

BIOL 266 – CELL BIOLOGY

Lectures:

Tuesdays and Thursdays 10:15 - 11:30, Room SP-S110

Instructor:

Vladimir Titorenko

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WEB: <http://sites.google.com/site/titorenkolabwebsite20/>

Office hours:

Tuesdays and Thursdays from 14:00 to 15:00

Marking scheme:

(1) If your mark for the final exam is better than your worst mark for a midterm test:

80 % of your final grade for the course = your mark for the final exam

+ 20 % of your final grade for the course = your best mark for a midterm test

Example:

If:

1st midterm: 16 % out of 20 % (80 points out of 100)

2nd midterm: 5 % out of 20 % (25 points out of 100)

Final exam: 75 points out of 100

Then:

Final grade for the course = 16 % + (80 % x 75) = 16 % + 60 % = 76 %

Marking scheme:

(2) If your mark for the final exam is not better than your worst mark for a midterm test:

60 % of your final grade for the course = your mark for the final exam

+ 20 % of your final grade for the course = your mark for the 1st midterm test

+ 20 % of your final grade for the course = your mark for the 2nd midterm test

Example:

If:

1st midterm: 16 % out of 20 % (80 points out of 100)

2nd midterm: 18 % out of 20 % (90 points out of 100)

Final exam: 75 points out of 100

Then:

Final grade for the course = 16 % + 18 % + (60 % x 75) = 16 % + 18 % + 45 % = 79 %

In the first term test you will be examined on materials covered in lectures 1-5.

In the second term test you will be examined on materials covered in lectures 6-12.

The final examination deals with the entire course with approximately half of the questions coming from the last seven lectures of the course.

If you miss a term test for medical or other serious reasons, you must provide documentation within a week. Otherwise your mark will be zero.

If you miss the final examination, you must contact the Examination Office to schedule a deferred examination.

Questions:

Total number = 40

13 (32.5%) - Short answer questions

27 (67.5%) – Multiple choice questions

Grades will be assigned as follows:

90-100 % = A+

66-69.9 % = C+

85-89.9 % = A

63-65.9 % = C

80-84.9 % = A-

60-62.9 % = C-

76-79.9 % = B+

56-59.9 % = D+

73-75.9 % = B

53-55.9 % = D

70-72.9 % = B-

50-52.9 % = D-

Recommended texts:

(1) *Essential Cell Biology* by Alberts et al. (4th Edition), Garland Publishing, 2014

(2) *Cell and Molecular Biology: Concepts and Experiments* by Gerald Karp (7th Edition), John Wiley & Sons, 2013

- **Although either of the two textbooks is recommended, none of them is required**
- **Examination questions will be based on lecture materials**

Course outline and lecture notes:

are available on the WEB

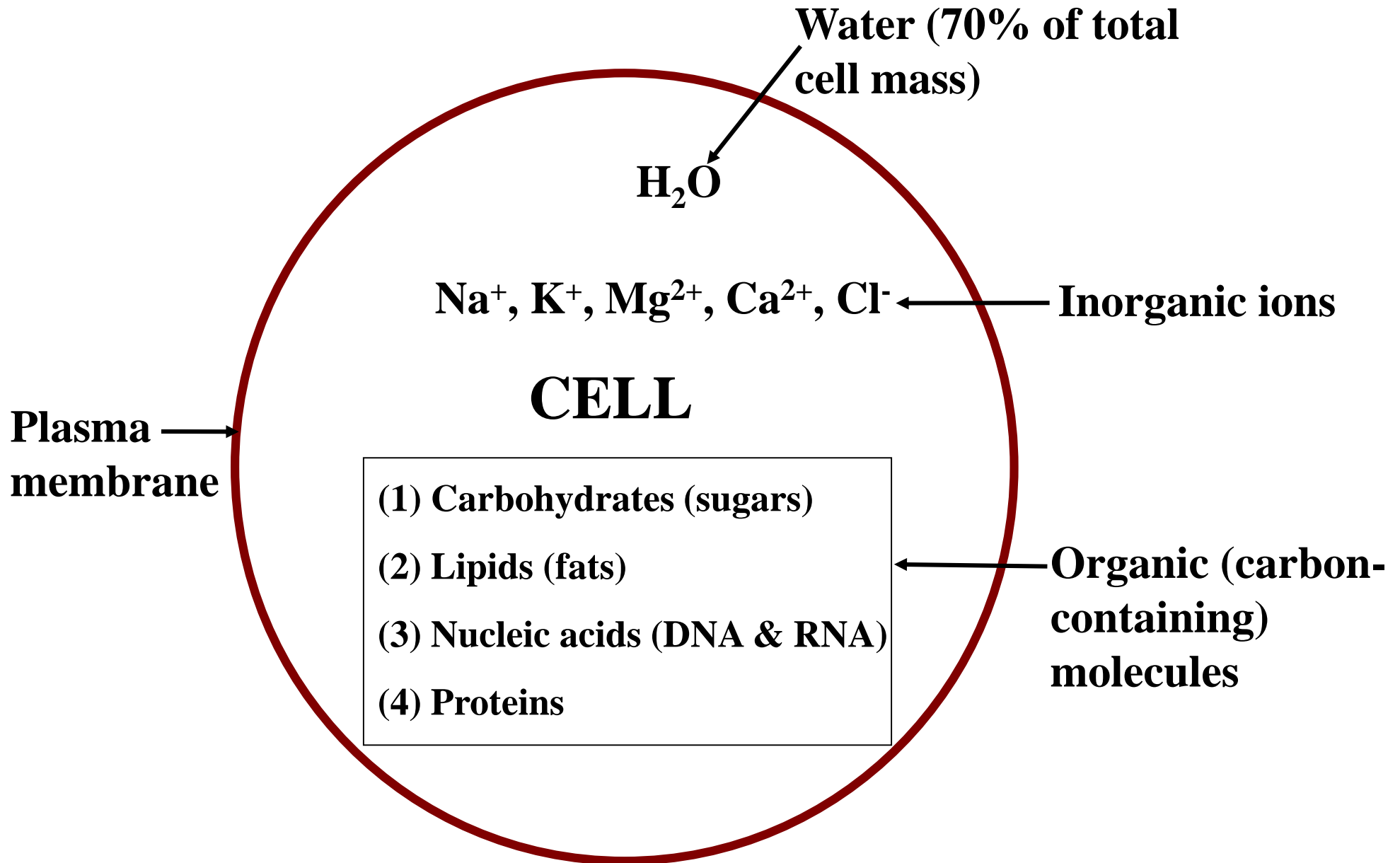
<http://moodle.concordia.ca/>

BIOL 266 – CELL BIOLOGY

Lecture 1:

Cells and Organelles (I)

Cells are small membrane-bounded units filled with a concentrated aqueous solution of chemicals



Unity and diversity of cells

All cells contain *DNA* (deoxyribonucleic acid) as a store of genetic information

Two types of cells:

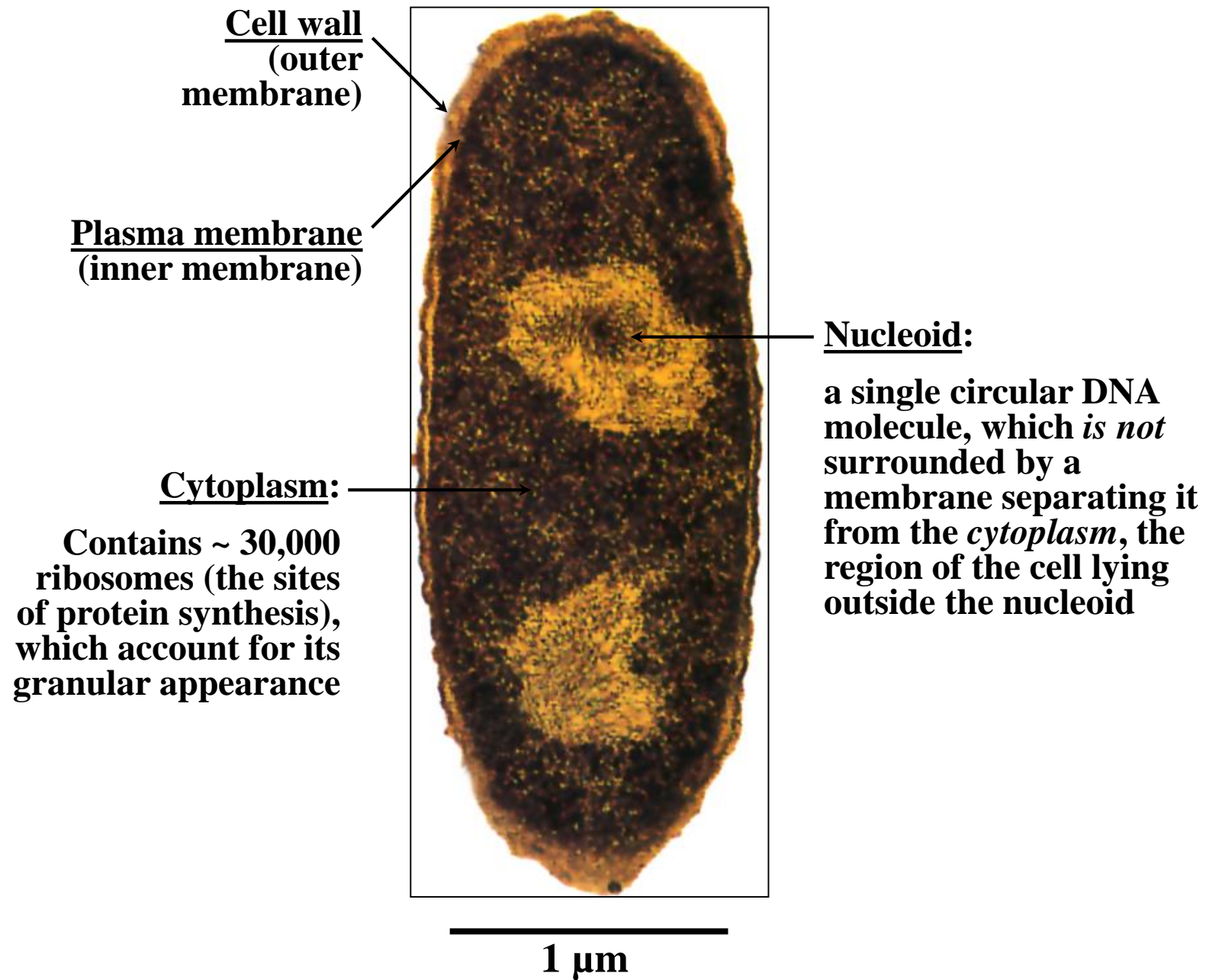
Prokaryotic cells

- > From the Greek words *pro*, meaning “before”, and *karyon*, meaning “nucleus”
- > *DNA is not segregated* within a defined nucleus
- > Do not contain internal membranes

Eukaryotic cells

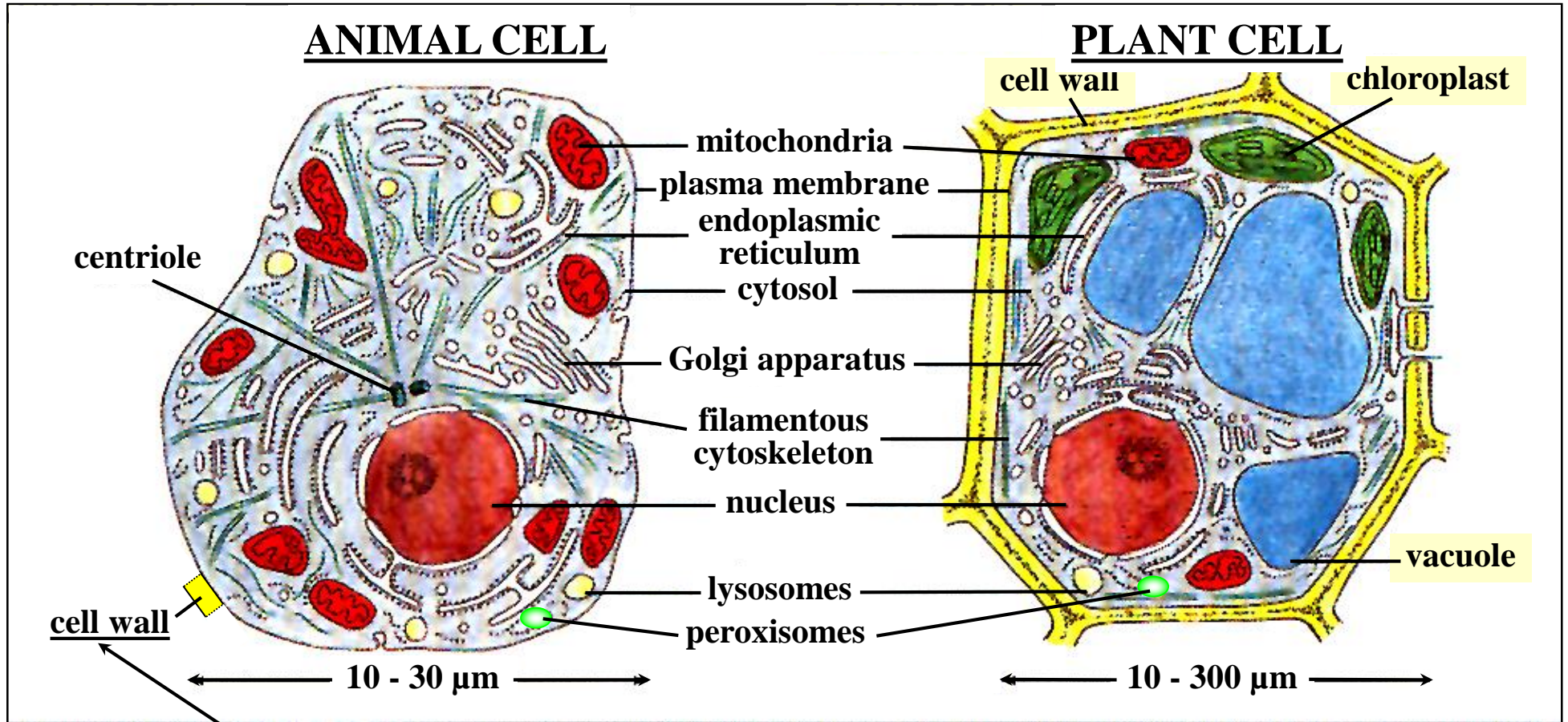
- > From the Greek words *eu*, meaning “truly” or “well”, and *karyon*, meaning “nucleus”
- > *DNA is segregated* within a defined nucleus
- > Contain extensive internal membranes that enclose specific compartments (*the organelles*) and separate them from the rest of the cell (*the cytosol*)

Prokaryotes comprise a single membrane-limited compartment

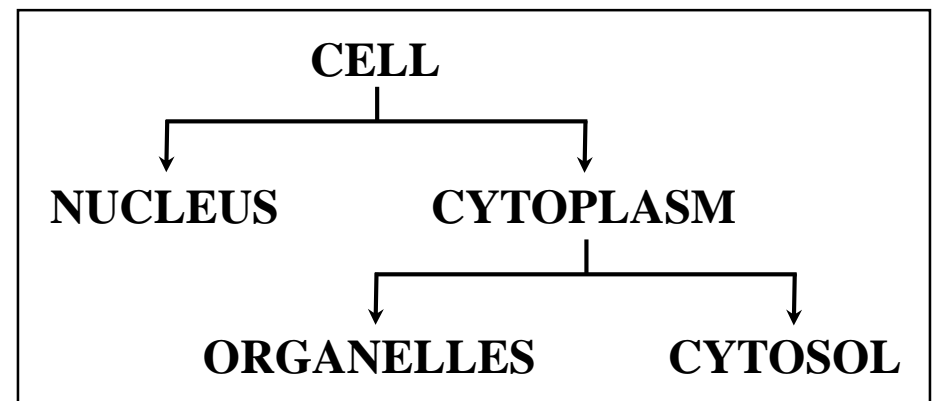


Electron micrograph of a typical prokaryotic cell, the bacterium *Escherichia coli*

Eukaryotic cells contain many organelles and a complex cytoskeleton

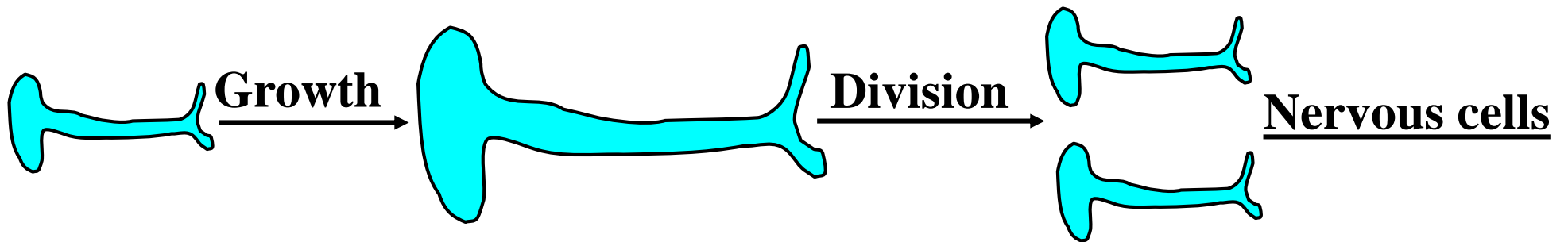


Single-celled or unicellular eukaryotes
(YEAST and FUNGI)

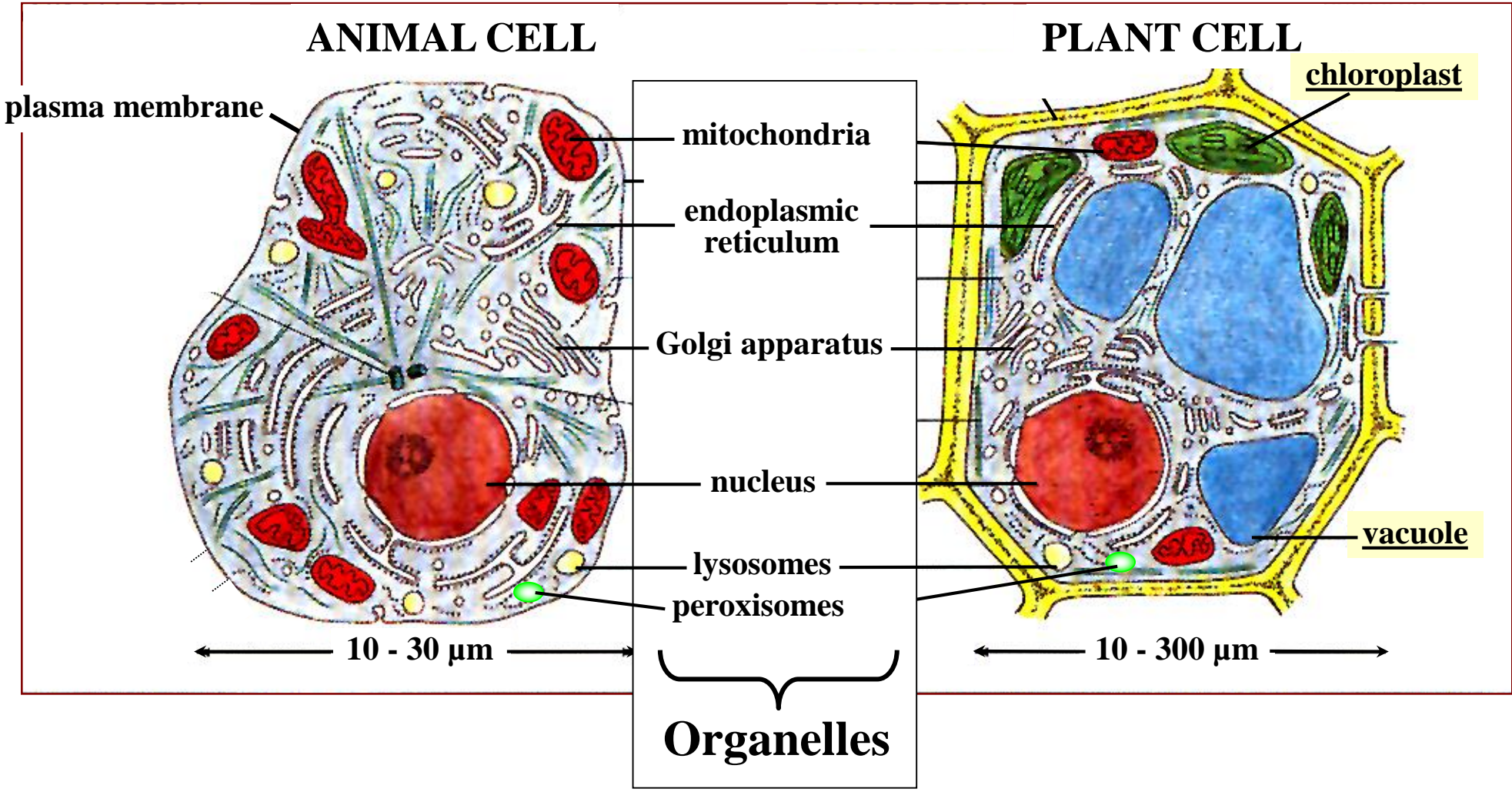


Cells are the smallest units exhibiting the characteristics of life:

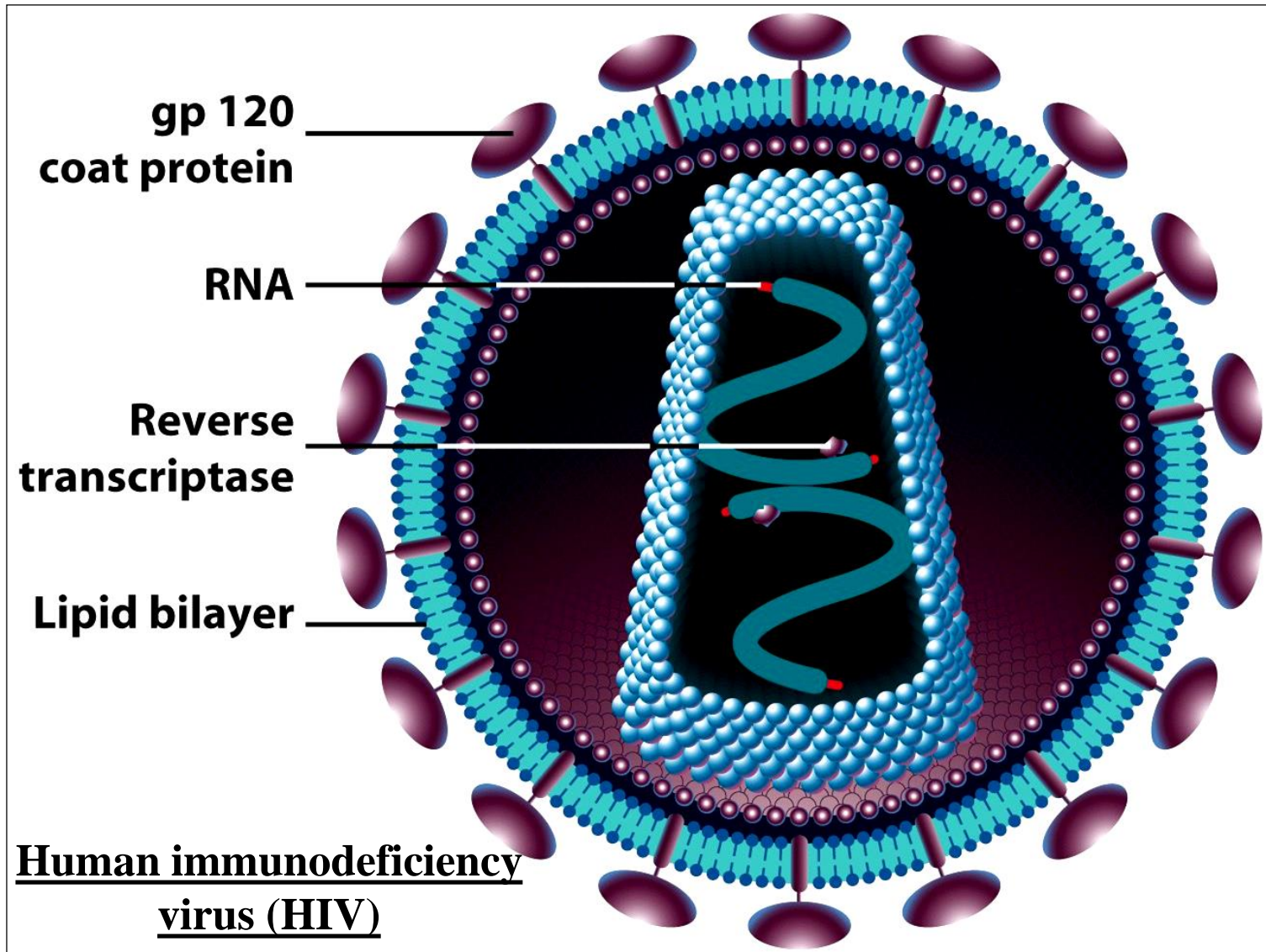
they are able to reproduce themselves by their own efforts



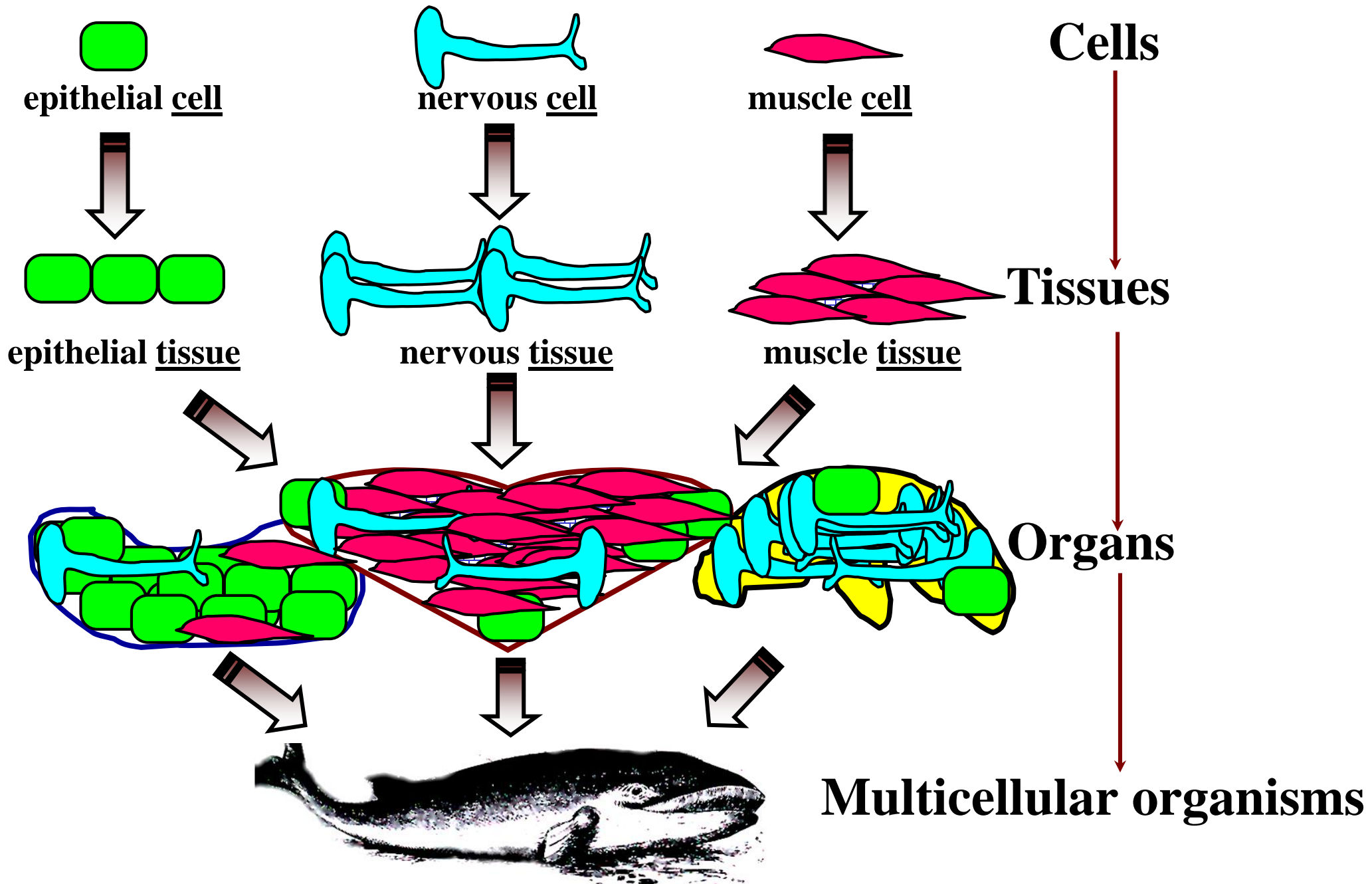
Organelles are not the smallest units exhibiting the characteristics of life:
they are not able to reproduce themselves by their own efforts, outside of their host cells



Viruses are not the smallest units exhibiting the characteristics of life:
they are not able to reproduce themselves by their own efforts;
they use the reproductive machinery of cells that they invade



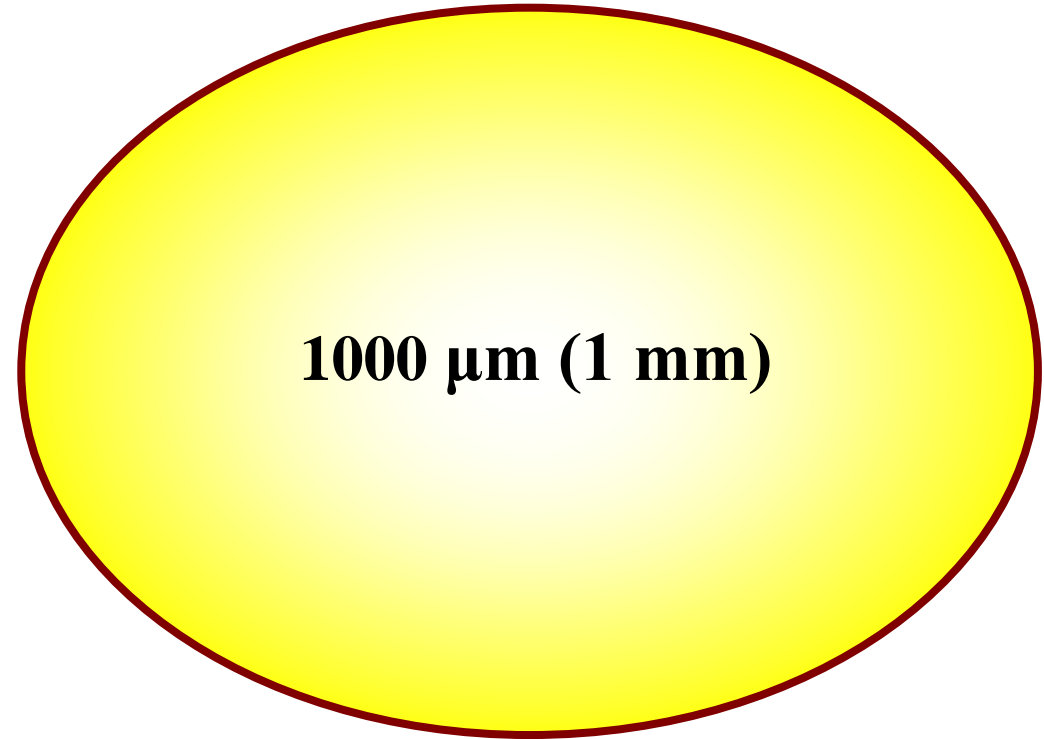
The cell is the fundamental unit of life, the underlying building block from which all organisms are constructed



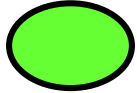
**Cell Biology investigates how cells
grow, divide, operate, communicate,
control their activities, and die**

Cells are small: typically 5 – 20 μm (0.005 – 0.02 mm) in diameter

A frog's egg



A bacterium



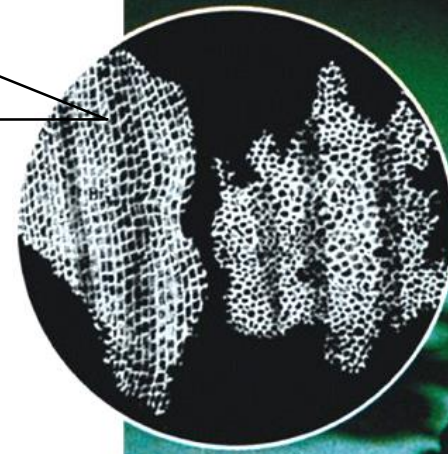
1 μm (0.001 mm)

Cells are invisible to the naked eye, so scientists did not know of their existence prior to the invention of the light microscope in the 17th century

Robert Hooke's light microscope (1665):

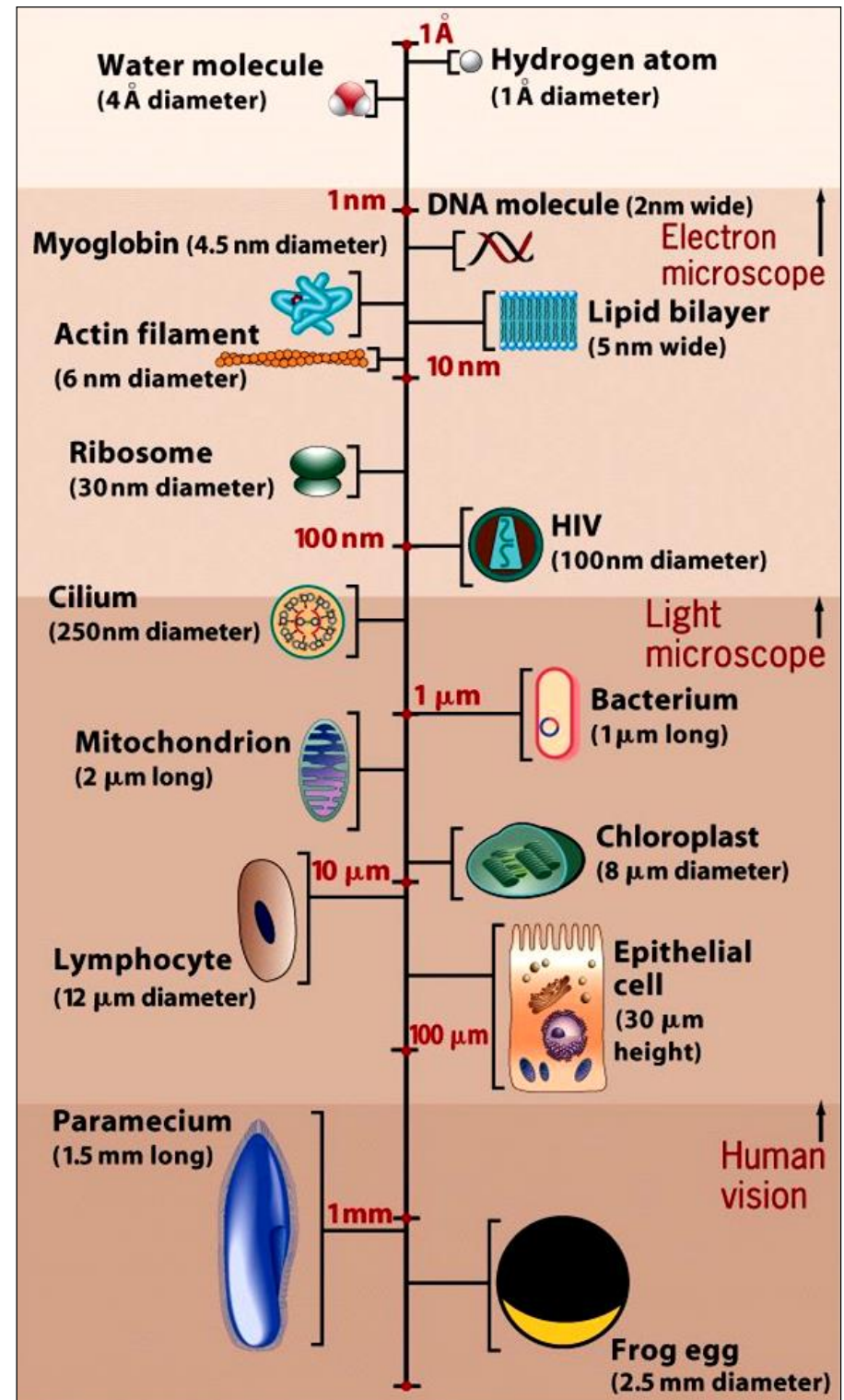
A thin slice of cork, showing
the network of pores a.k.a.
“cells”

Hook called the pores *cells*
because they reminded him
of the cells inhabited by
monks living in a monastery



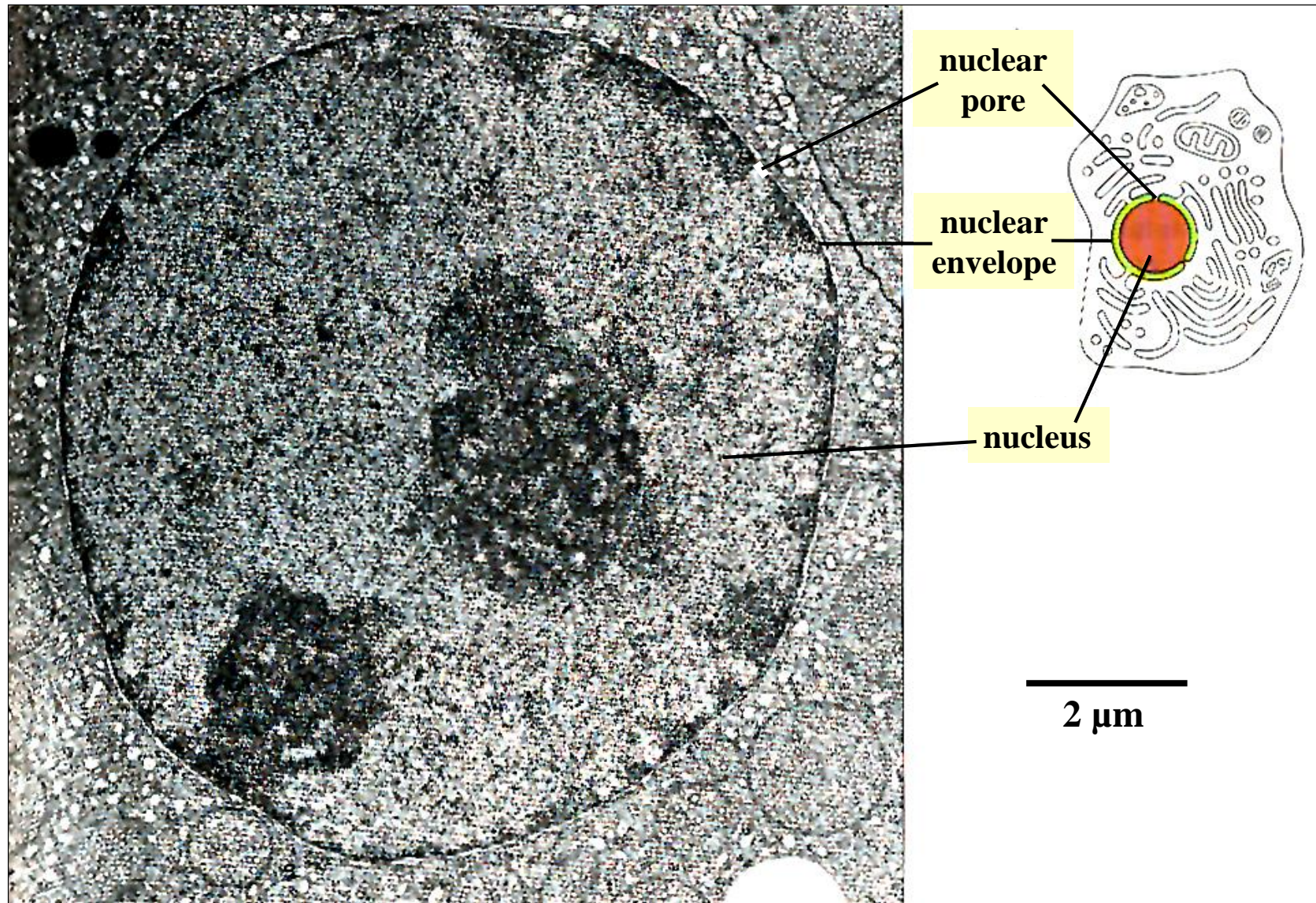
Relative sizes of cells and cell component, and the units in which they are measured

$$1 \text{ m} = 10^6 \mu\text{m} = 10^9 \text{ nm} = 10^{10} \text{ angstrom } (\text{\AA})$$

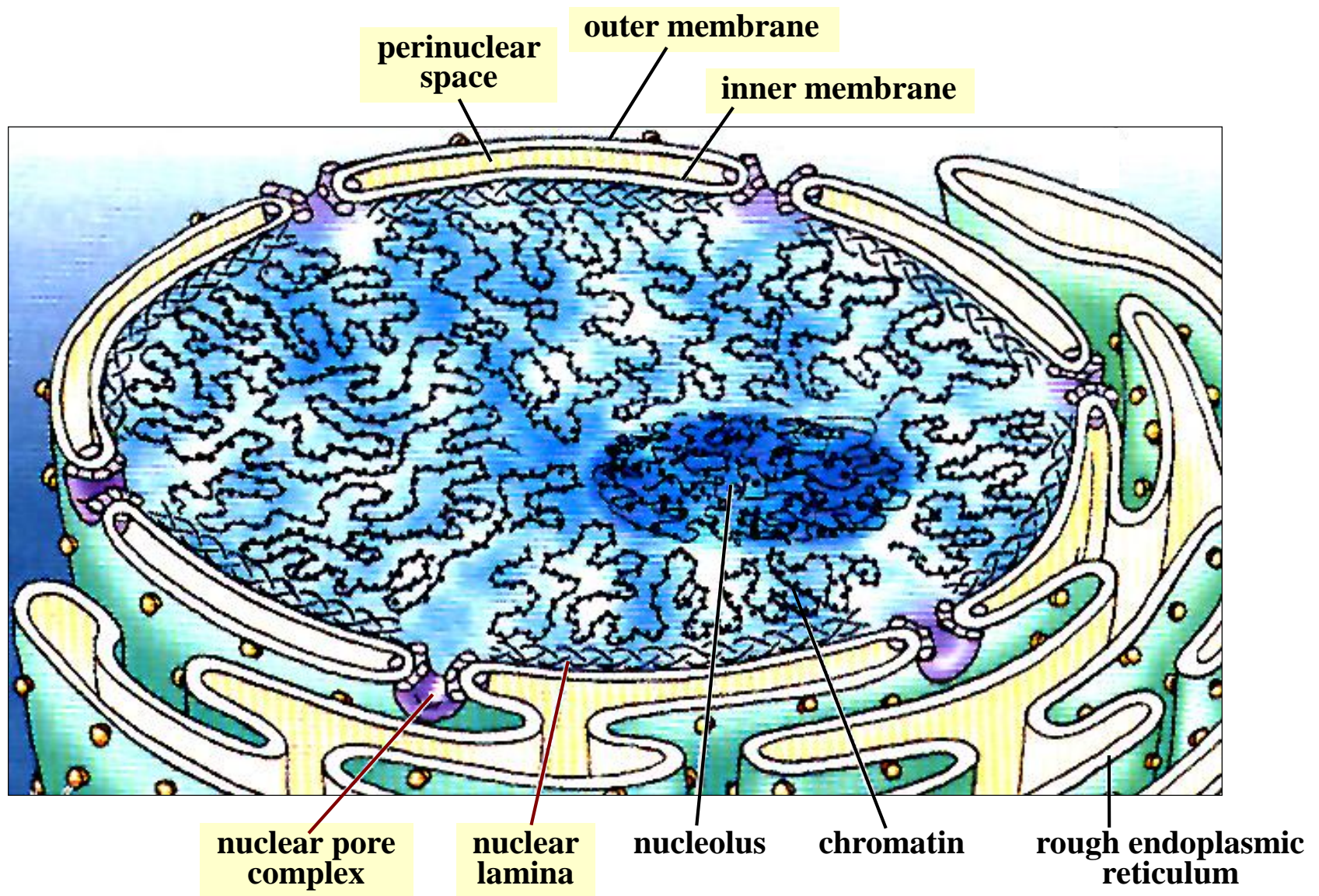


**The nucleus is the information store of
the cell**

**The nucleus contains molecules of *DNA*
(deoxyribonucleic acid) - extremely
long polymers that encode the genetic
specification of the organism**



- The nucleus is surrounded by a double membrane, called the nuclear envelope
- The nucleus communicates with the cytosol via nuclear pores that perforate the envelope



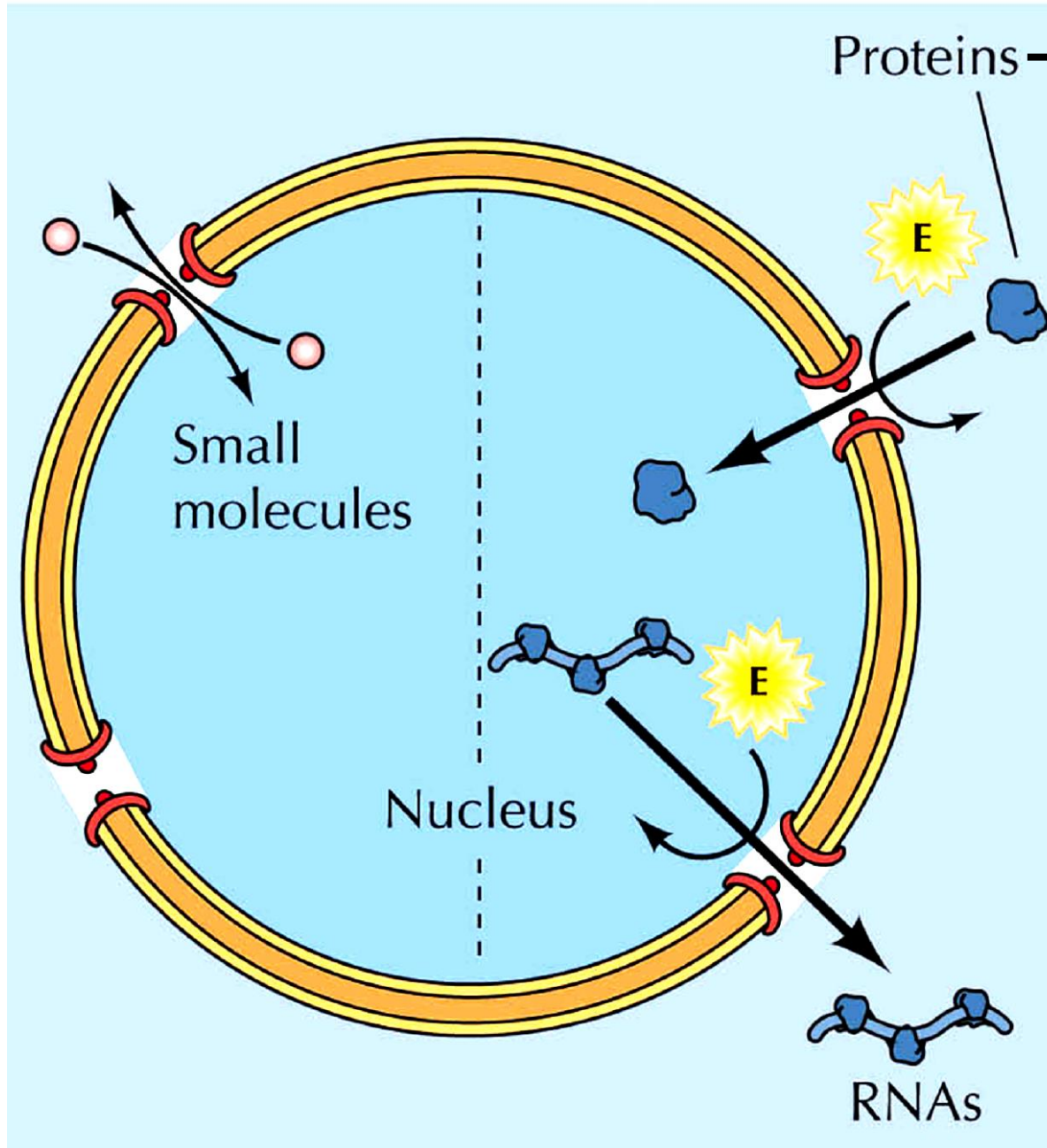
The *nuclear envelope* consists of:

- Two concentric membranes, called the inner and outer nuclear membranes
- The nuclear lamina, a fibrous network that provides structural support to the nucleus
- The nuclear pore complexes, the only channels through which molecules are able to travel between the nucleus and the cytoplasm

Molecular traffic through nuclear pore complexes (NPCs)

Passive diffusion

Energy-dependent
transport



1) **DNA-binding proteins:**

- histones, nonhistone proteins, activators and repressors of transcription

2) **Messenger RNA (mRNA)-binding proteins**

3) **Components of the nucleus (lamins)**

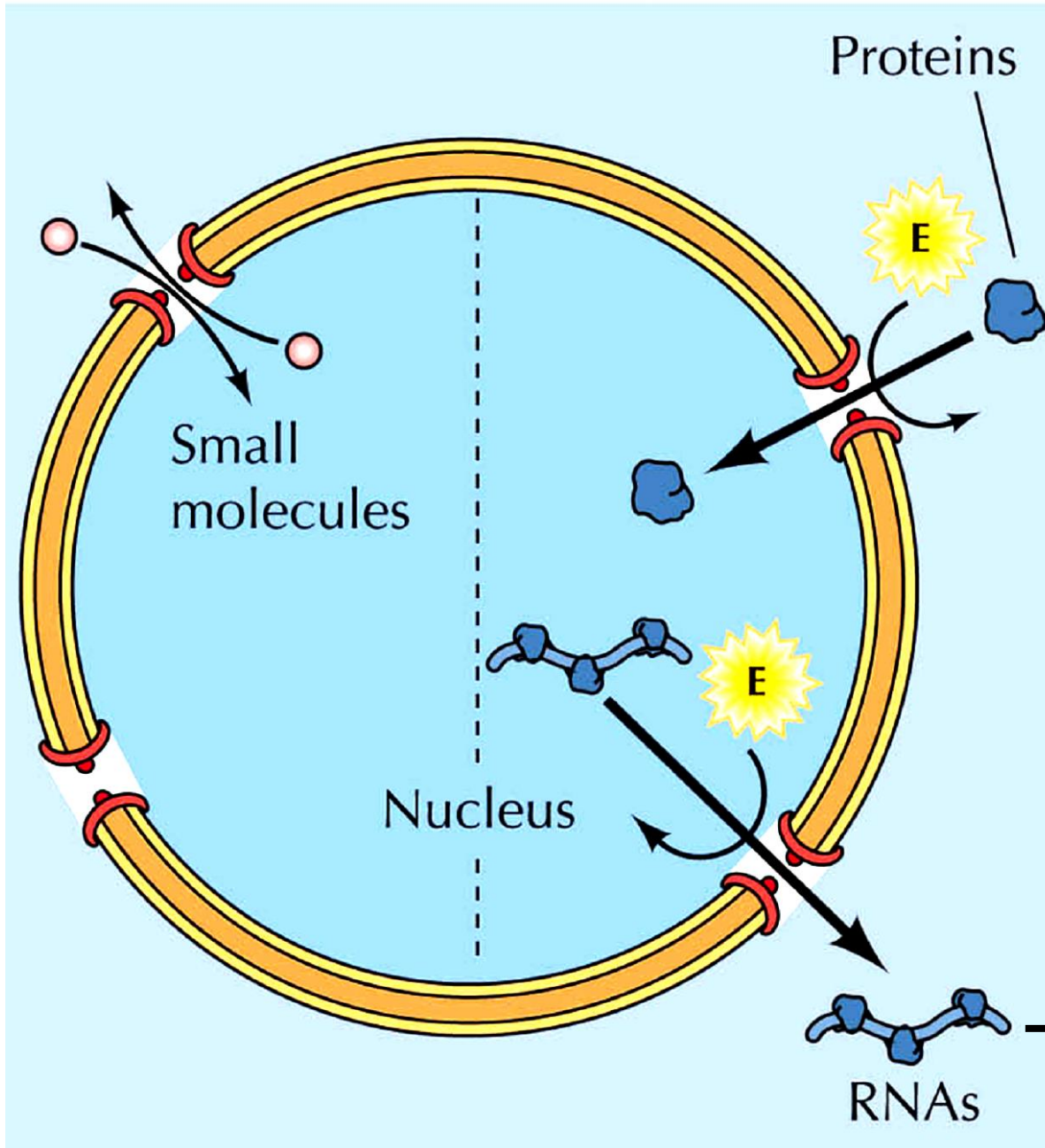
4) **Ribosomal proteins**

5) **Shuttling nuclear transport receptors (importins): deliver other proteins to the nucleus**

Molecular traffic through nuclear pore complexes (NPCs)

Passive diffusion

Energy-dependent
transport



1) mRNAs (together with mRNA-binding proteins)

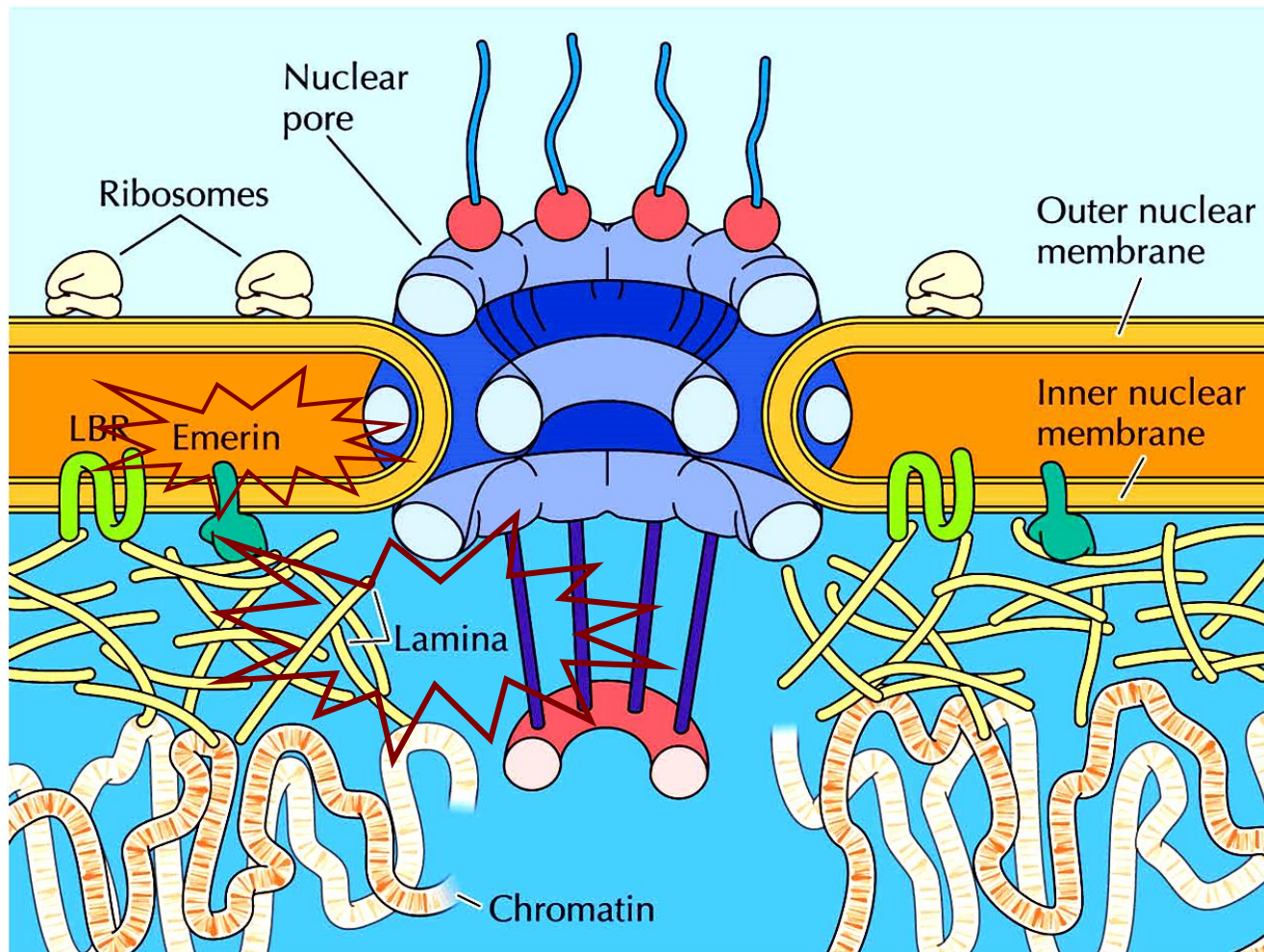
2) tRNAs (transport RNAs)

3) 40S and 60S ribosomal subunits (complexes of ribosomal RNAs [rRNAs] and ribosomal proteins]

RNAs

Molecular medicine: Nuclear lamina diseases

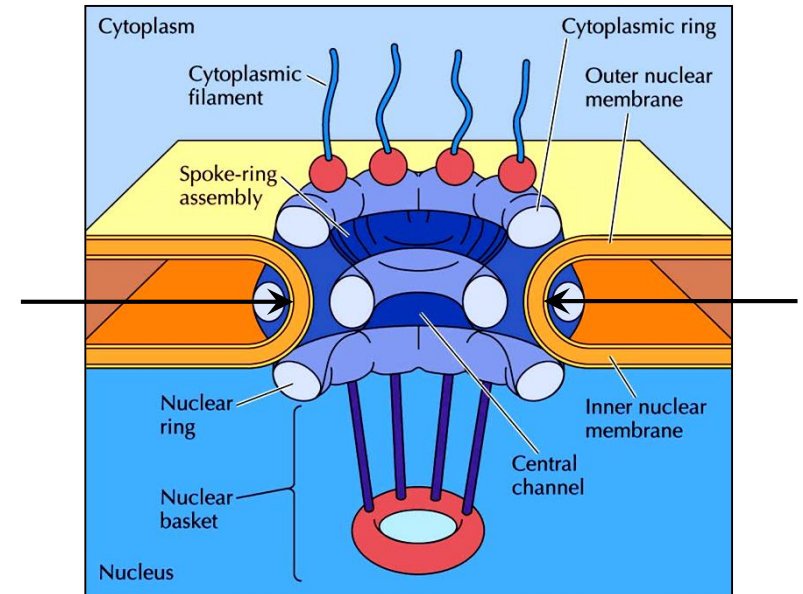
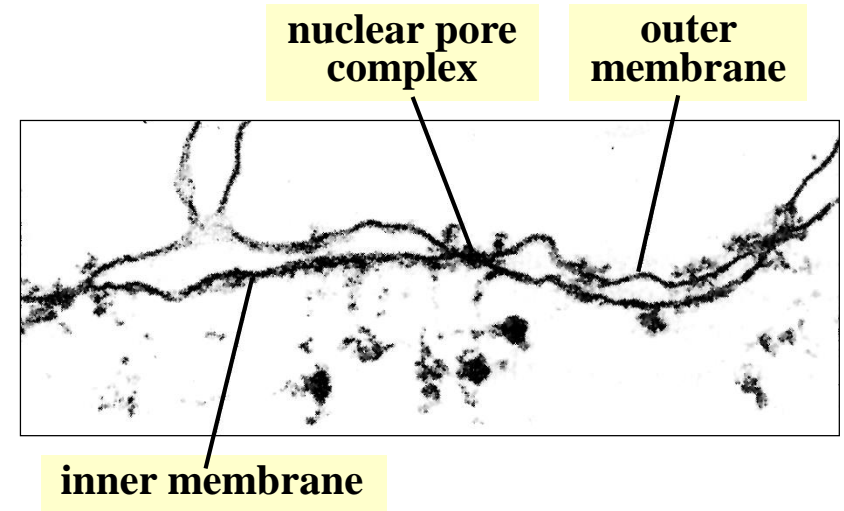
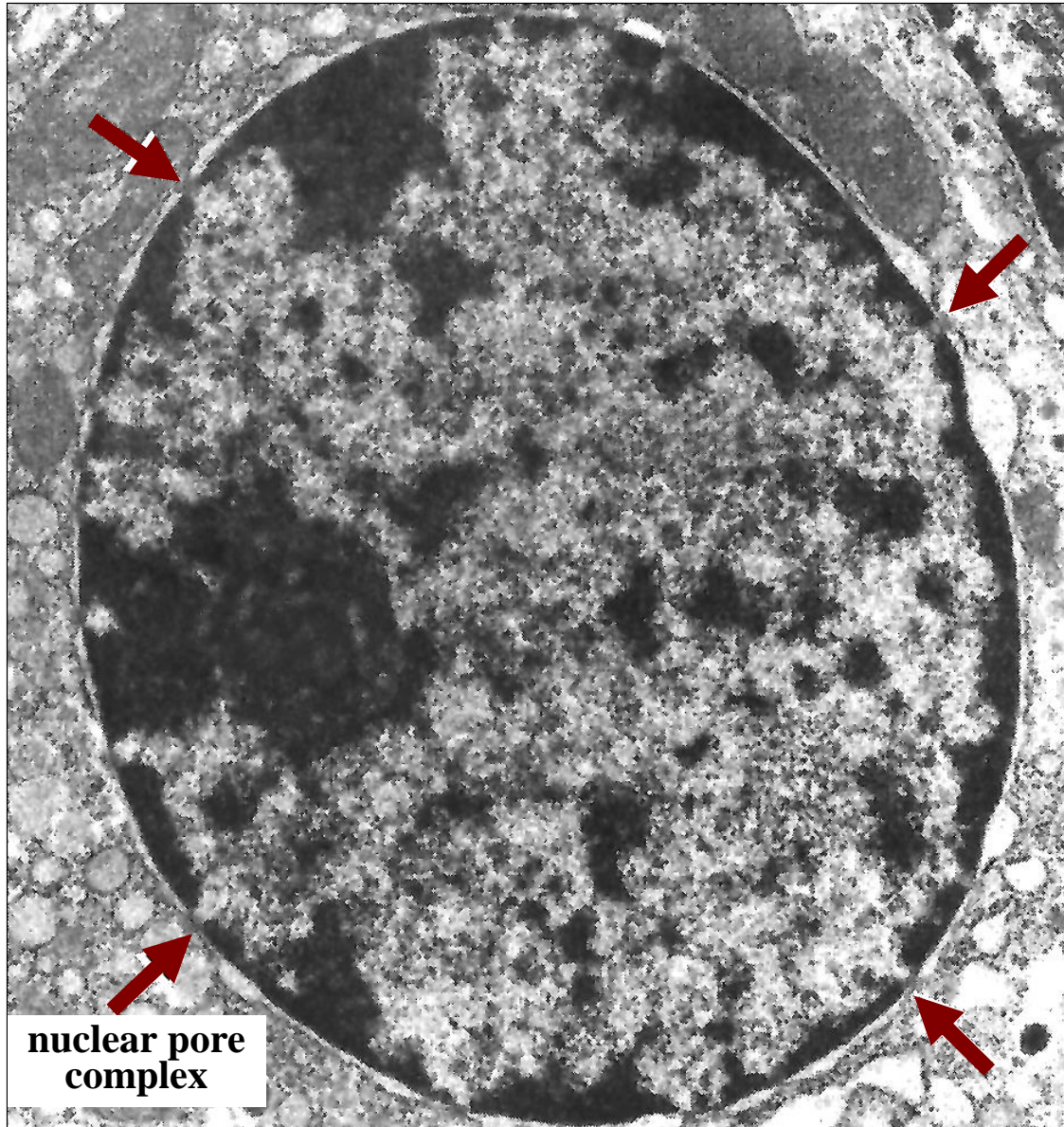
- **Emery-Dreifuss muscular dystrophy** (the elbows, neck and heels become stiff, heart problems) & **Hutchinson-Gilford progeria** (premature aging)
- **Is caused by mutations in two genes:**
 - 1) coding for emerin (an inner nuclear membrane protein, a lamin-binding protein)
 - 2) coding for lamin (a major component of the nuclear lamina)



• **Possible mechanism:**

The correct interaction of lamins with the nuclear envelope is essential for normal tissue-specific transcription of certain genes)

LBR = lamin B receptor



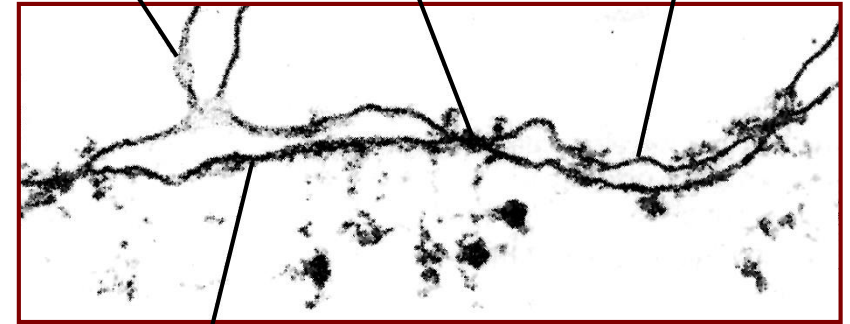
The inner and outer nuclear membranes *are joined* at nuclear pore complexes

The outer nuclear membrane is *continuous* with the rough endoplasmic reticulum (ER), and the space between the inner and outer nuclear membranes is *continuous* with the lumen of the rough ER

endoplasmic reticulum

nuclear pore complex

outer membrane

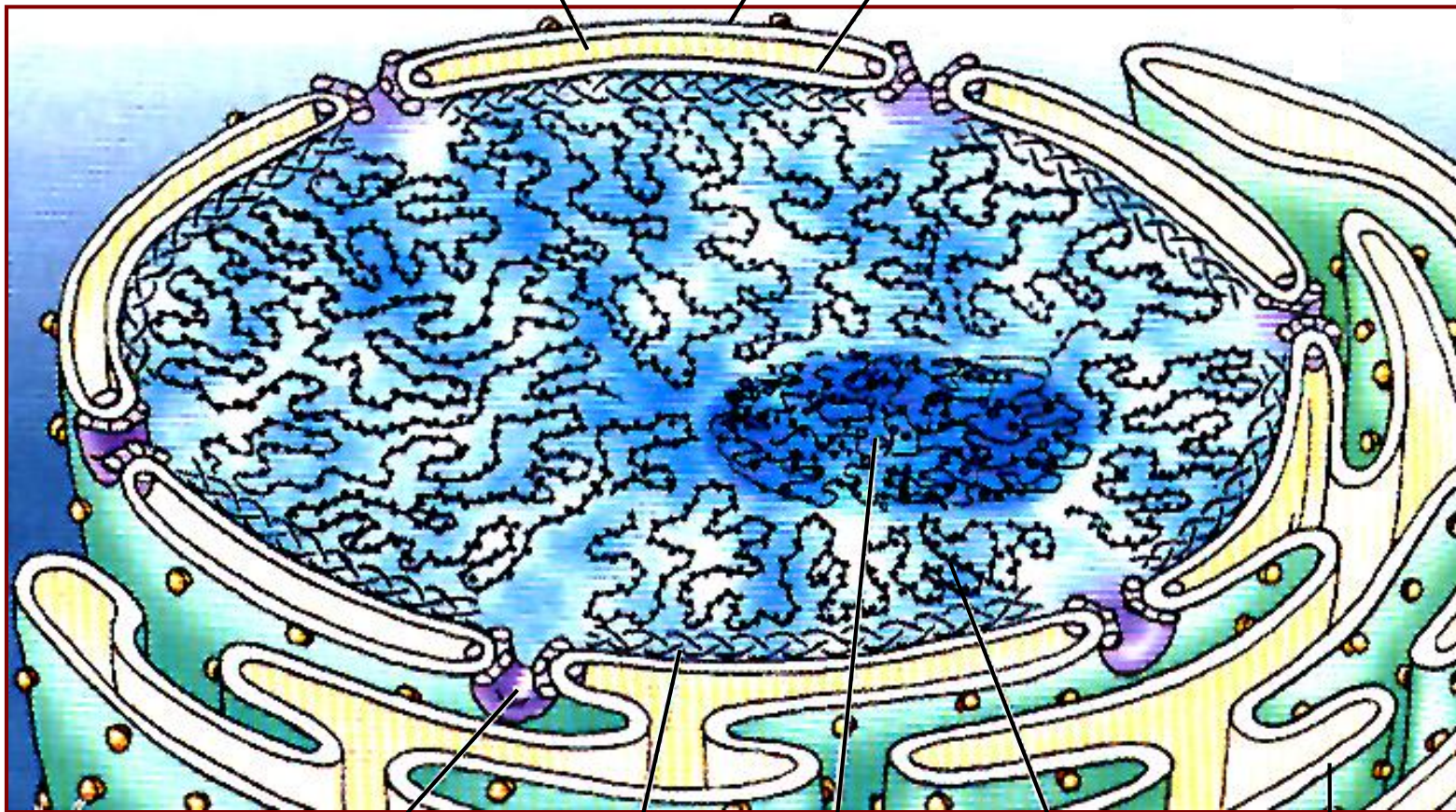


inner membrane

perinuclear space

outer membrane

inner membrane



nuclear pore complex

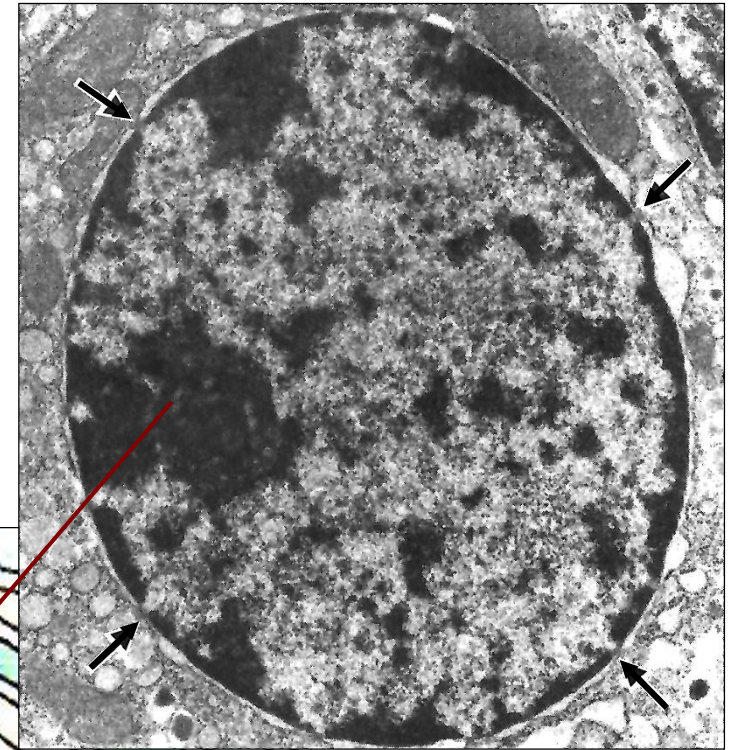
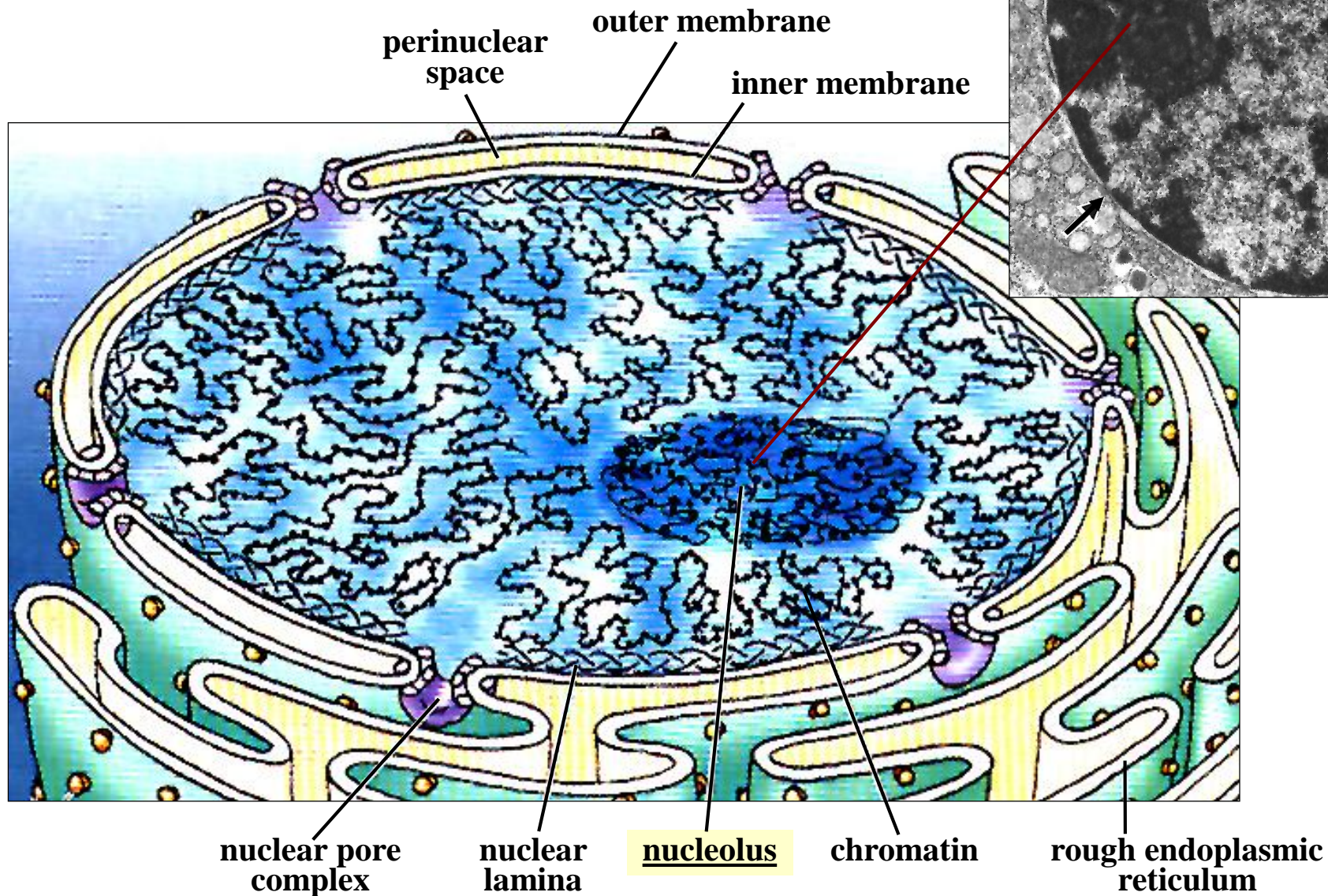
nuclear lamina

nucleolus

chromatin

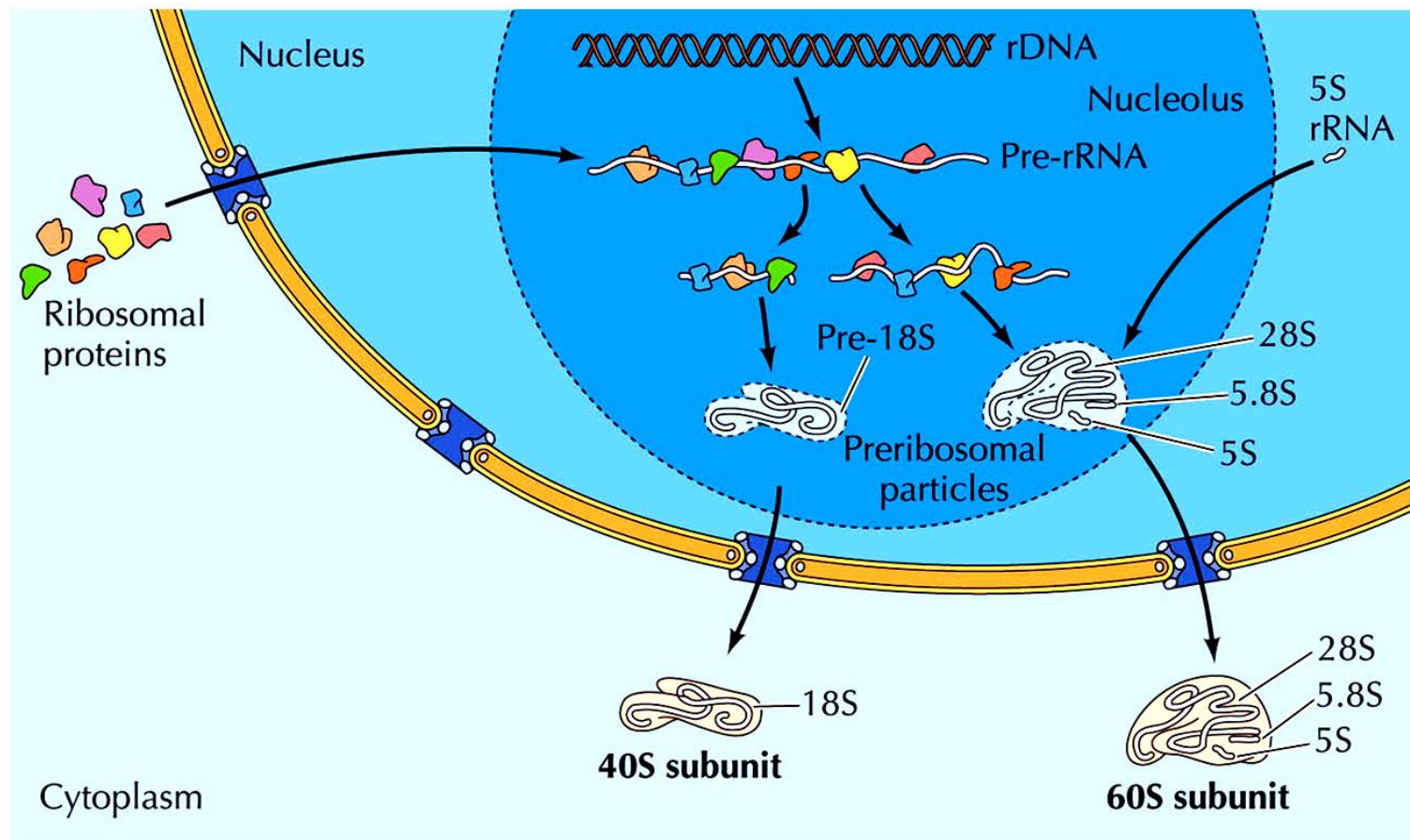
rough endoplasmic reticulum

A *suborganelle* of the nucleus, the nucleolus, is a factory where the cell's *ribosomes* are assembled

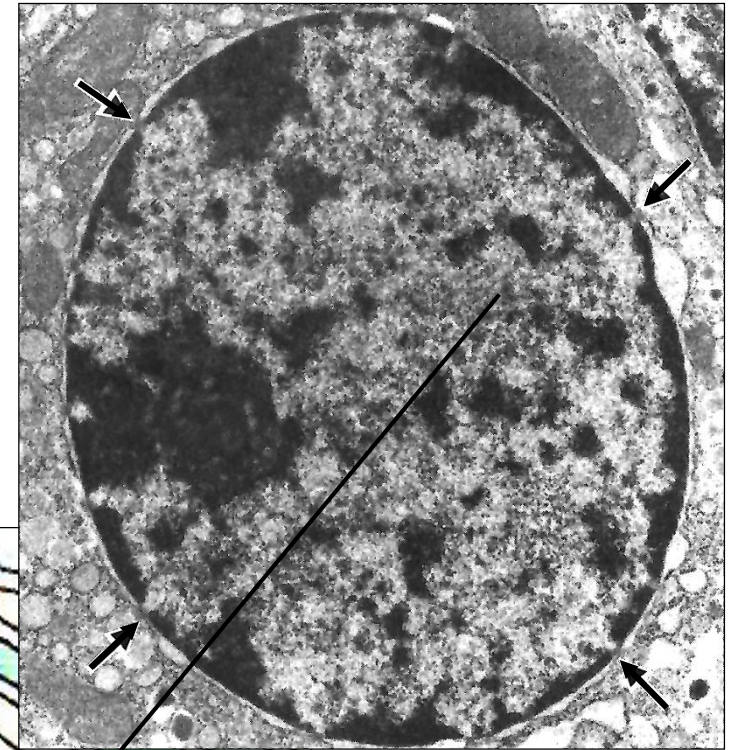
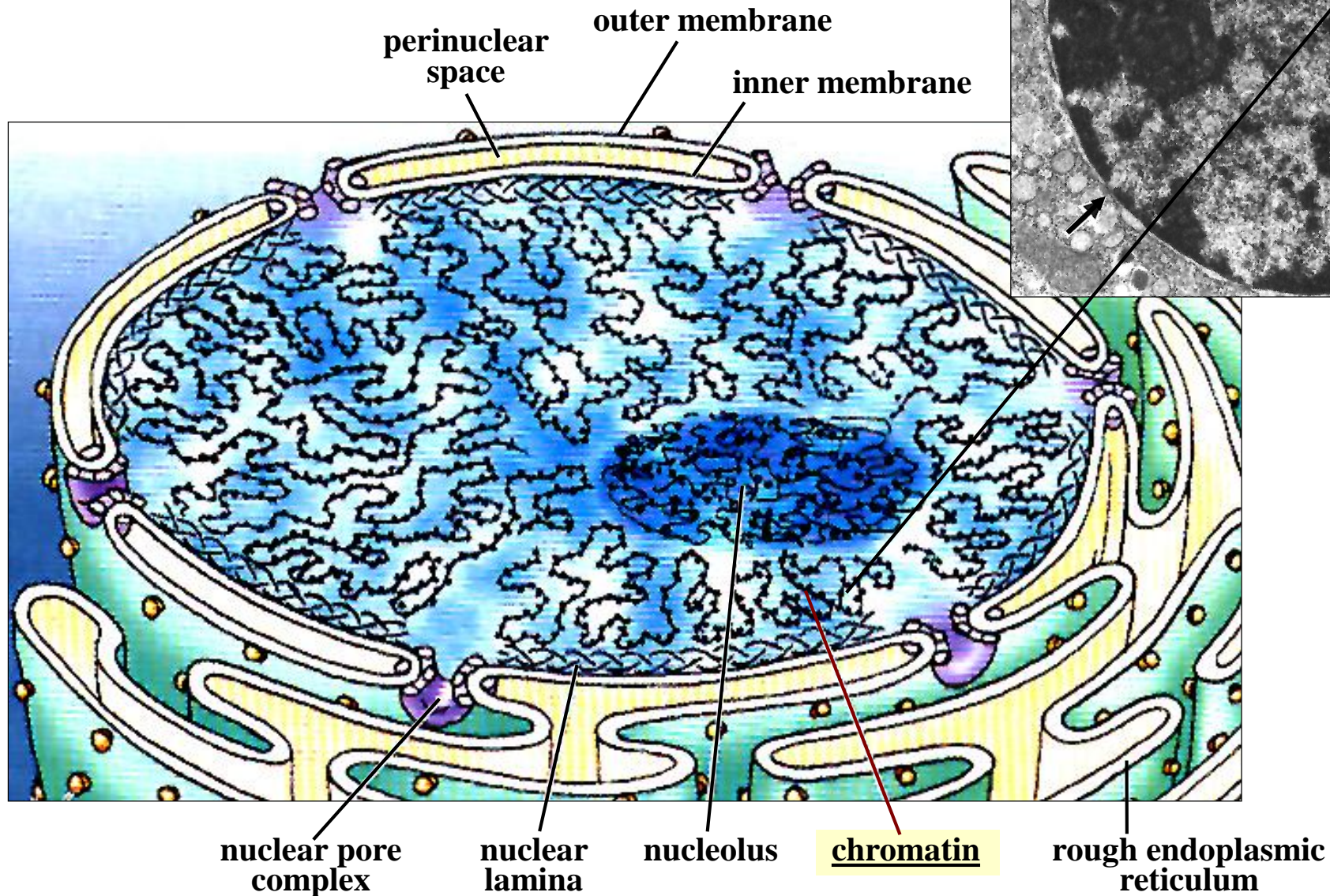


Ribosome assembly in the nucleolus:

- 1) Ribosomal proteins are imported to the nucleus from the cytoplasm
- 2) These ribosomal proteins are then delivered to the nucleolus and assemble on pre-rRNA (pre-ribosomal RNA)
- 3) The pre-rRNA is cleaved to form several rRNAs
- 4) Ribosomal proteins and rRNAs assemble to form the 40S and 60S ribosomal subunits
- 5) These subunits are exported to the cytoplasm

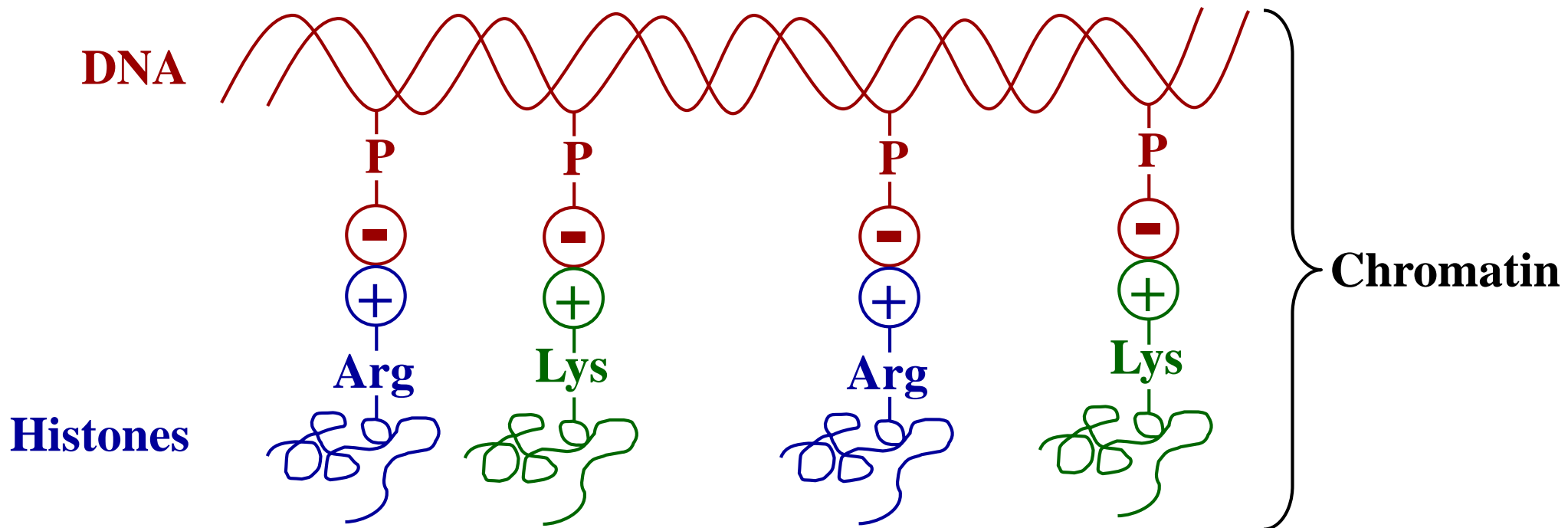


The chromosomal DNA is packed into chromatin fibers with the aid of specialized proteins



Chromatin

- The complexes between eukaryotic DNA and proteins are called chromatin
- Chromatin contains about twice as much protein as DNA
- The major proteins of chromatin are the histones
- Histones are small proteins (11 to 23 kDa) containing a high proportion of basic amino acids (arginine and lysine) that facilitate binding to the negatively charged DNA molecule



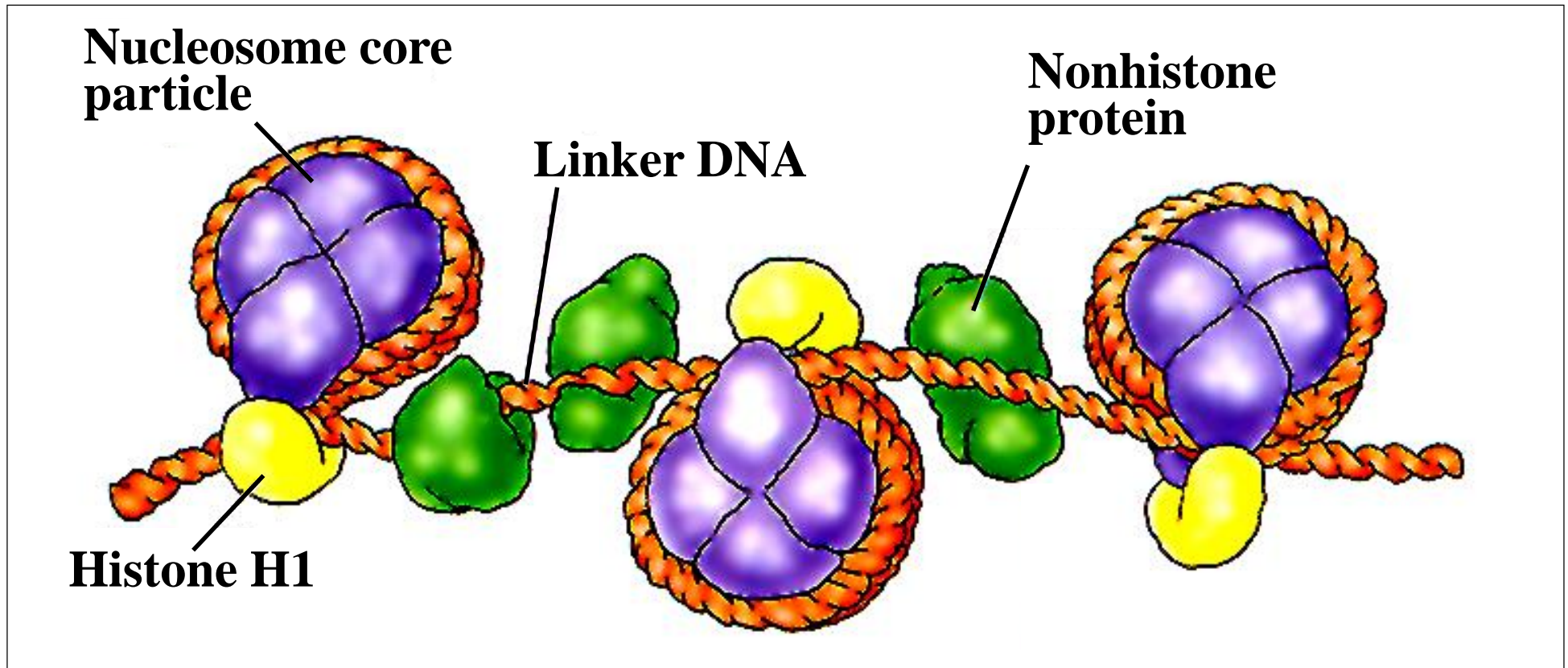
There are 5 major types of histones:

H1 H2A H2B H3 H4

These histones are very similar among different species of eukaryotes

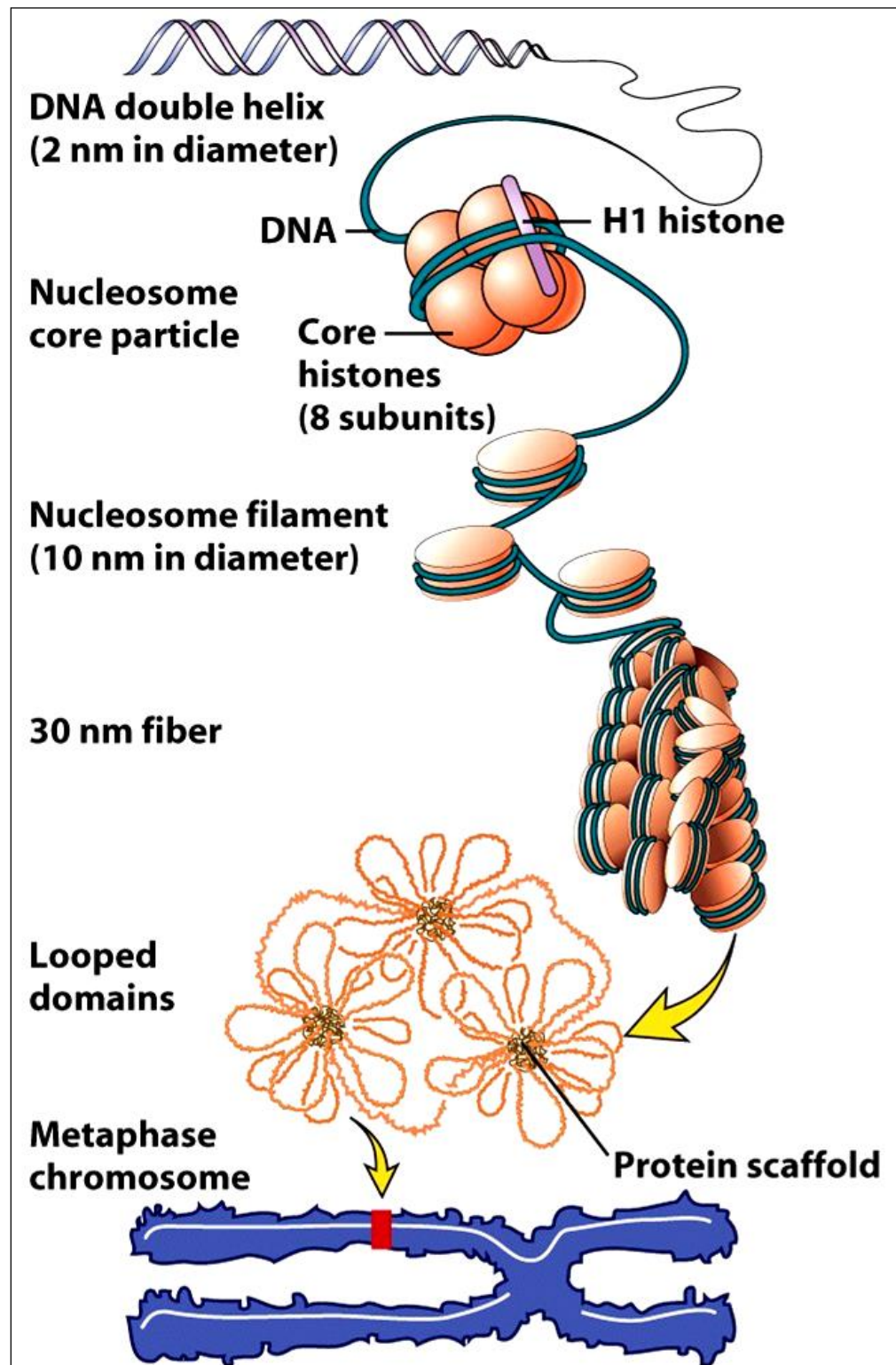
- In addition, chromatin contains an approximately equal mass of nonhistone chromosomal proteins
- There are more than a 1000 different types of the nonhistone chromosomal proteins

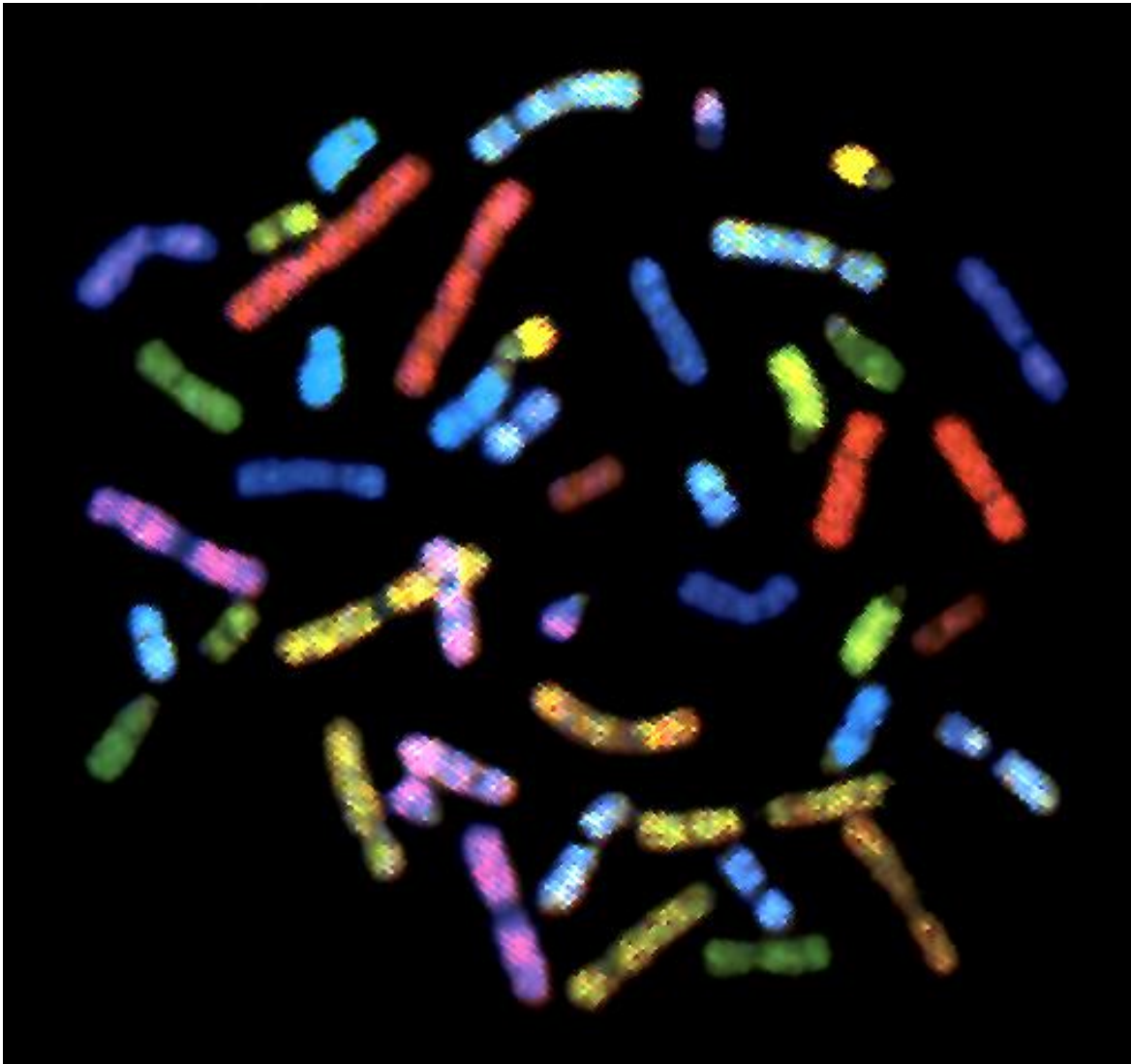
The basic structural unit of chromatin is called the *nucleosome*



- The DNA is wrapped around histones H2A, H2B, H3 and H4 in *nucleosome core particle* and sealed by histone H1
- Nonhistone proteins bind to the *linker DNA* between nucleosome core particles

Levels of organization of chromatin





As a cell prepares to divide into two daughter cells, its chromatin condenses into chromosomes that can be distinguished in the light microscope

Light micrograph of the 46 human chromosomes (23 pairs of chromosomes)

A specific technique permits each of the chromosome pairs to be shown in a different color

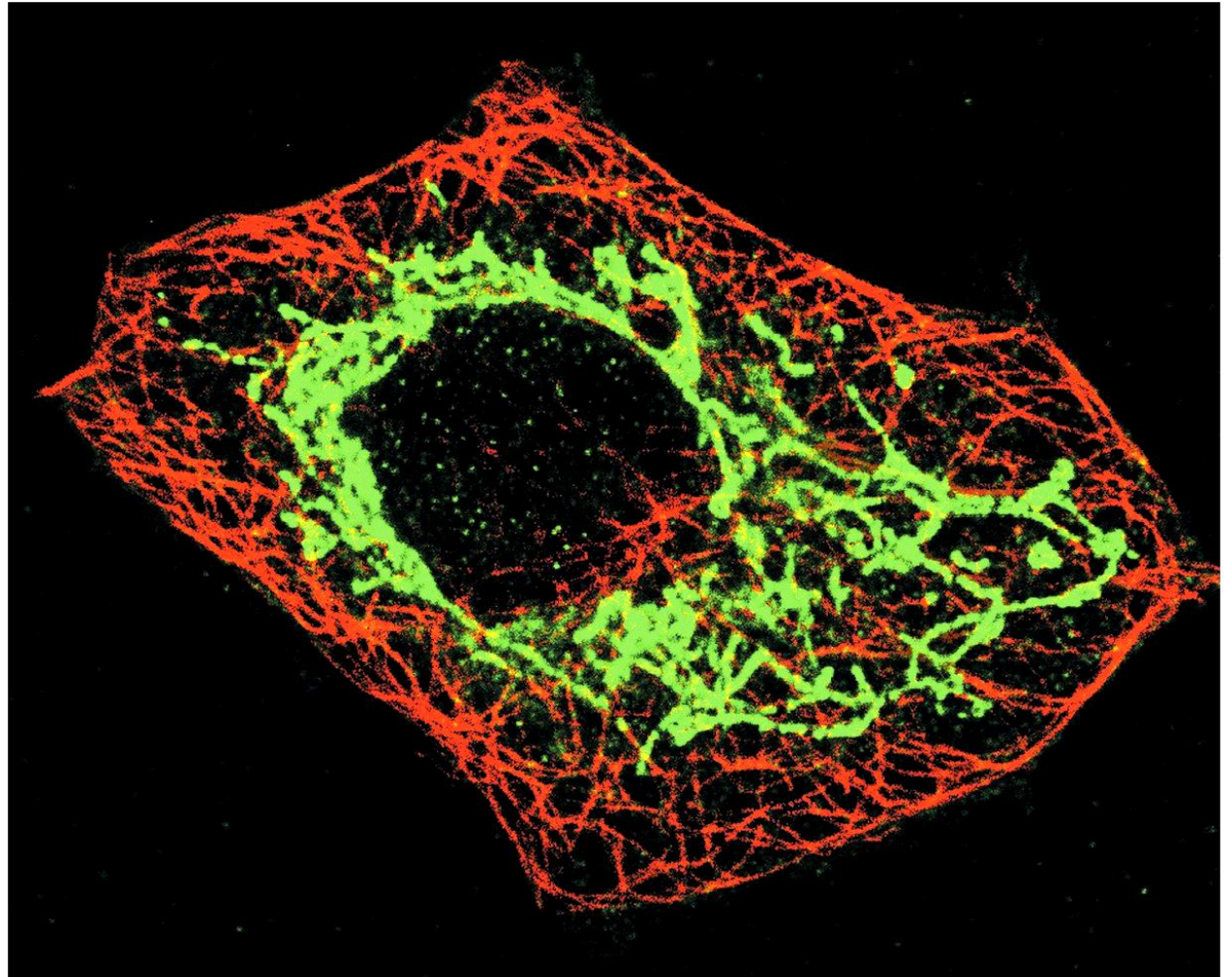
Mitochondria play a critical role in the generation of metabolic energy in eukaryotic cells

- **These organelles oxidize carbohydrates and lipids to produce the basic chemical fuel adenosine triphosphate (ATP) by the process of oxidative phosphorylation**
- **ATP is then used to drive a variety of energy-requiring reactions within cells**
- **Because mitochondria consume oxygen and release carbon dioxide in the course of ATP production, the entire process is called cellular respiration, from its similarity to breathing**

- Depending upon the cell type and physiological conditions, mitochondria can have a very different overall structure

Mitochondria can appear as a highly branched, interconnected tubular network

Micrograph of a mammalian fibroblast that has been fixed and stained with fluorescent antibodies to a mitochondrial (green) and a cytoskeletal (red) proteins

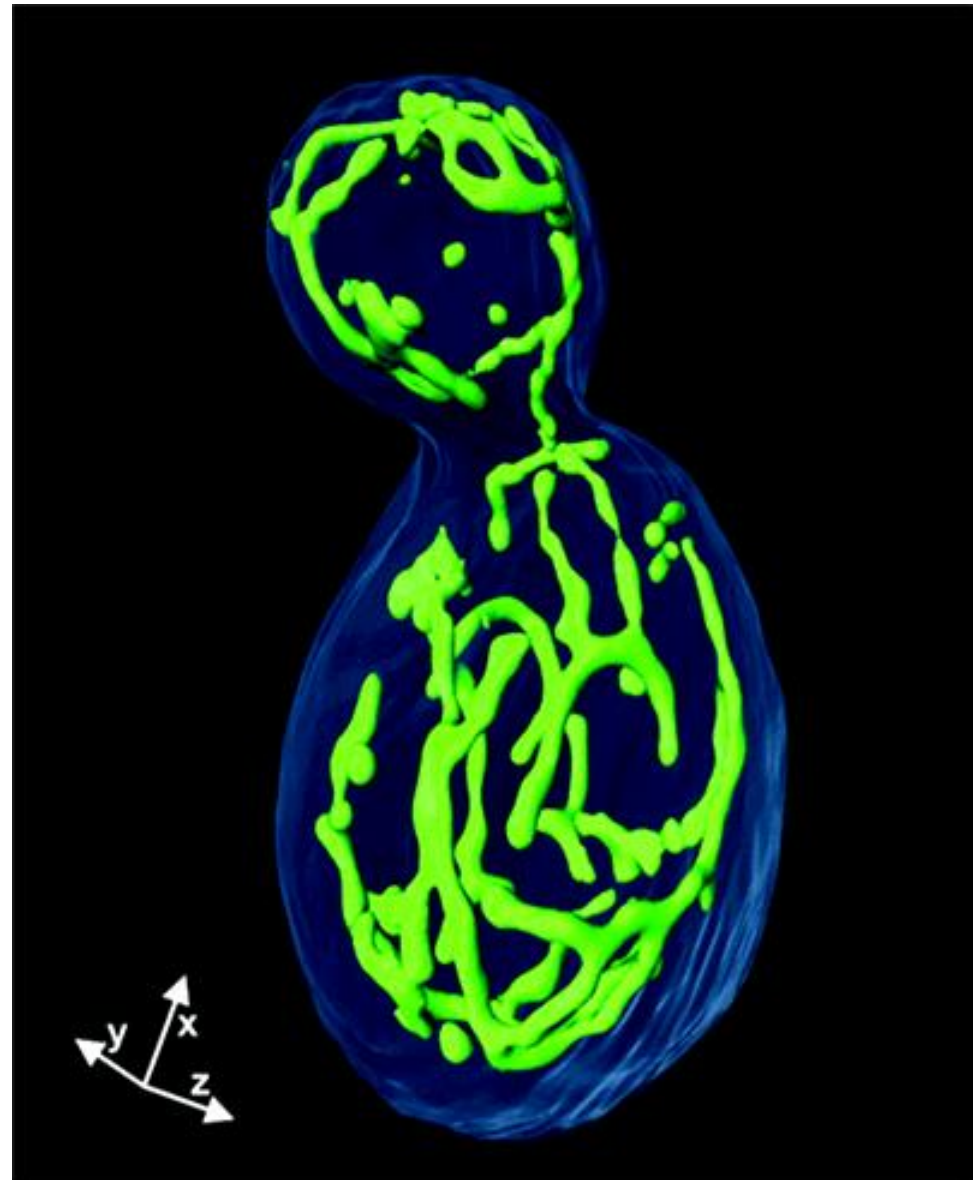


- **The balance between fusion and fission is a major determinant of mitochondrial morphology**

Observations of fluorescently labeled mitochondria within living cells have shown them to be dynamic organelles capable of dramatic changes in shape

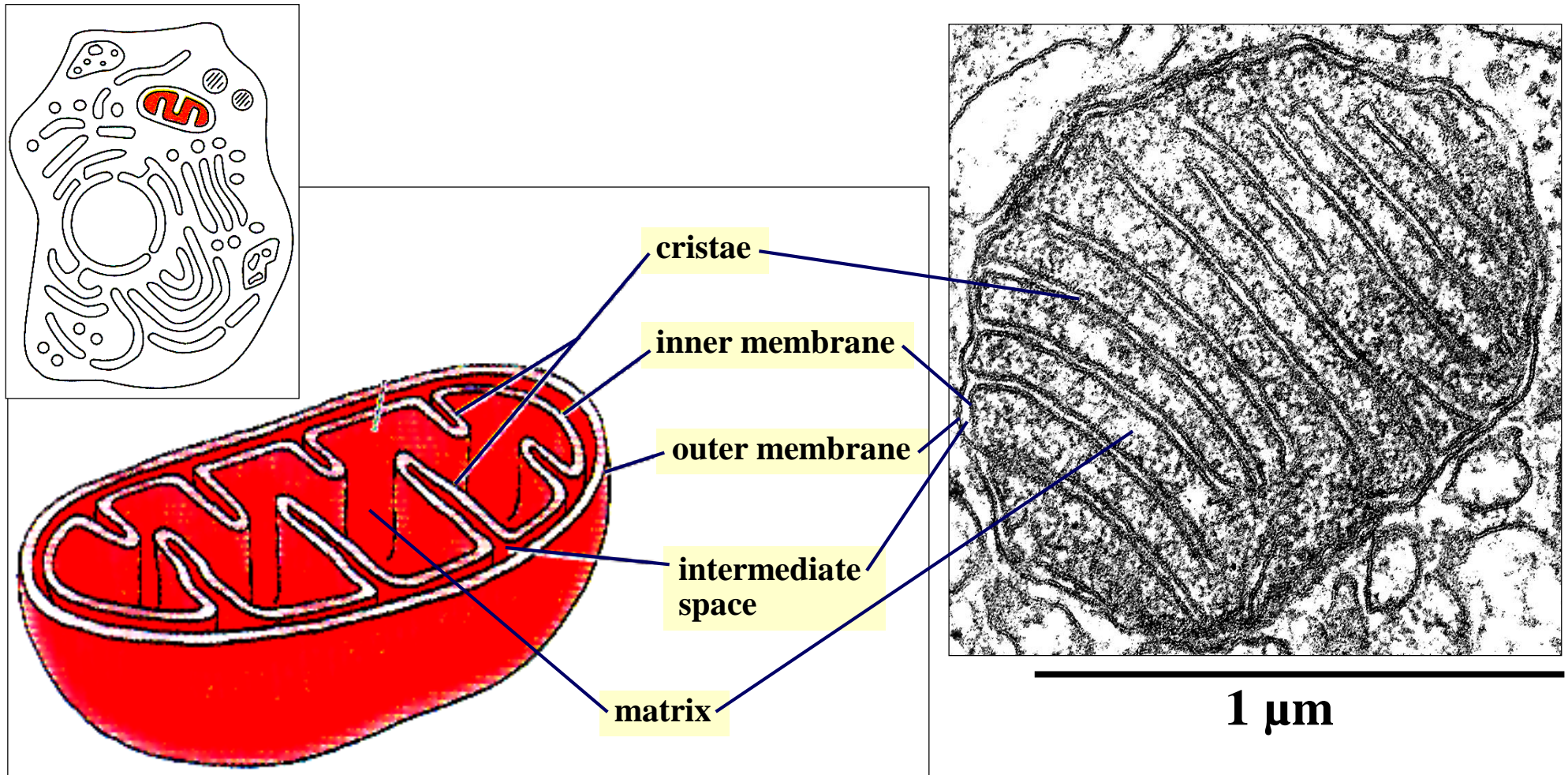
Mitochondria can fuse with one another, or split in two

Branched mitochondrial network in a budding yeast cell expressing mitochondria-targeted GFP



Organization of mitochondria

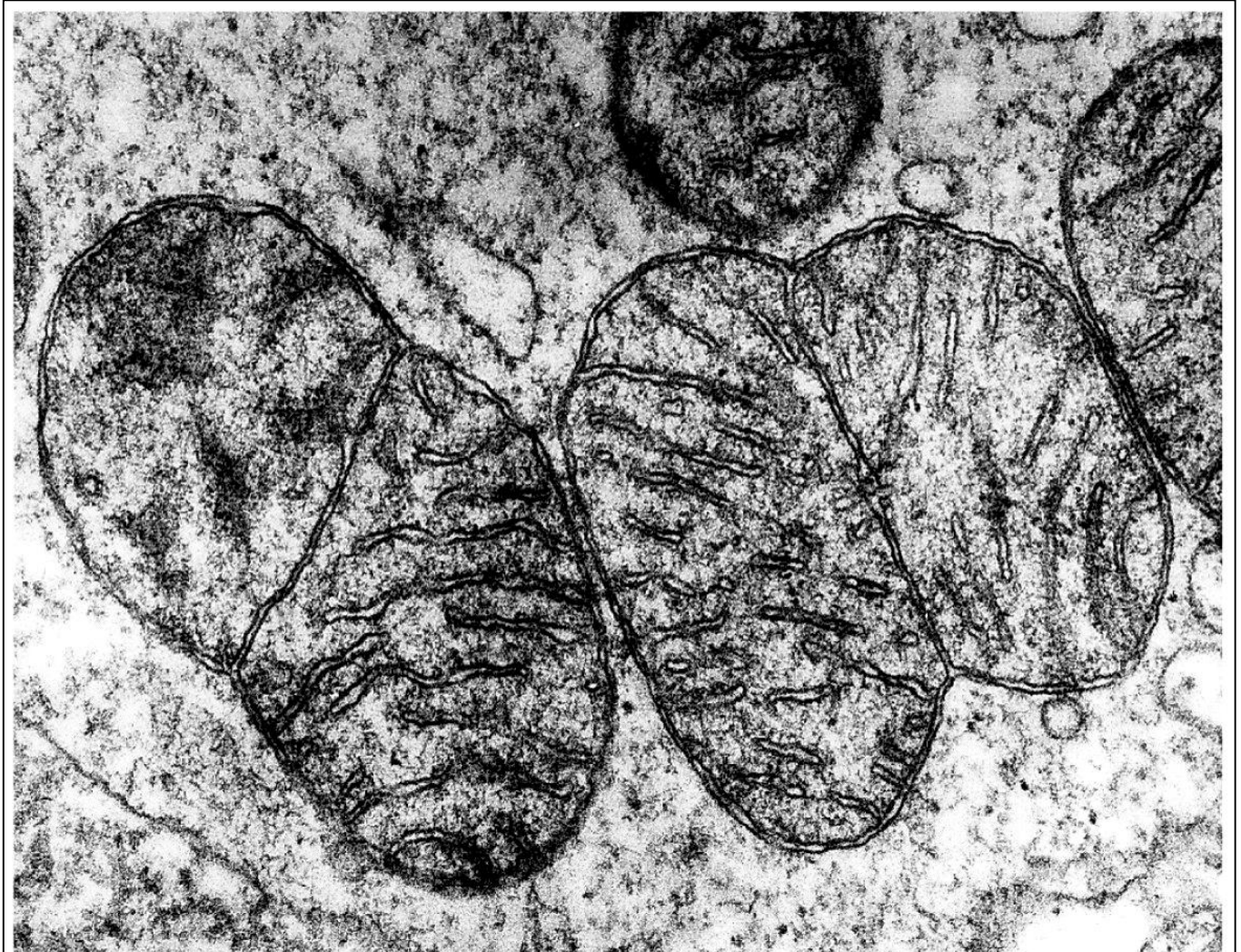
- Mitochondria are surrounded by a double-membrane system, consisting of inner and outer mitochondrial membranes separated by an intermembrane space
- The inner membrane forms numerous folds (cristae), which extend into the interior (or matrix) of the organelle. Its *surface area* is substantially increased by its folding into cristae



- **Mitochondria arise from preexisting mitochondria by a process called fission**

**Transmission
electron
micrograph of an
insect cell**

**Two mitochondria
in the process of
fission**

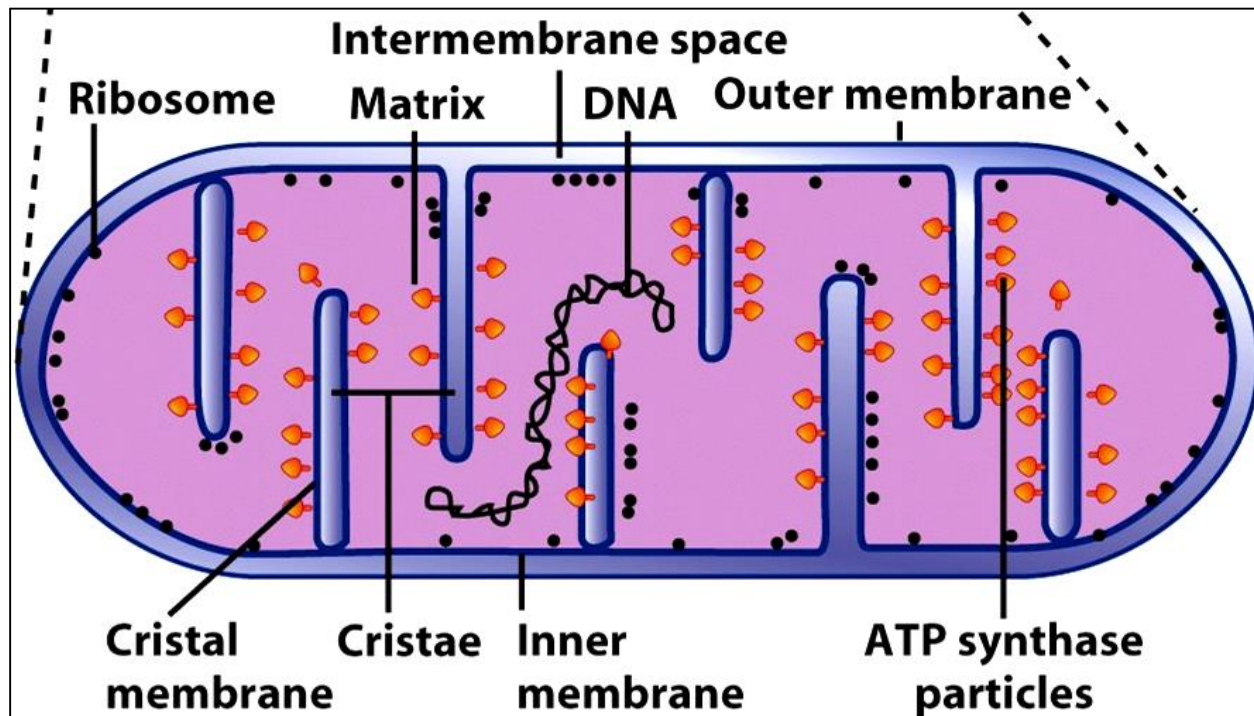


0.4 μm

Functional roles of mitochondrial compartments

The mitochondrial matrix contains:

- Enzymes responsible for the oxidative breakdown of carbohydrates and lipids via the citric acid cycle
- Several identical copies of circular DNA molecules (mitochondrial genome)
- Special mitochondrial ribosomes
- Various enzymes required for expression of the mitochondrial genes



Functional roles of mitochondrial compartments

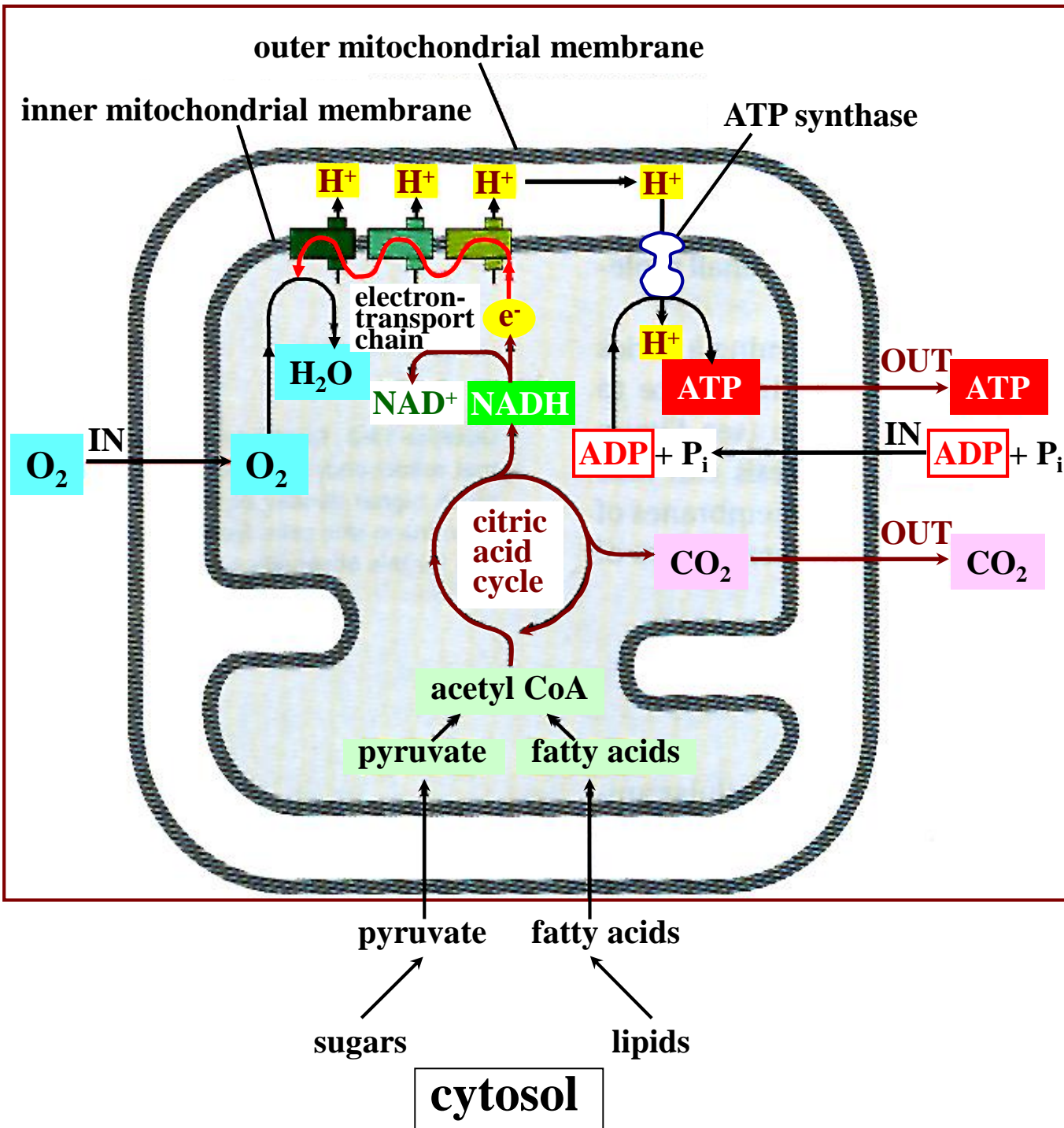
Inner mitochondrial membrane:

- **The principal site of ATP synthesis**

Outer membrane:

- **Contains enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix**

Energy-generating metabolism in mitochondria



This electron transport generates a proton gradient across the inner membrane, which is used to drive the production of ATP by ATP synthase

In the process of oxidative phosphorylation, high-energy electrons from **NADH** and **FADH₂** are then passed along the electron-transport chain in the inner membrane to oxygen (**O₂**)

Pyruvate and fatty acids enter the mitochondrion, are broken down to acetyl CoA, and are then metabolized by the citric acid cycle, which produces **NADH** and **FADH₂**

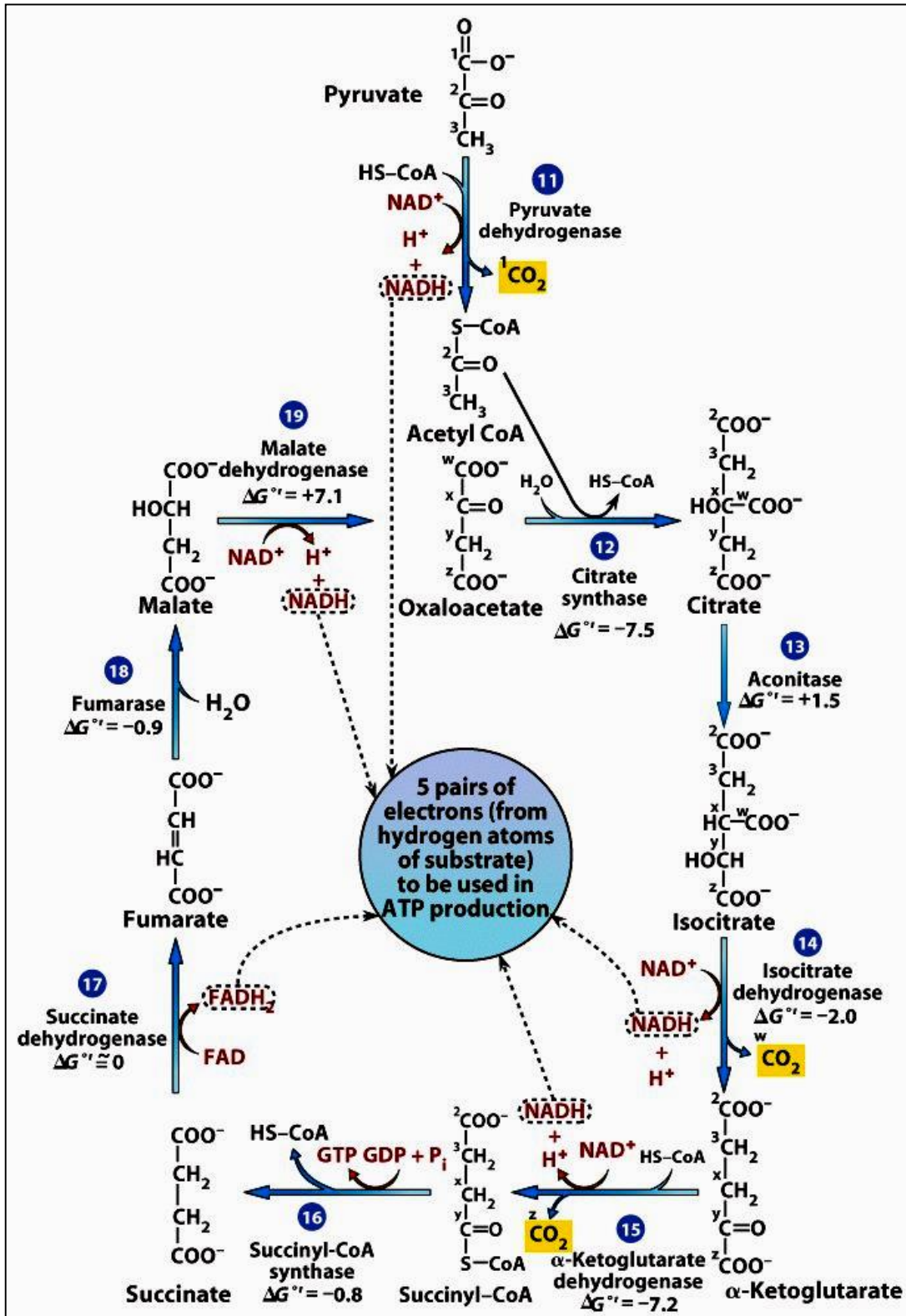
The tricarboxylic acid (TCA) cycle

a.k.a. the Krebs cycle after the person who formulated it

a.k.a. the citric acid cycle after the 1st compound formed in it

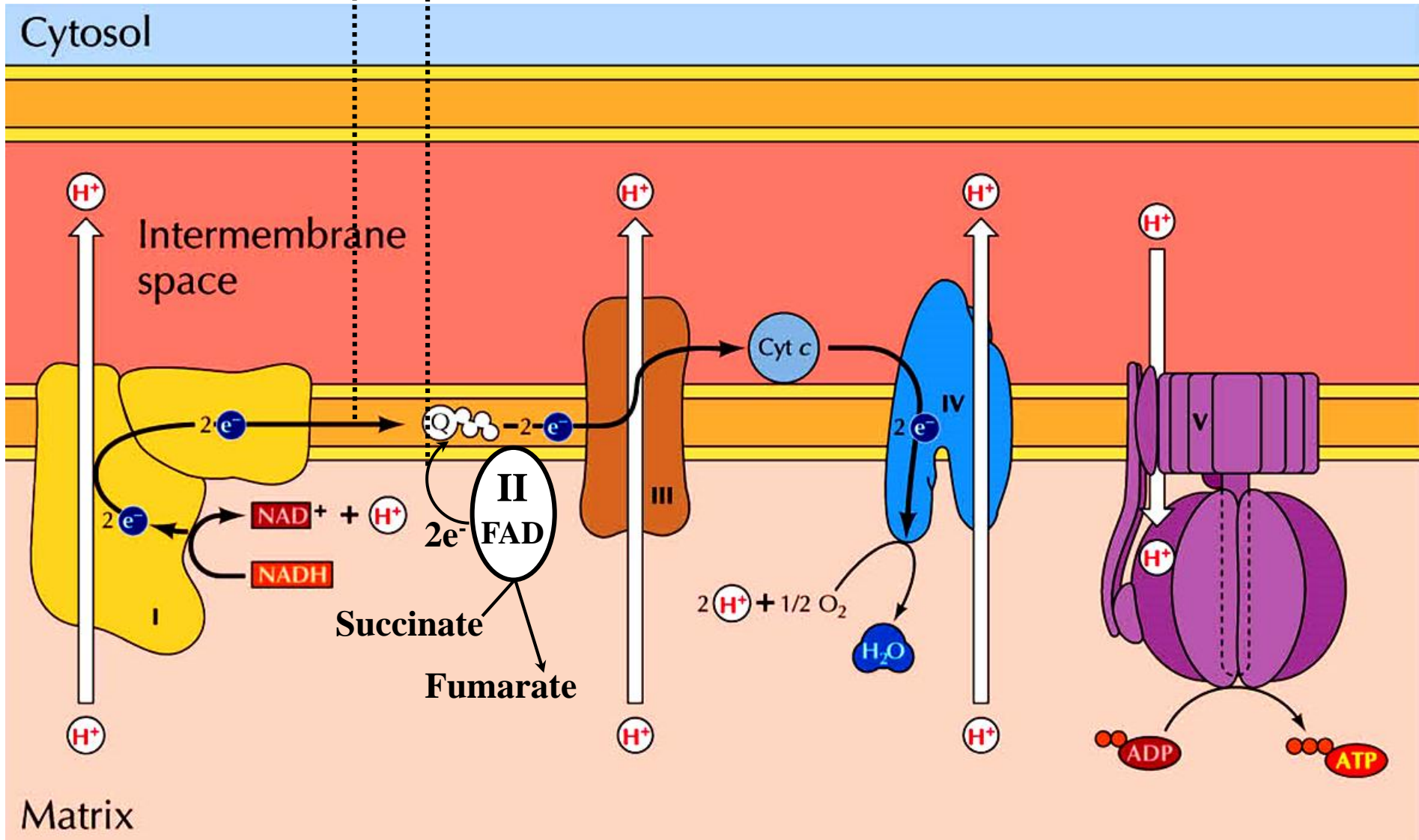
Five pairs of electrons are removed from substrate molecules by pyruvate dehydrogenase and the enzymes of the TCA cycle

These high-energy electrons are transferred to NAD^+ or FAD and then passed down the electron-transport chain for use in ATP production



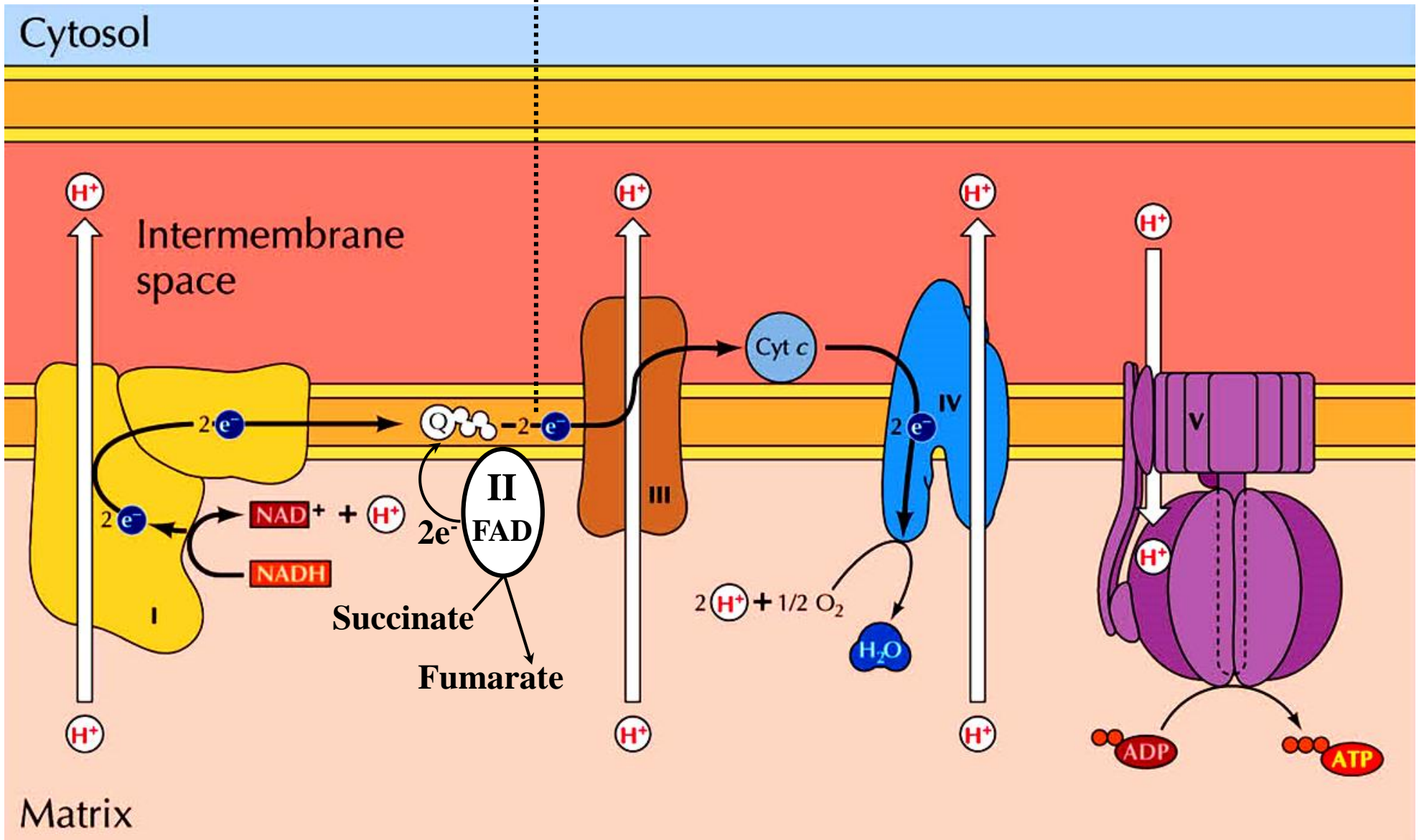
The electron transport chain

1) Electrons derived from either NADH (via complex I or NADH dehydrogenase) or FADH₂ (complex II or succinate dehydrogenase) are passed to ubiquinone (Q or UQ), a lipid-soluble molecule



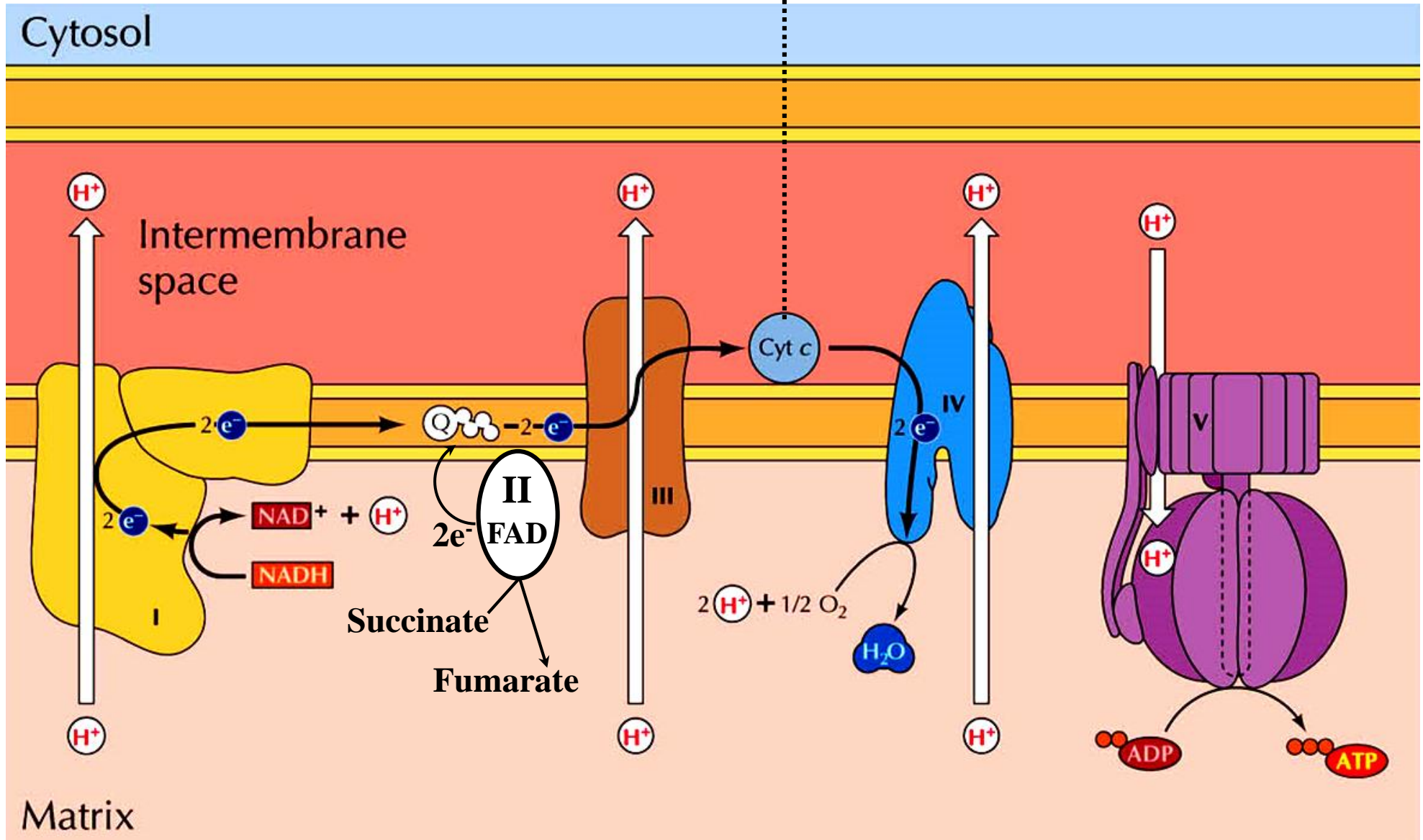
The electron transport chain

2) The electrons are then passed from coenzyme Q (a.k.a. ubiquinone) to complex III (a.k.a. the cytochrome b-c₁ complex)



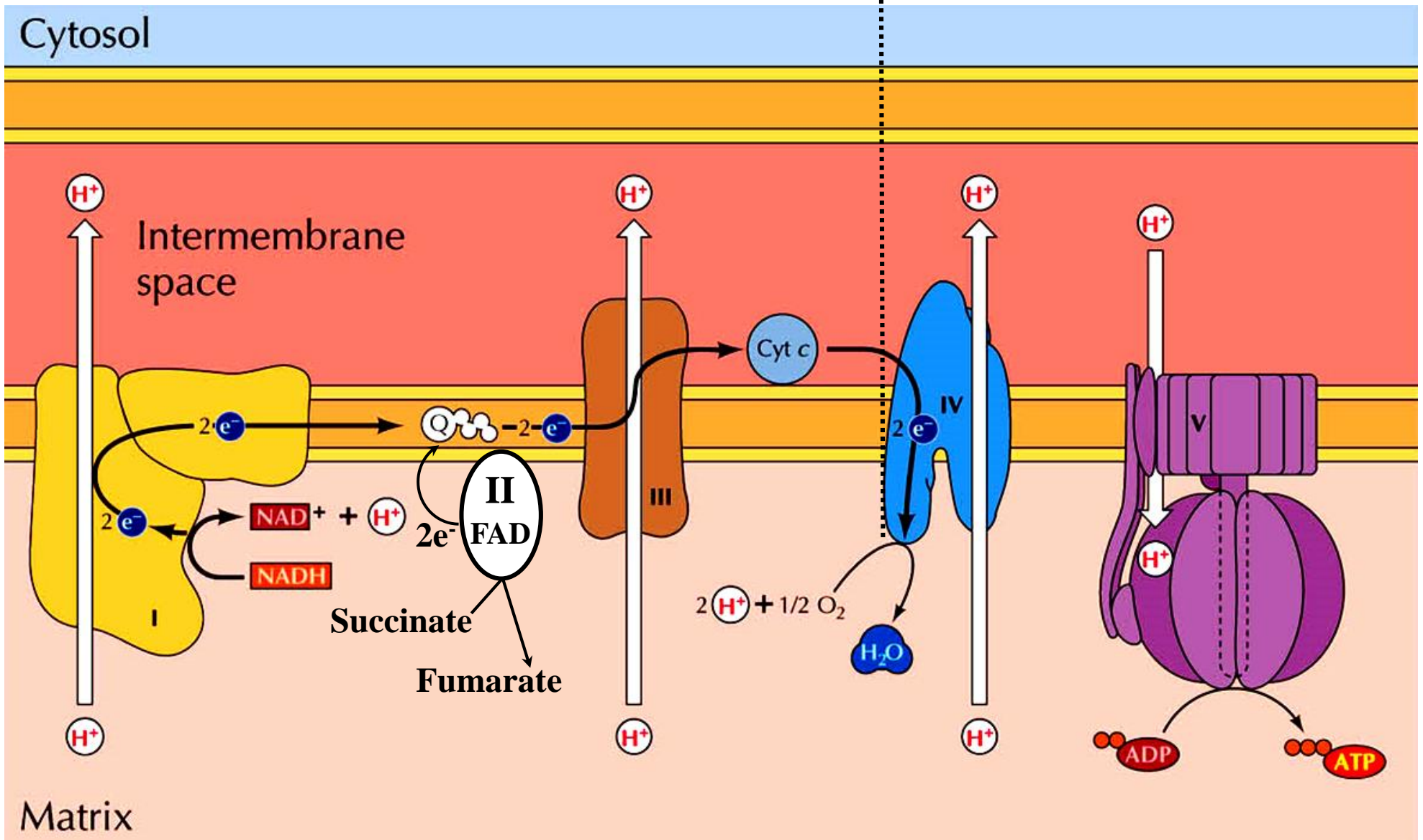
The electron transport chain

3) Electrons are then transferred to cytochrome c, a peripheral membrane protein, which carries electrons to complex IV (aka the cytochrome oxidase complex)



The electron transport chain

4) Complex IV transfers electrons to molecular oxygen to form H₂O within the matrix

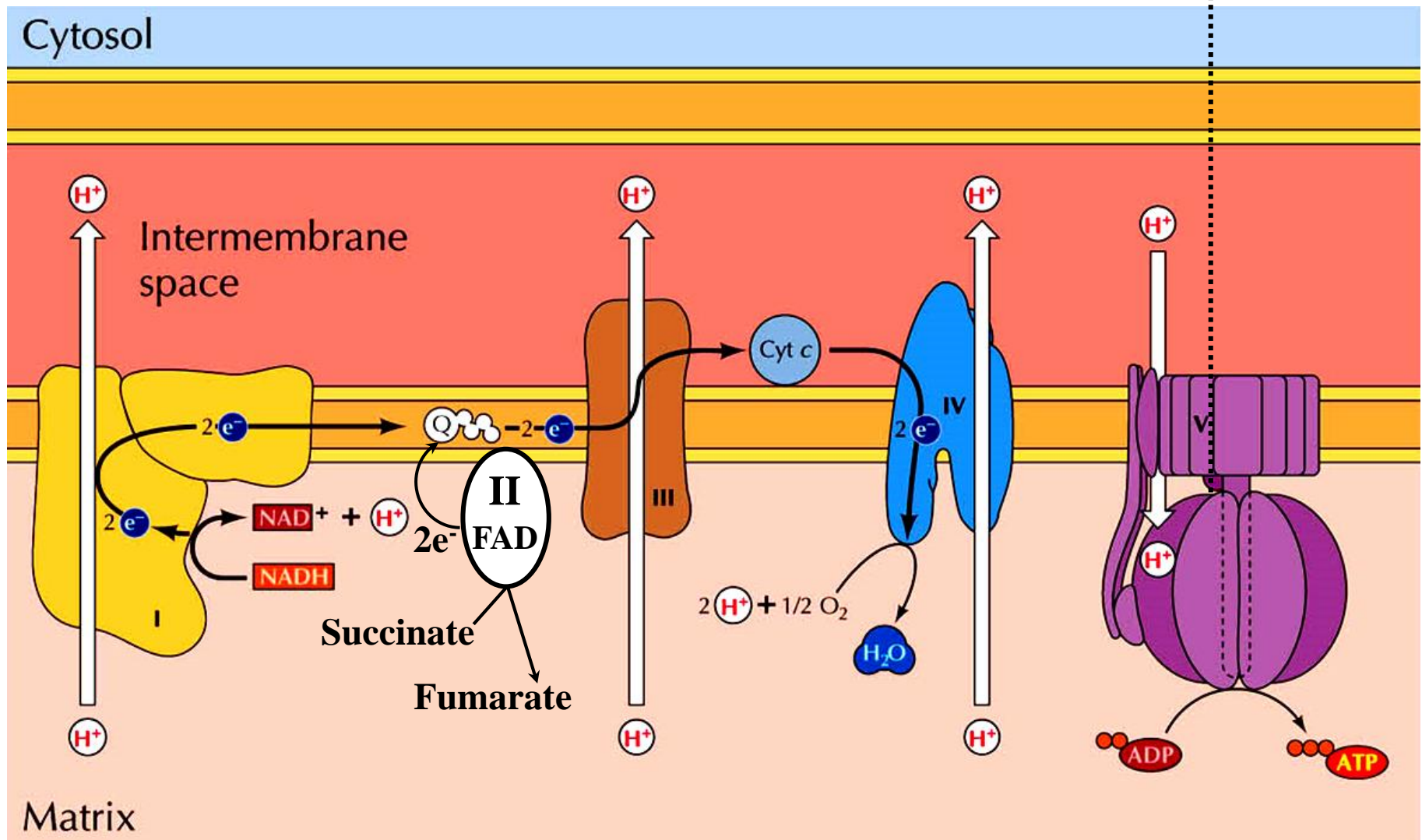


The electron transport chain

5a) The electron transfers in complexes I, III and IV generate energy, which is used to pump protons from the matrix to the intermembrane space

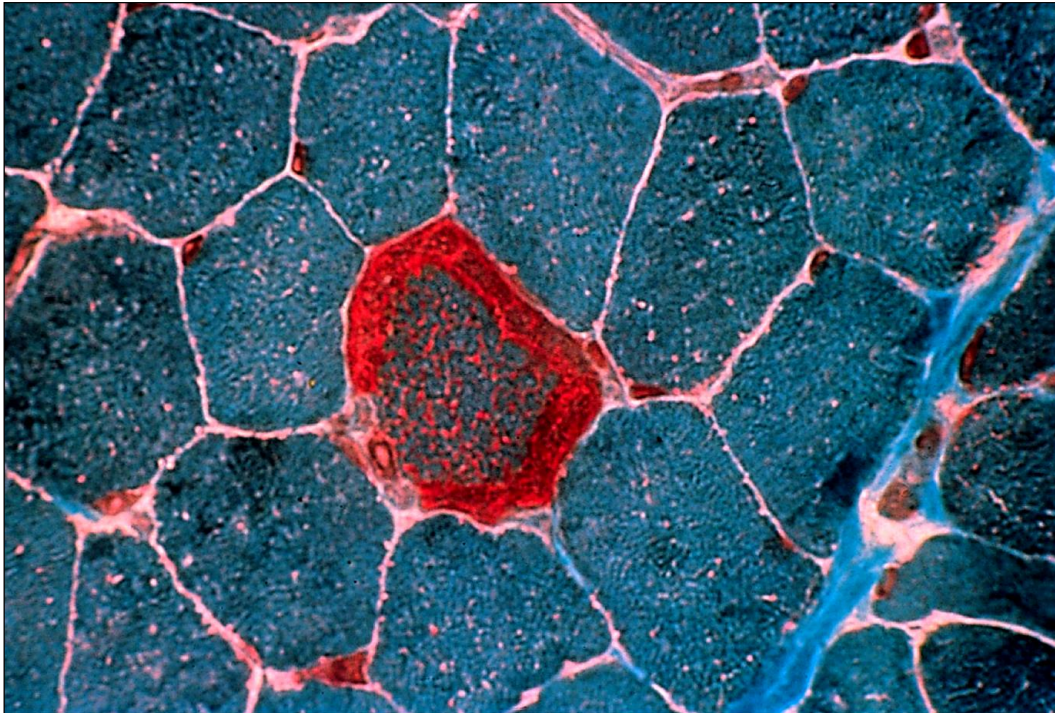
5b) this establishes a proton gradient across the inner membrane

5c) the energy stored in the proton gradient is then used to drive ATP synthesis as the protons flow back to the matrix through complex V (a.k.a. ATP synthase)



Mitochondrial disorders

- Disorders that are due to abnormalities in mitochondrial structure and function
- Most seriously impact muscle and nerve tissues because these are the tissues with the highest demand for ATP

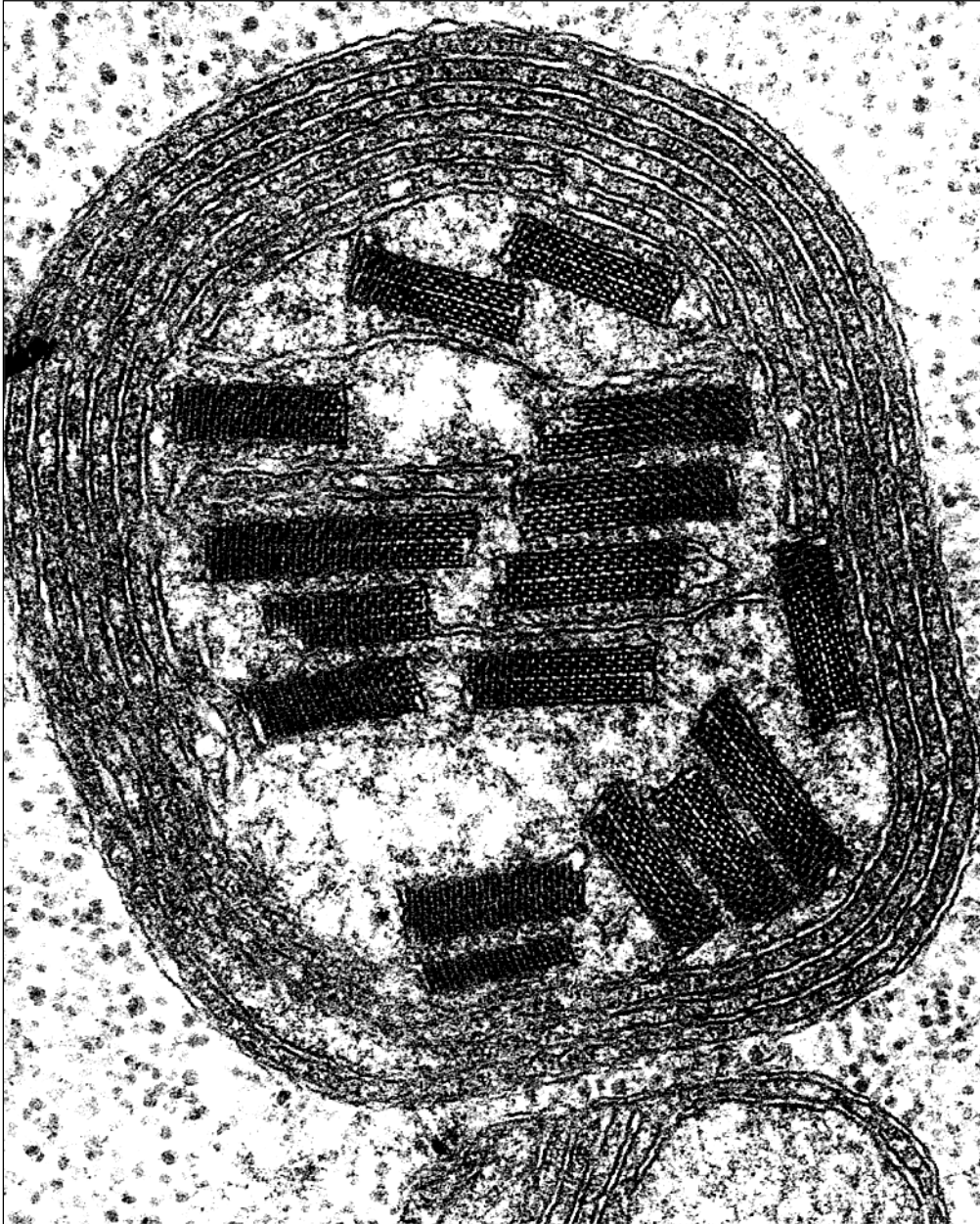


Ragged-red fibers in skeletal muscle of a patient suffering from the Myoclonic Epilepsy and Raged Red Fibers (MERRF) disorder

The red-stained “blotches” just beneath the cells’ plasma membrane are due to abnormal proliferation of mitochondria that have decreased cytochrome c oxidase (complex IV) activity

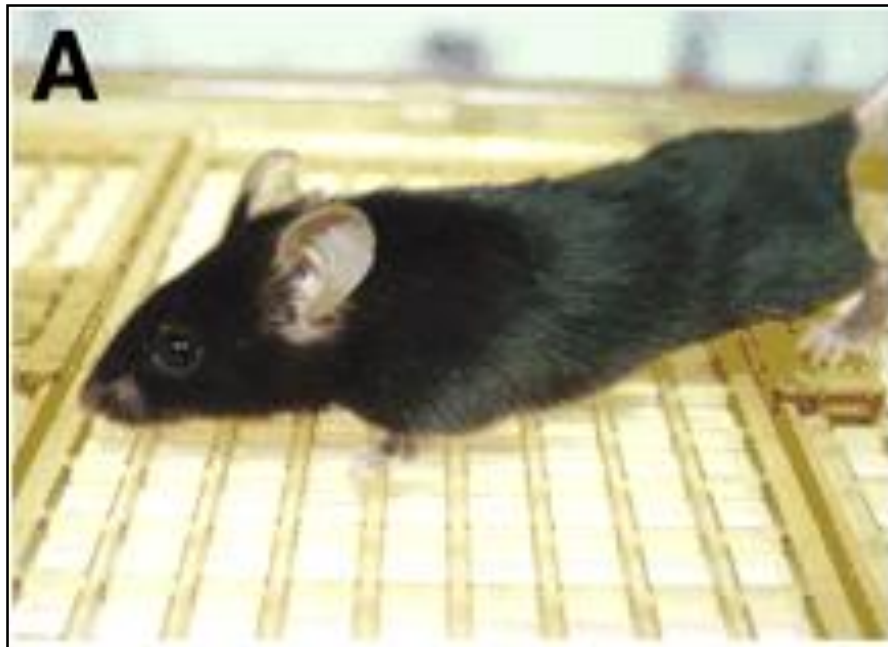
Mitochondrial disorders

Myoclonic Epilepsy and Raged Red Fibers (MERRF) disorder



Electron microscopy reveals large numbers of abnormal inclusions that may cause the mitochondria to resemble a “parking lot”

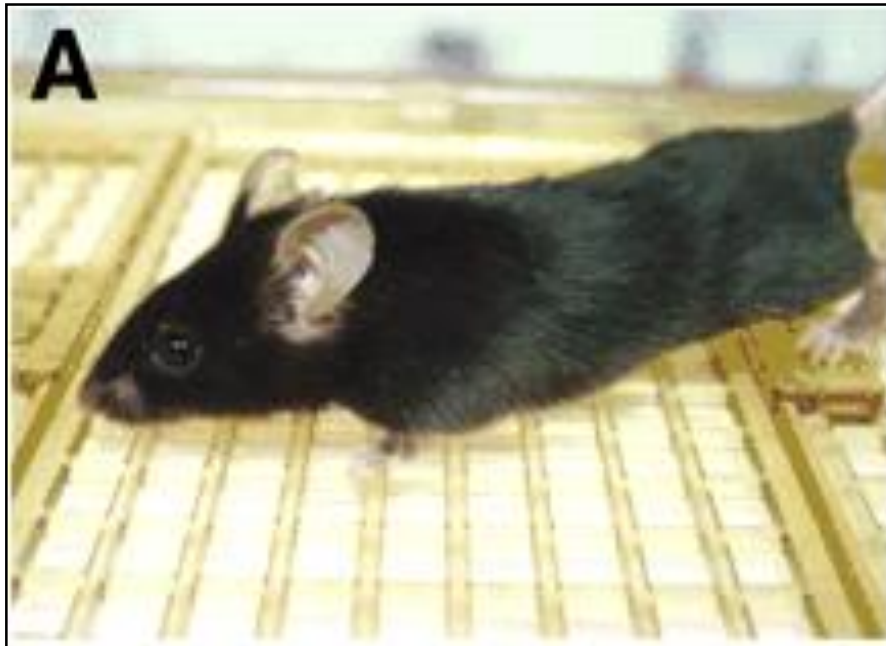
Accumulation of mutations in mitochondrial DNA promotes aging



13-month-old wild-type mouse



13-month-old “knock-in” mouse that has a proofreading-deficient version of the mitochondrial DNA polymerase gamma (POLG)



Wild-type mouse



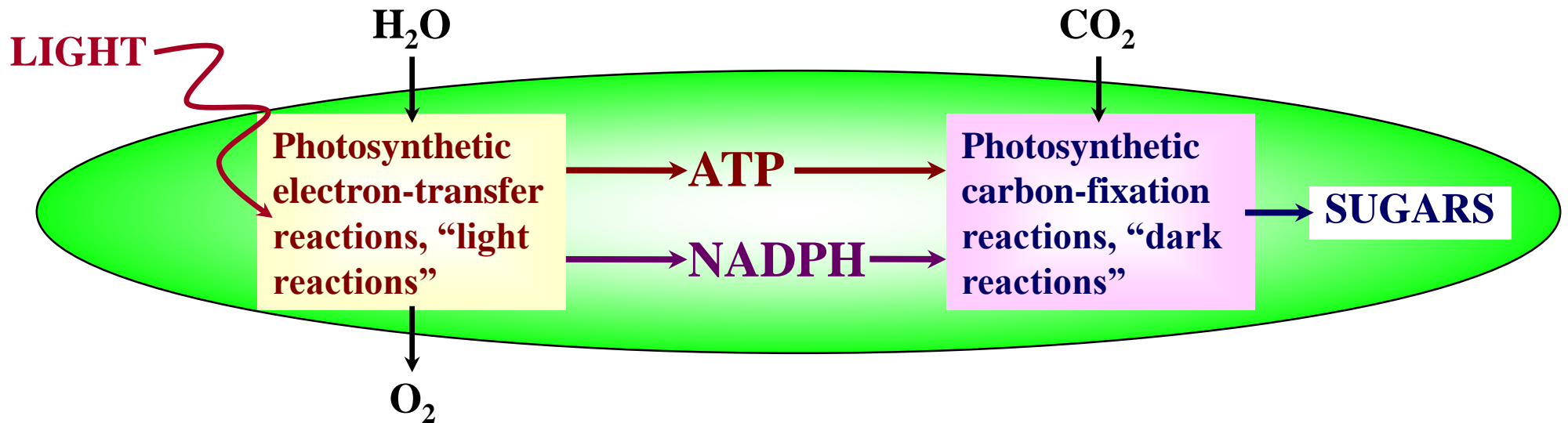
POLG-deficient mouse

Phenotype of the POLG-deficient mouse:

- **A frequency of mutations in mtDNA is 3-8 times as high as in wild-type mouse**
- **Premature aging (progeria): hair loss, graying, and a reduced life span (460 days vs. 850 days for wild-type mouse)**
- **Enhanced apoptosis (death) of post-mitotic (non-dividing), irreplaceable cells**

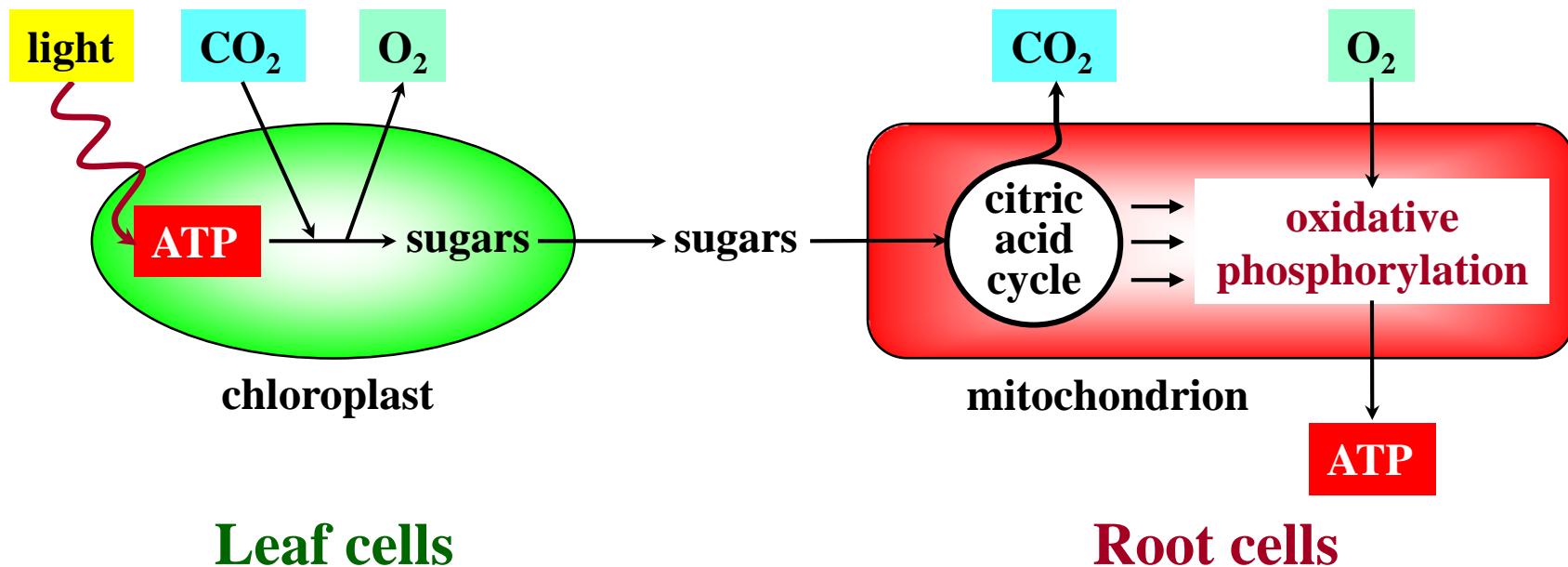
Chloroplasts:

- are the largest and most characteristic organelles in the cells of plants
- are the sites of photosynthesis – the series of light-driven reactions that creates organic molecules from atmospheric carbon dioxide (CO_2)
- perform photosynthesis during the day light hours and thereby produce ATP and NADPH, which in turn are used to convert CO_2 into sugars inside the chloroplast



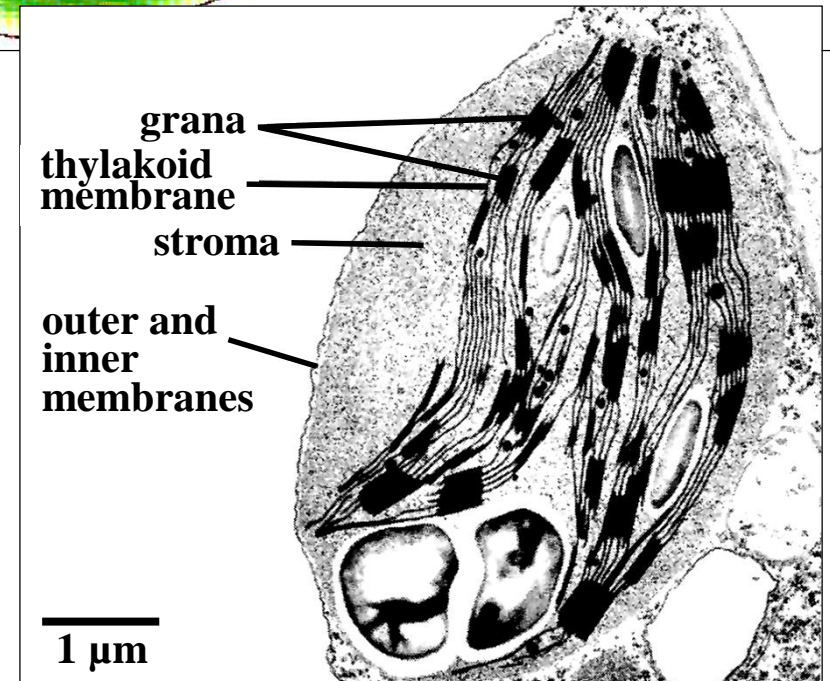
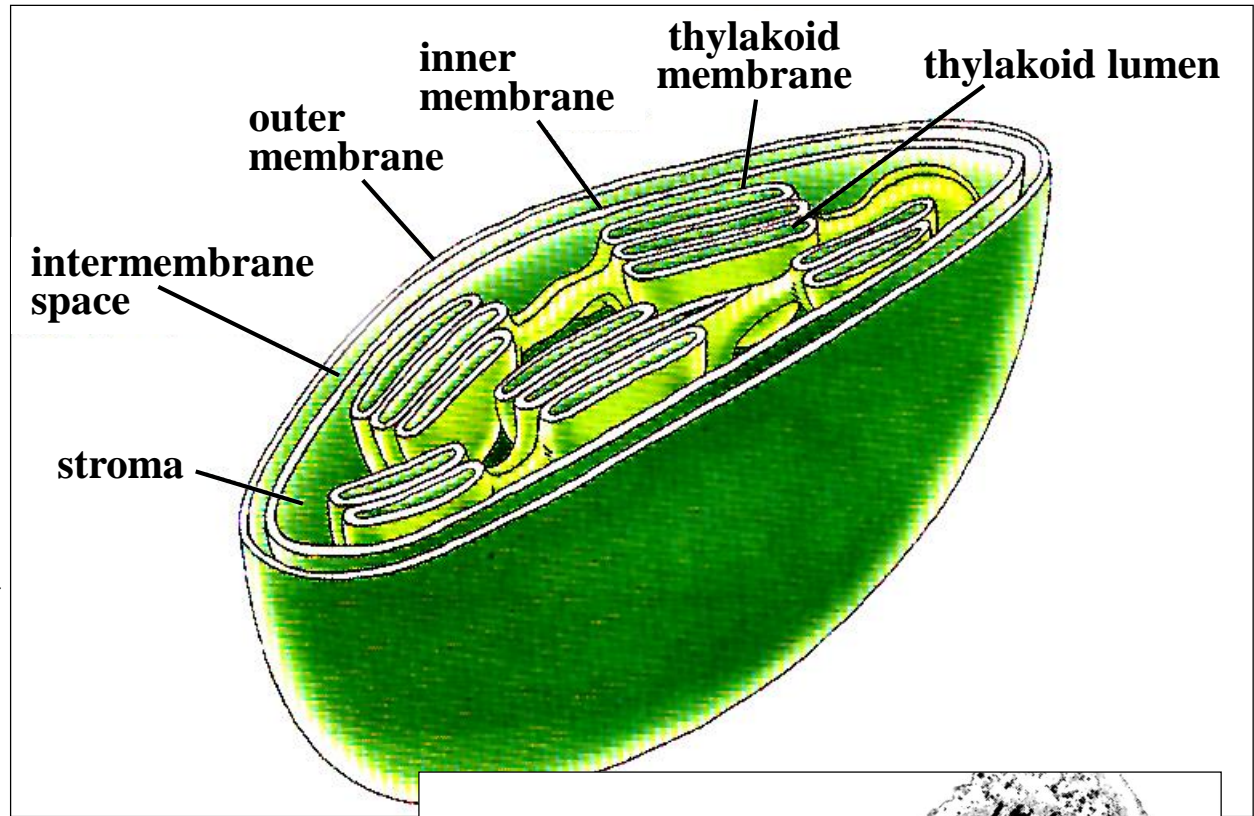
In plants, the chloroplasts and mitochondria collaborate to supply cells with metabolites and ATP

- The plant contains many cells that lack chloroplasts and therefore cannot produce their own sugars and ATP
- Therefore for most of its ATP production, the plant relies on an export of sugars from its chloroplasts to the mitochondria that are located in all cells of the plants
- Most of the ATP needed by the plant is synthesized in these mitochondria



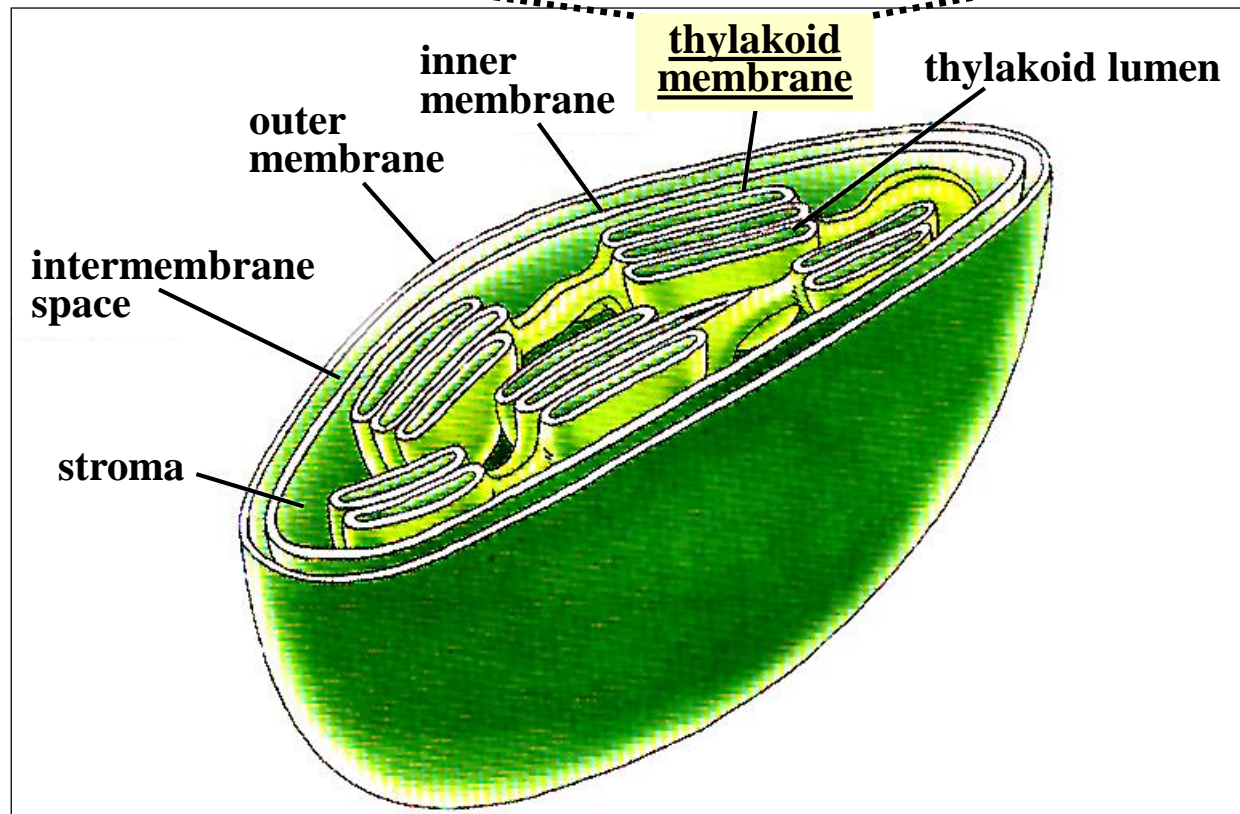
Chloroplasts contain three membrane-limited compartments

- Like the mitochondria, the chloroplast is surrounded by an outer and an inner membrane
- Chloroplasts also contain an extensive internal system of interconnected membrane-limited sacs called thylakoids, which are flattened to form disks; these are grouped in stacks called grana and embedded in a matrix, the stroma
- Their three membranes divide chloroplasts into three distinct internal compartments:
 - (1) the intermembrane space between the inner and outer membranes
 - (2) the stroma, which lies inside the envelope but outside the thylakoid membrane
 - (3) the thylakoid lumen



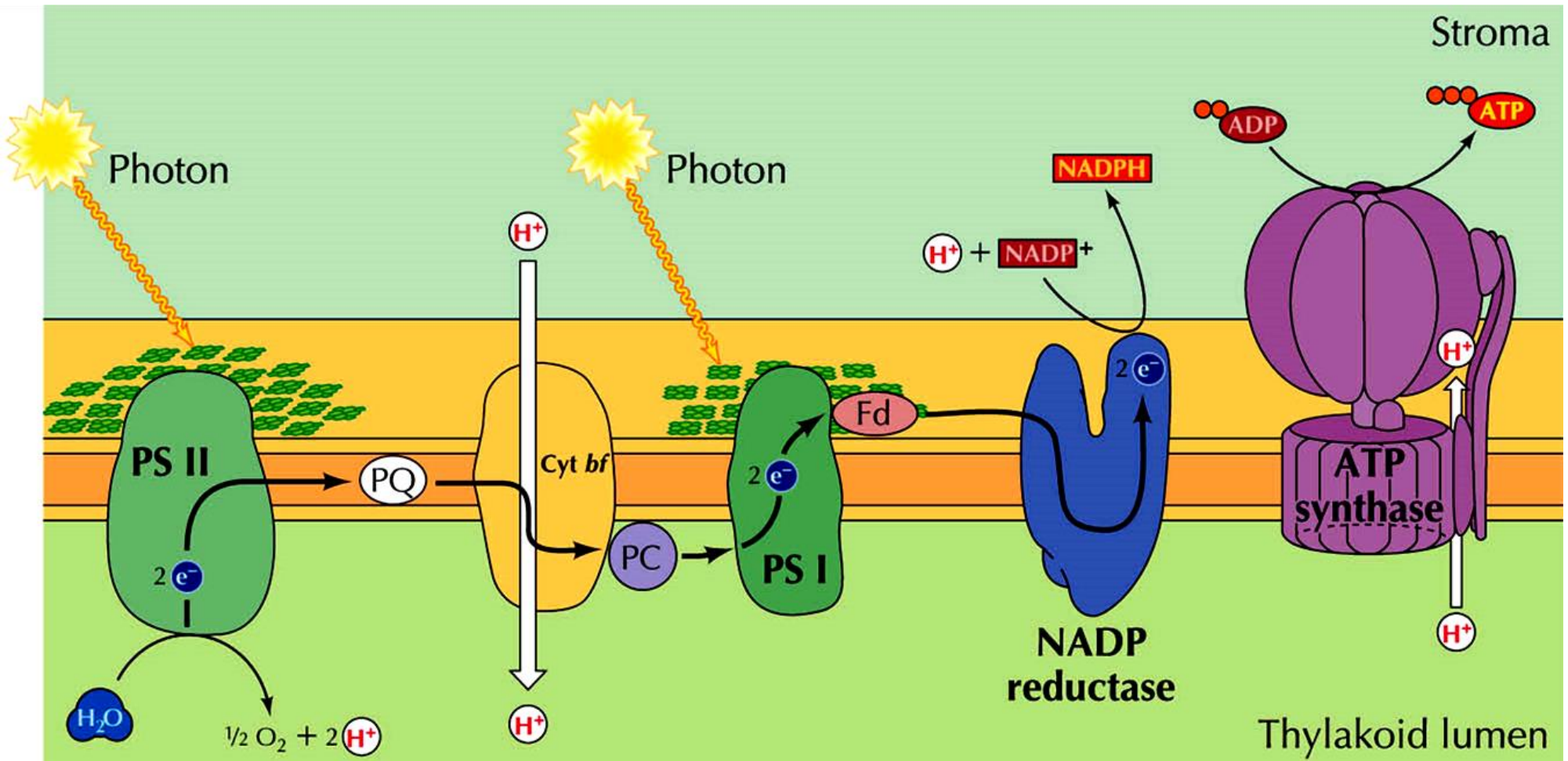
Three of four stages in photosynthesis (the “light reactions”):

- Absorption of light by green pigments (chlorophylls) attached to proteins
- Electron transport
- Synthesis of ATP and NADPH



Electron transport and ATP synthesis during photosynthesis

- Four protein complexes in the thylakoid membrane function in electron transport and the synthesis of ATP and NADPH



PQ = plastoquinone; PC = plastocyanin

- The last stage of photosynthesis (the “dark reactions”): the conversion of CO_2 to carbohydrates (CO_2 fixation)
- Several identical copies of the chloroplast DNA genome, special chloroplast ribosomes and various enzymes required for expression of chloroplast genes

