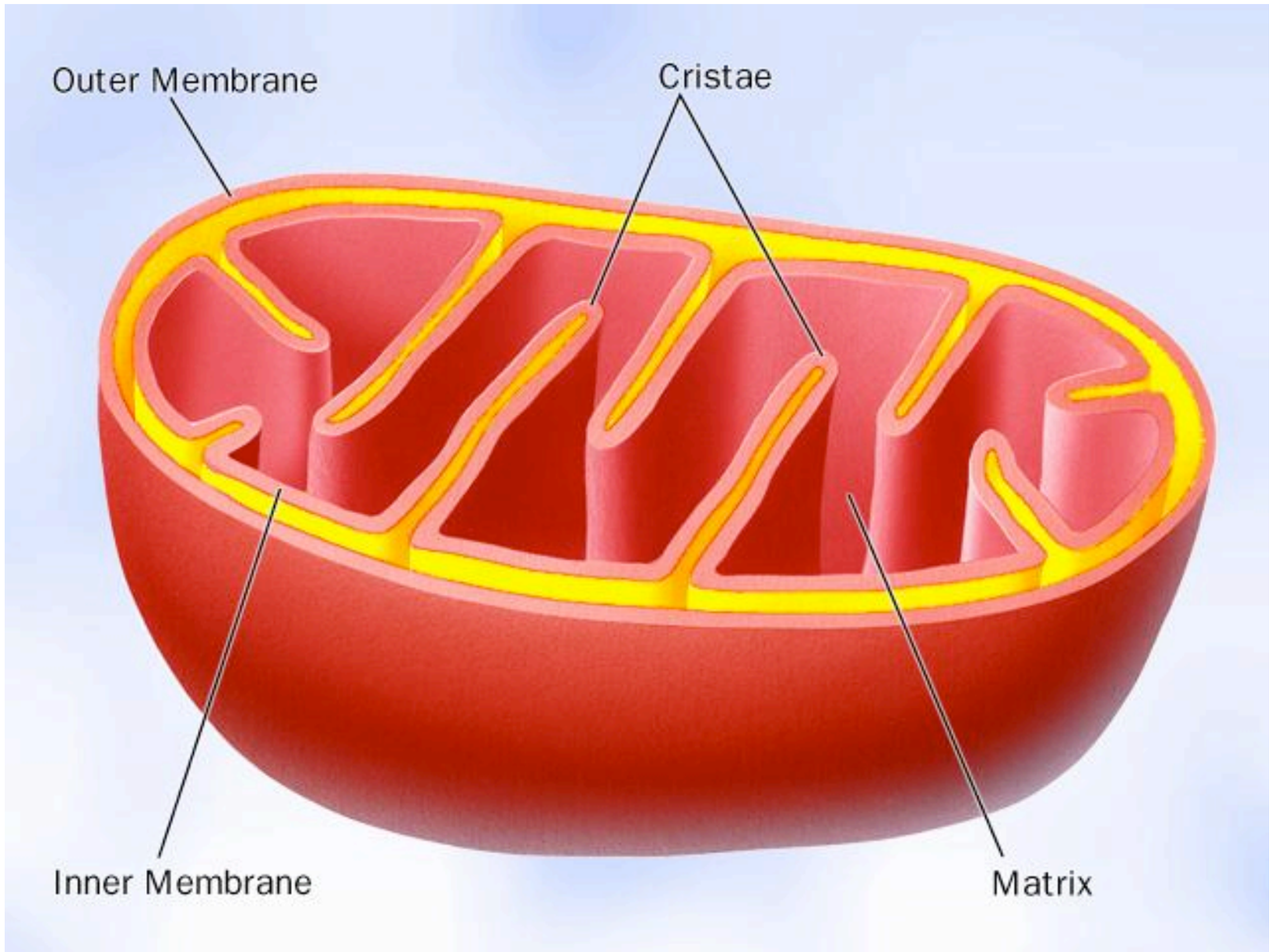
So mitochondria have four compartments

outer mitochondrial membrane (OMM)

intermembrane space (IMS)

inner mitochondrial membrane (IMM)

matrix



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2) the oxidation of fatty acids to acetyl coA (animals only) in β-oxidation

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4) the oxidation of reduced cofactors by oxygen forming water and releasing energy (respiratory electron transfer)

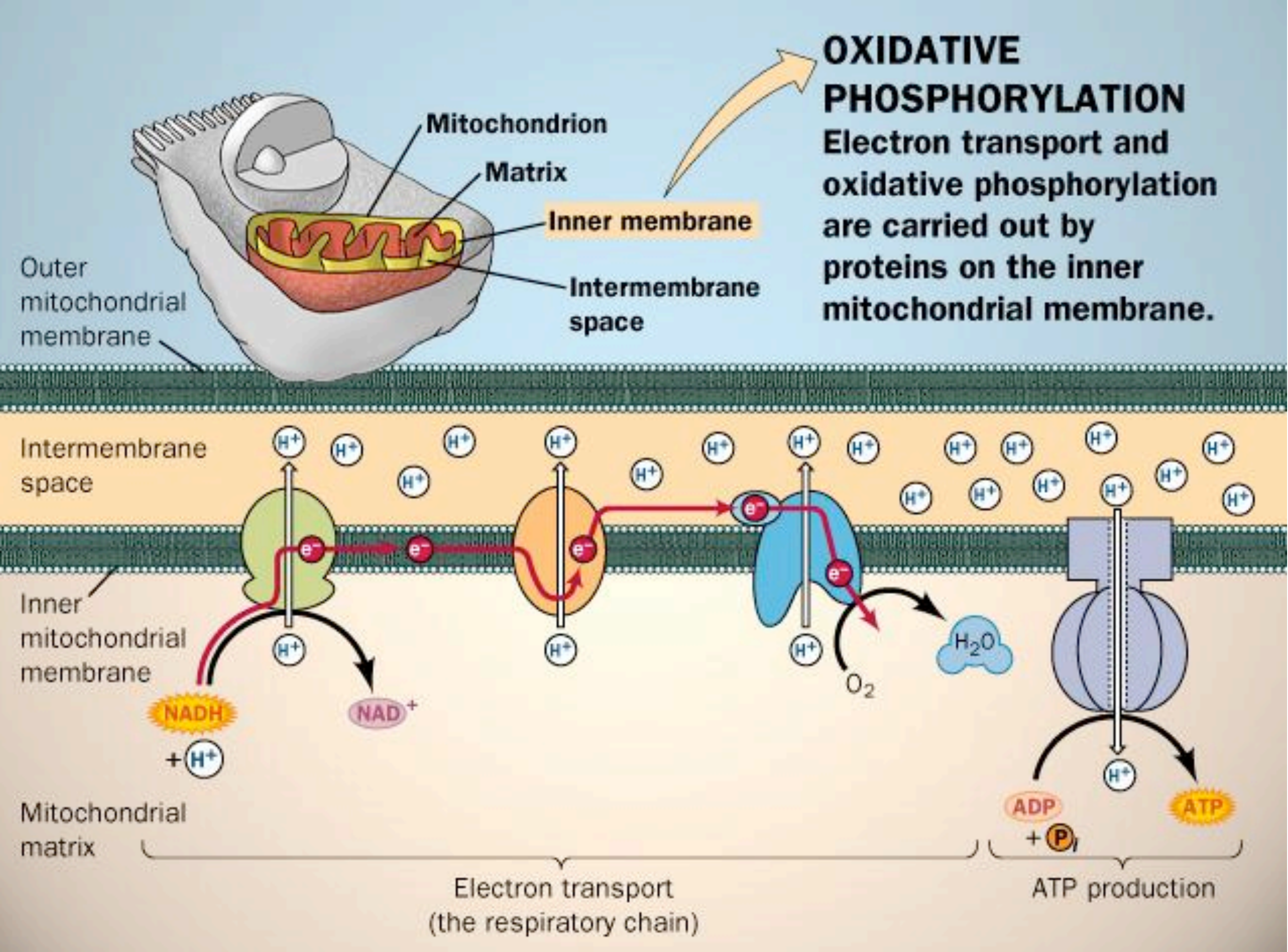
5) the synthesis of ATP from ADP and phosphate using energy released during electron transfer (oxidative phosphorylation) There is also transamination of amino-acids to produce acetyl coA or intermediates of TCA cycle.

Functions 1-3 above are located in matrix, Functions 4 and 5 are located in IMM. so IMM is **coupling membrane** coupling electron transfer in membrane to ATP synthesis. Label P (positive)[IMS] and N (negative)[matrix] phases depending on direction of proton (+ve charge) pumping across membrane.

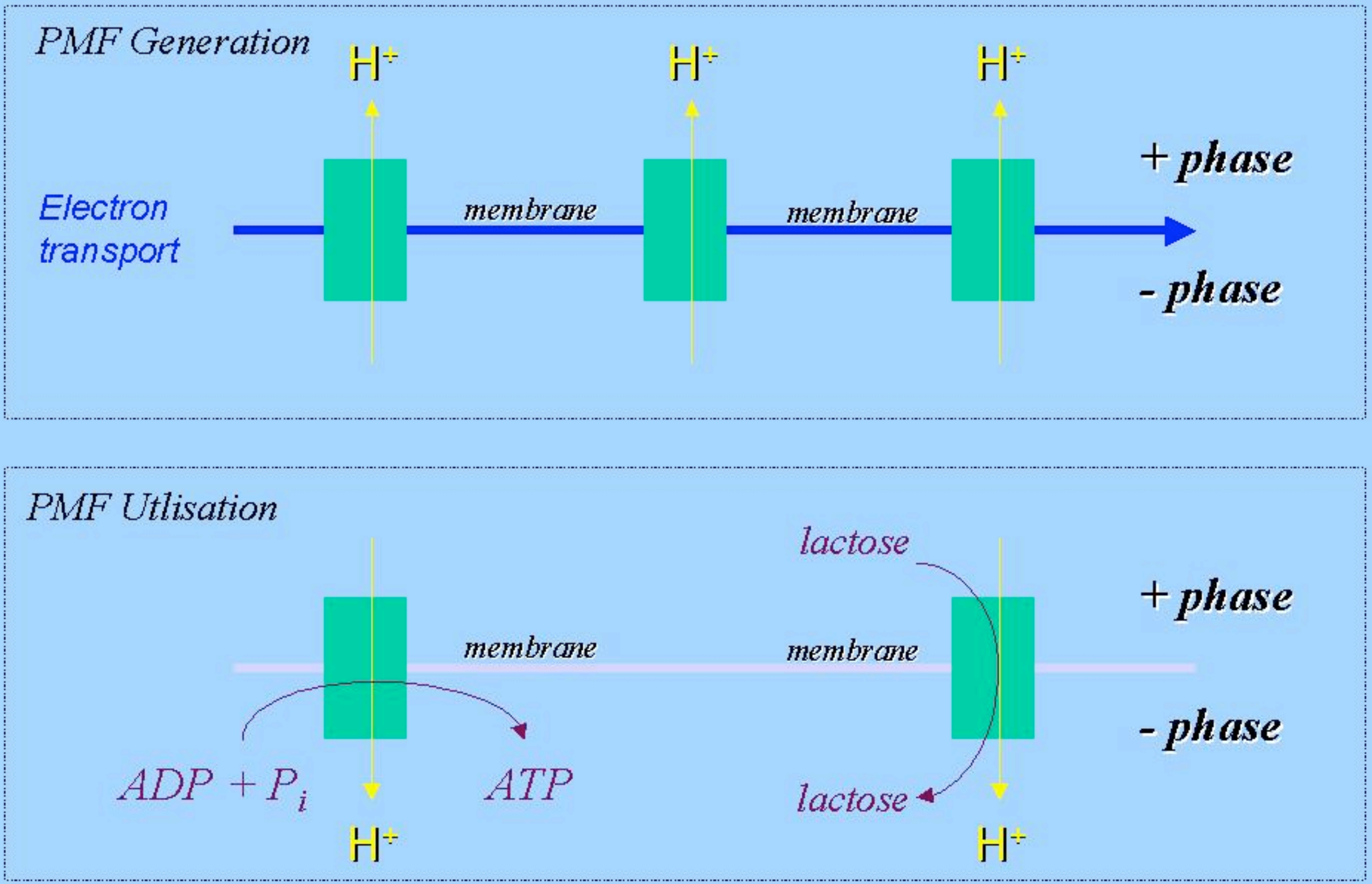
But the IMM is also the **compartment membrane,** since it is impermeable to small charged molecules. So controls import of substrates for catabolic reactions and export of ATP synthesised, because this occurs via specifiic transport proteins, many of which catalyse active transport

OXIDATIVE PHOSPHORYLATION

Electron transport and oxidative phosphorylation are carried out by proteins on the inner mitochondrial membrane.



Chemiosmotic Coupling



Cytosol

Proton concentration outside the matrix is higher than inside due to proton pumping.

Outer mitochondrial membrane

Mobile electron carriers

Intermembrane space

H^+

ATP synthase

Inner membrane

I

II

III

IV

Matrix

Membrane-bound protein complexes

Electrons from NADH

Electrons from $FADH_2$

ADP + P_i

ATP

Electron transport

Generates energy to pump protons across a membrane, creating a concentration gradient

ATP synthesis

Harnesses protons moving back across membrane to drive ATP production

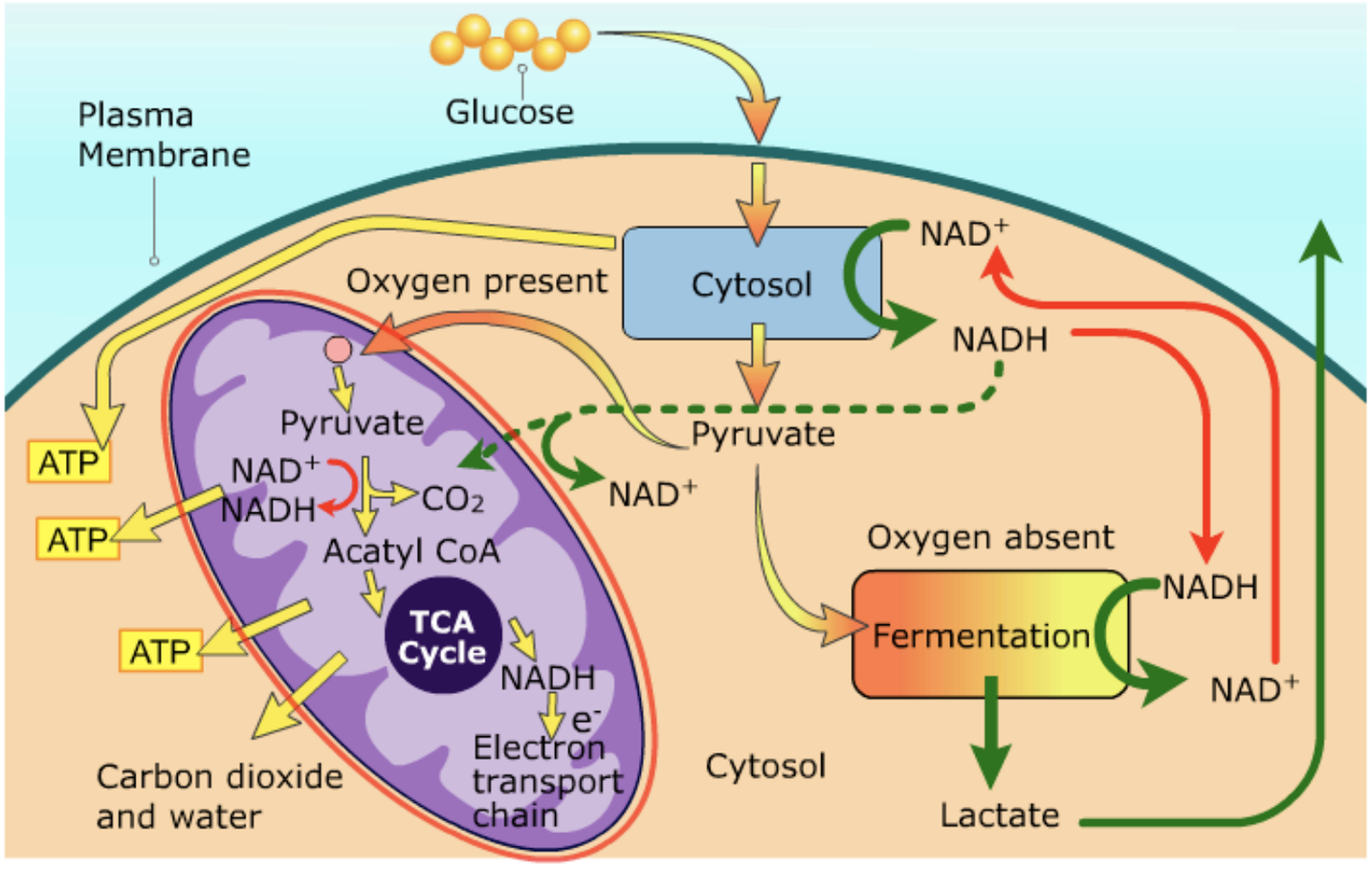
OMM freely permeable to molecules <12kDa r.m.m. because of presence of porins. Main function is to restrict swelling of IMM and protect IMM against enzymes of cytosol.

IMM is not permeable to anything except small uncharged molecules (because of hydrophobic core of lipid bilayer). So contains many transport proteins to connect mitochondria to cytosol. So IMM **compartment membrane** as well as coupling membrane.

IMM infolded into cristae to increase surface area for electron transfer and ATP synthesis. Inside cristae continuous with IMS. Find IMM greater proportion of mitochondria in mitochondria specialised for ATP synthesis (i.e. in heart muscle) than in mitochondria specialised for catabolism (in liver cells).

Aerobic Respiration

Glycolysis converts glucose (C6) into two molecules of pyruvate (C3). If oxygen is present, pyruvate enters mitochondria and its free energy is utilized to make ATP via the TCA cycle and oxidative phosphorylation.



Click the mitochondrion to examine the TCA cycle and oxidative phosphorylation.

Membrane Biochemistry

Next lecture....

The respiratory chain

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