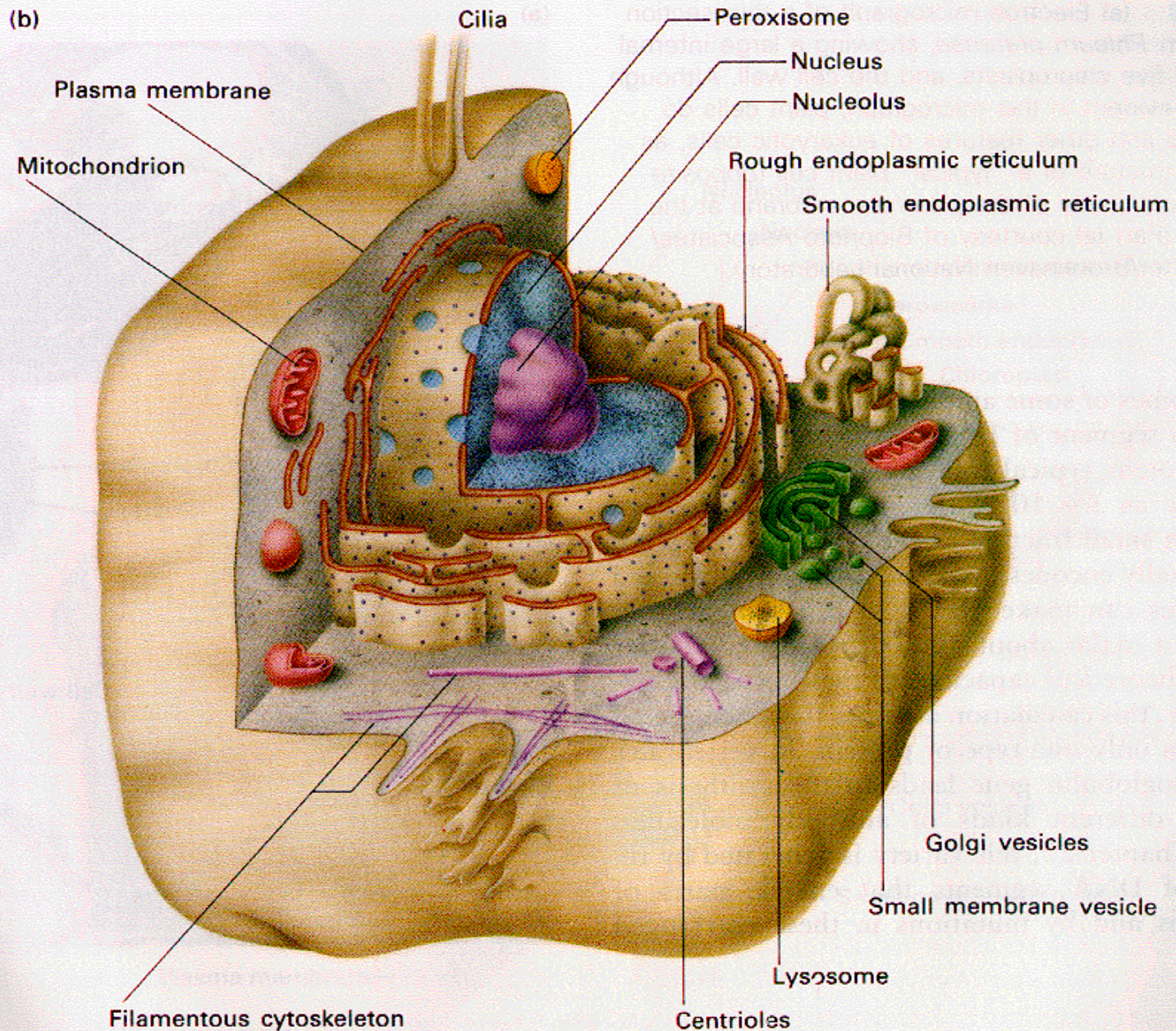
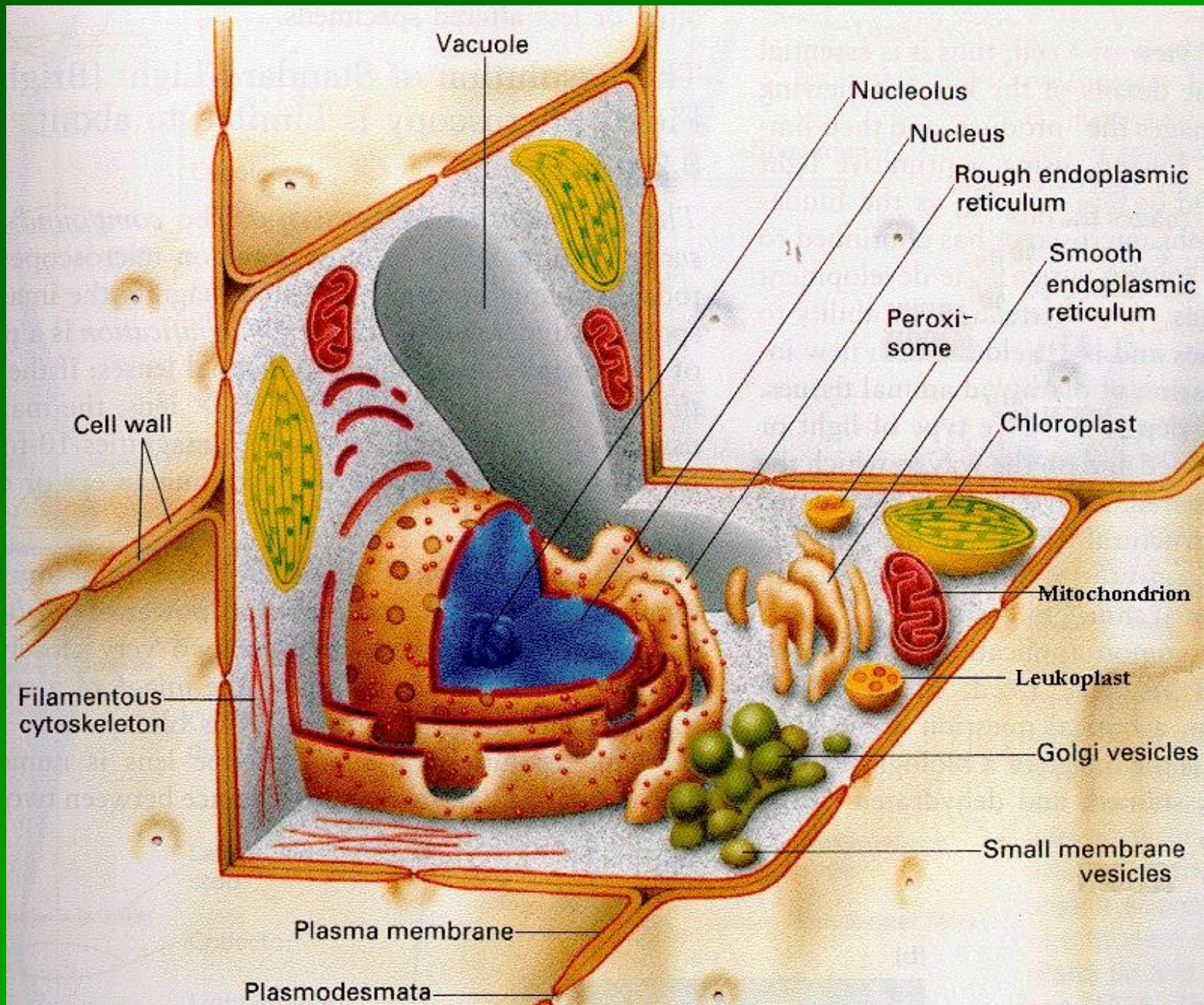


II. INTERNAL ORGANIZATION OF EUKARYOTIC CELLS

Structure of a „typical” animal cell



Structure of a „typical” plant cell



II.1. Cell compartments

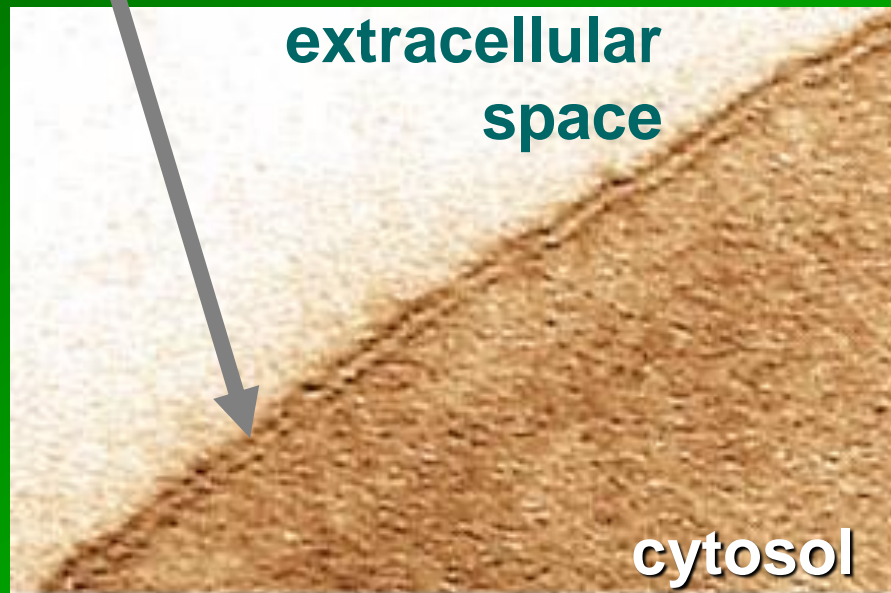
Compartments are aqueous spaces separated by intracellular membranes from each other, devoted to a particular set of functions. The compartments are unique in their chemical components, pH value and electrical potential.

Biological function: separation of the distinct cell functions (e.g. given biosynthetic pathways) in the space.

II.1.1. Biomembranes

Structure of the membranes

- In the electron microscope, biomembranes show a trilaminar structure (dark-light-dark)



- Ø 4 - 7 nm
- Membranes are always closed structures.

Components

Lipids, proteins and carbohydrates (as glycolipids, glycoproteins and proteoglycans)

Lipids

- are constitutive, principal components of all biomembranes.
- determine the physico-chemical properties of the membranes.

Biologically important properties of membrane lipids:

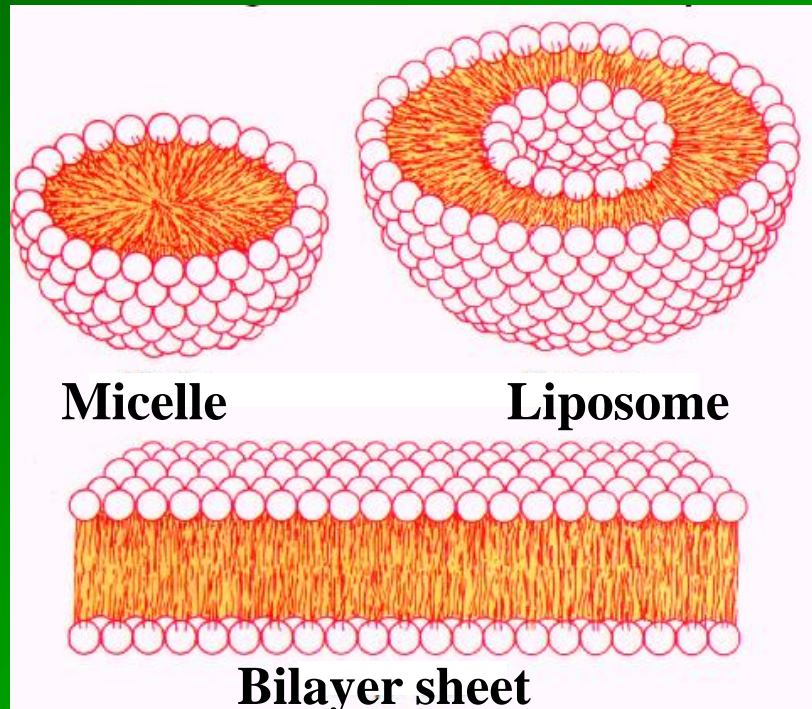
Fluidity: individual lipid molecules can diffuse freely within the lipid bilayer.

Amphipathic (amphiphilic) nature: lipid molecules have a hydrophilic (polar) and a hydrophobic (non-polar) end.

Variability: The variability of the structure of the lipid molecules arises from the combination of various hydrophobic parts (chain length, number of double bonds) with various hydrophilic parts

Self-organisation: lipids form bilayers spontaneously in aqueous solutions.

Three structure types:

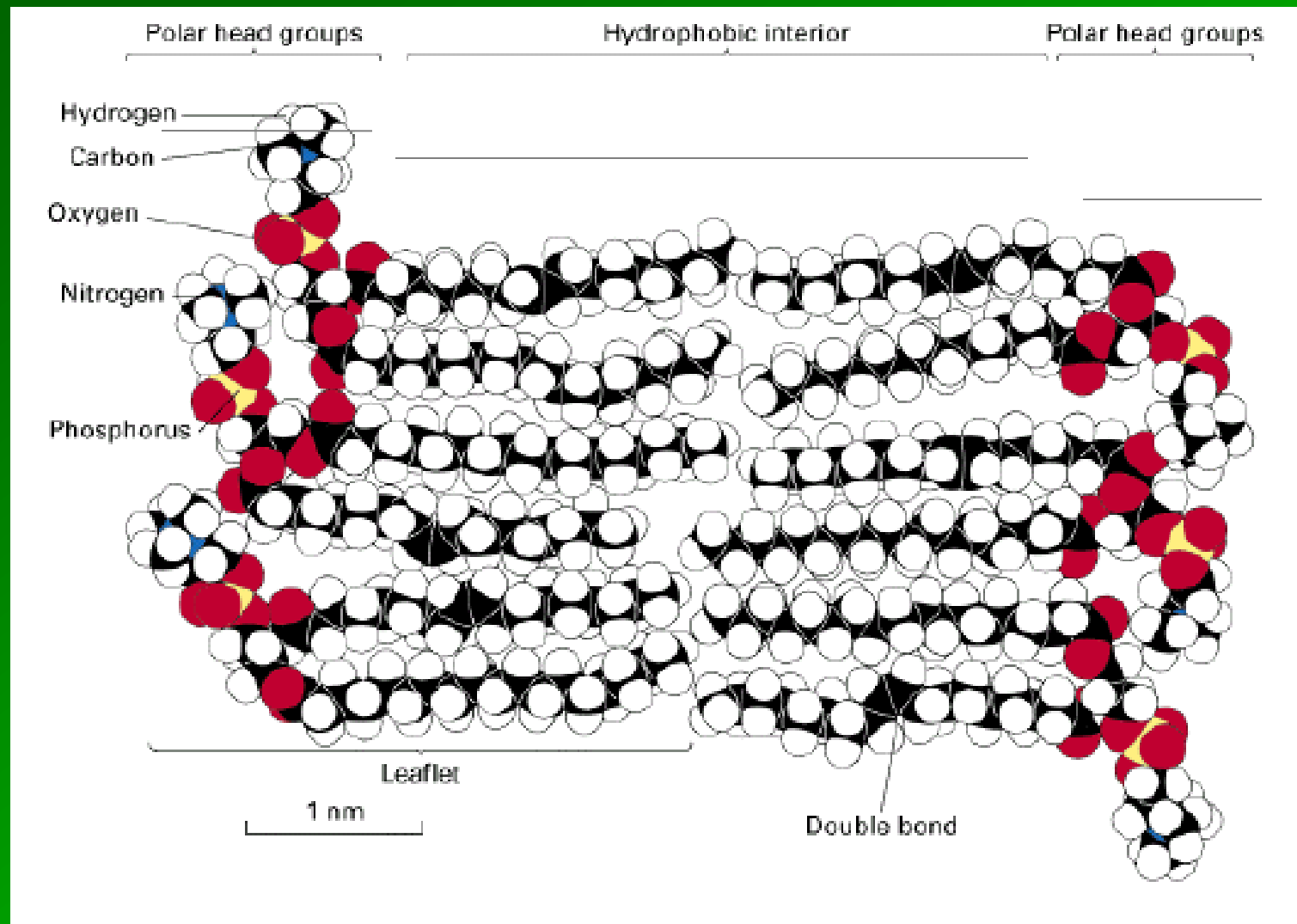


Fatty acyl chains aggregate by hydrophobic interactions and exclude water molecules.

The type of structure formed depends on the \Rightarrow chemical nature and concentration of the lipid(s)
 \Rightarrow temperature
 \Rightarrow ionic composition of the aqueous solution.

E. GORTER und F. GRENDEL proposed in 1925, that the bilayer sheet would be a good model to describe the properties of biomembranes.

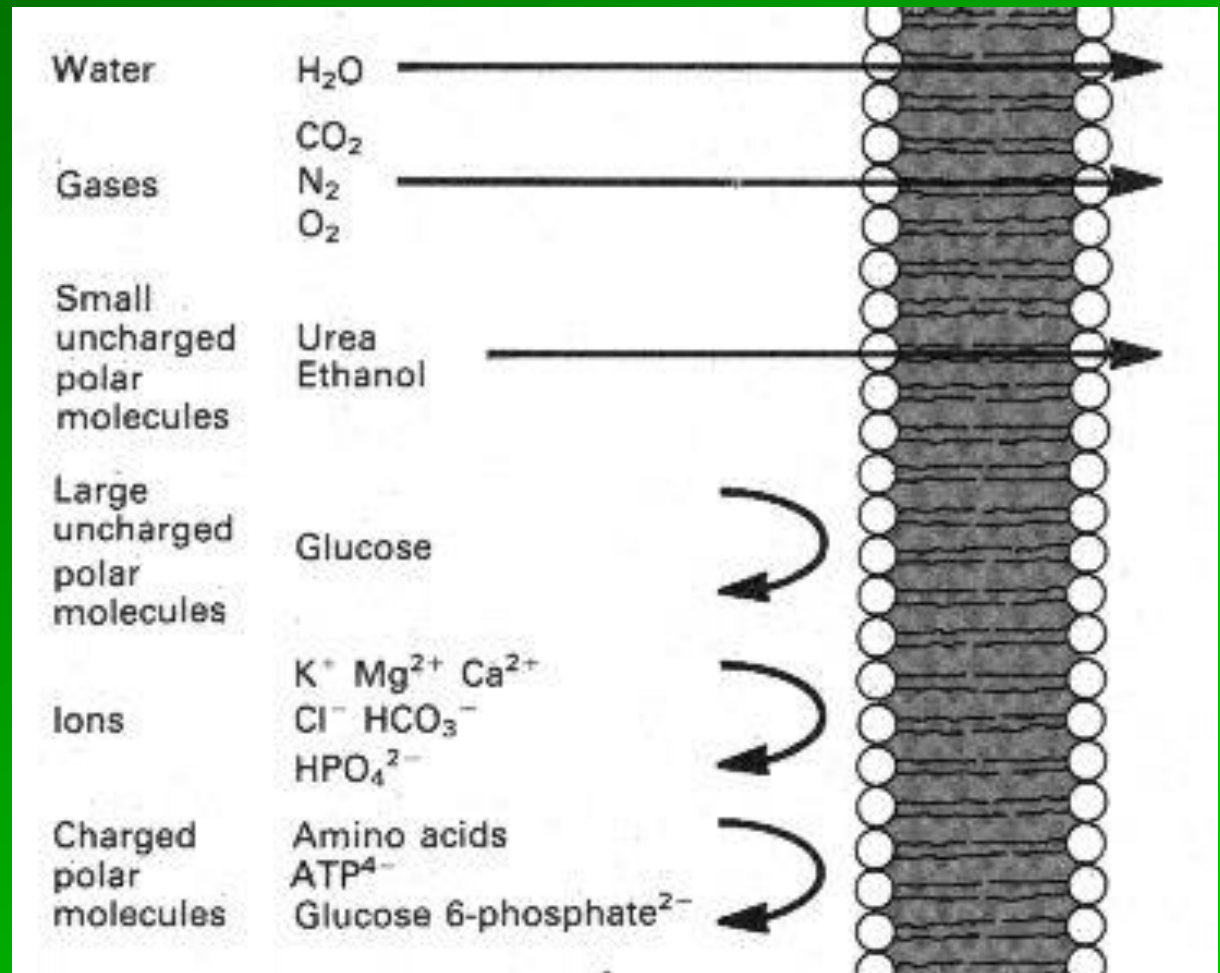
The lipid bilayer (molecular level)



Lipid bilayers as semipermeable barriers

Semipermeability:
pure lipid bilayers
allow free diffusion
of water , gases and
small uncharged
molecules, whereas
it is not permeable to
numerous molecules
of biological
importance.

This property is the
basis of the
generation of
membrane potential:
difference in the
electric charge on
the two sides of a
membrane.



Membrane proteins

What is their role?

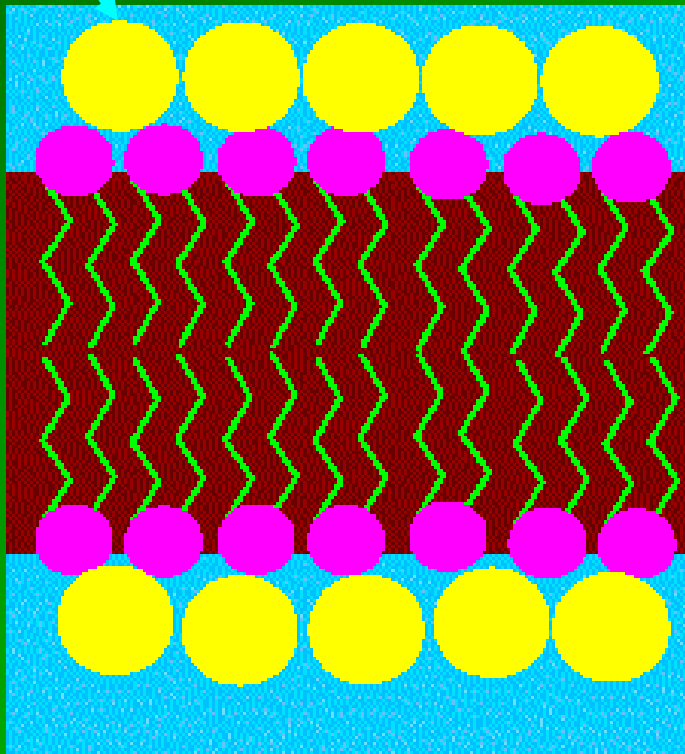
All biological functions of biomembranes (except the barrier- function) are determined by the proteins found in these membranes. Membrane proteins are controlling the flow of materials, energy and information of the cells.

How they are interacting with the lipid bilayer?

The membrane model of DAWSON and DANIELLI (1935)

Membrane lipids are forming a double layer.

Proteins are attached to the hydrophilic headgroups of the lipid bilayer on the inner and outer sides.

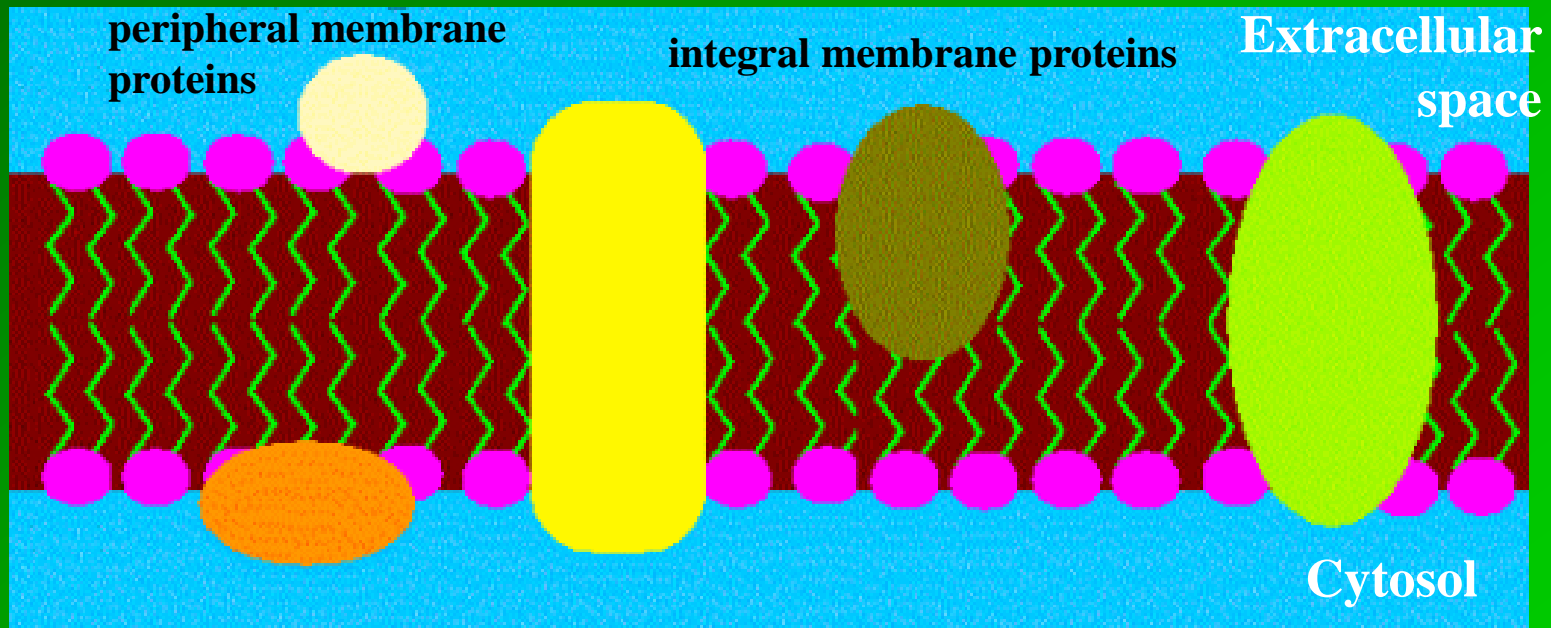


Robertson: protein-lined pores within the lipid bilayer

The fluid mosaic model

SINGER and NICHOLSON (1972)

The lipids are forming a two-dimensional viscous fluid, proteins are embedded in this medium. Some proteins are deeply integrated, others are interacting only with the surface layers (integrated and peripheral proteins). Both lipids and proteins are able to move freely in the plane of the membrane .

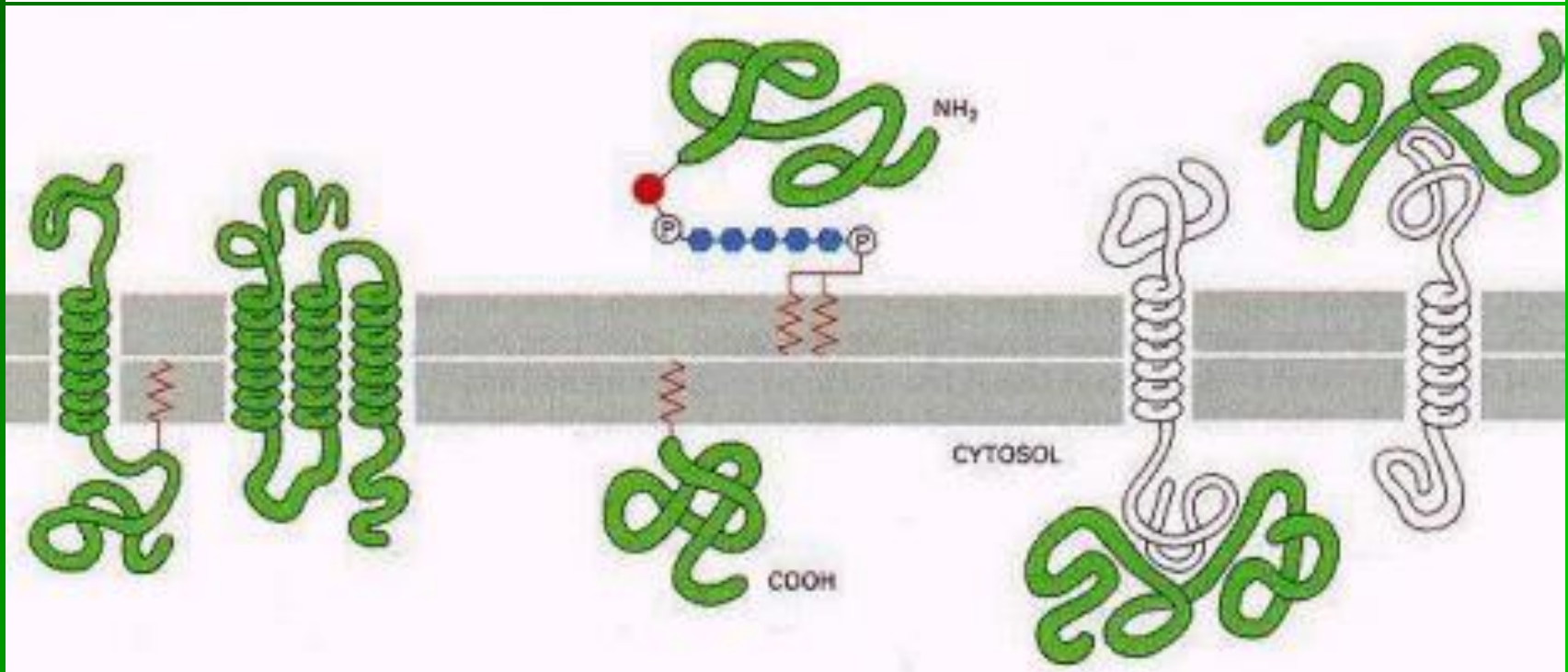


Association of membrane proteins with the lipid bilayer:

a. Transmembrane helices

b. association by covalently attached lipid

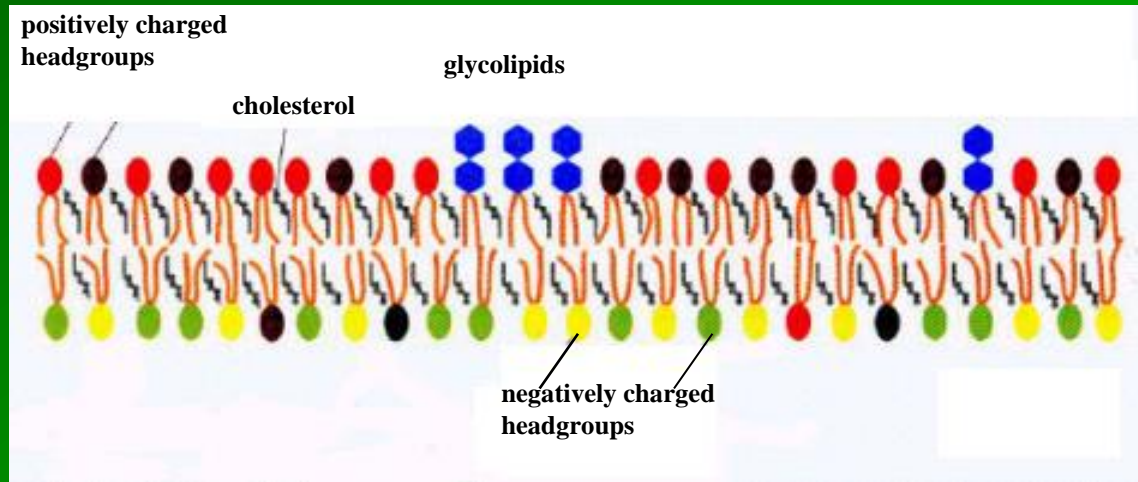
c. Interaction with trans-membrane protein



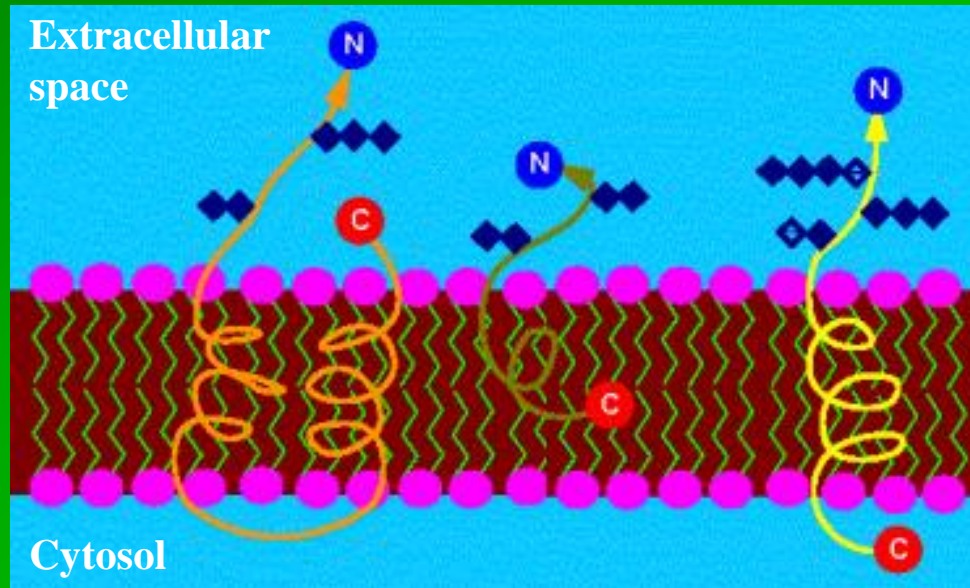
Biomembranes are asymmetric

(Example: plasma membrane)

Lipid
components



Proteins



Functions of biomembranes

Defining the boundaries of the cell: maintaining the essential differences between the cytosol and the extracellular environment.

Defining the boundaries of organelles: maintaining the characteristic differences between the cytosol and each organelle.

Energy storage: in the form of ion gradients (membrane potential)

Transmembrane transport of selected molecules.

Transfer of information (reacting to environmental cues)

II.1.2. Plasma membrane (Membrana cellularis)

EM image



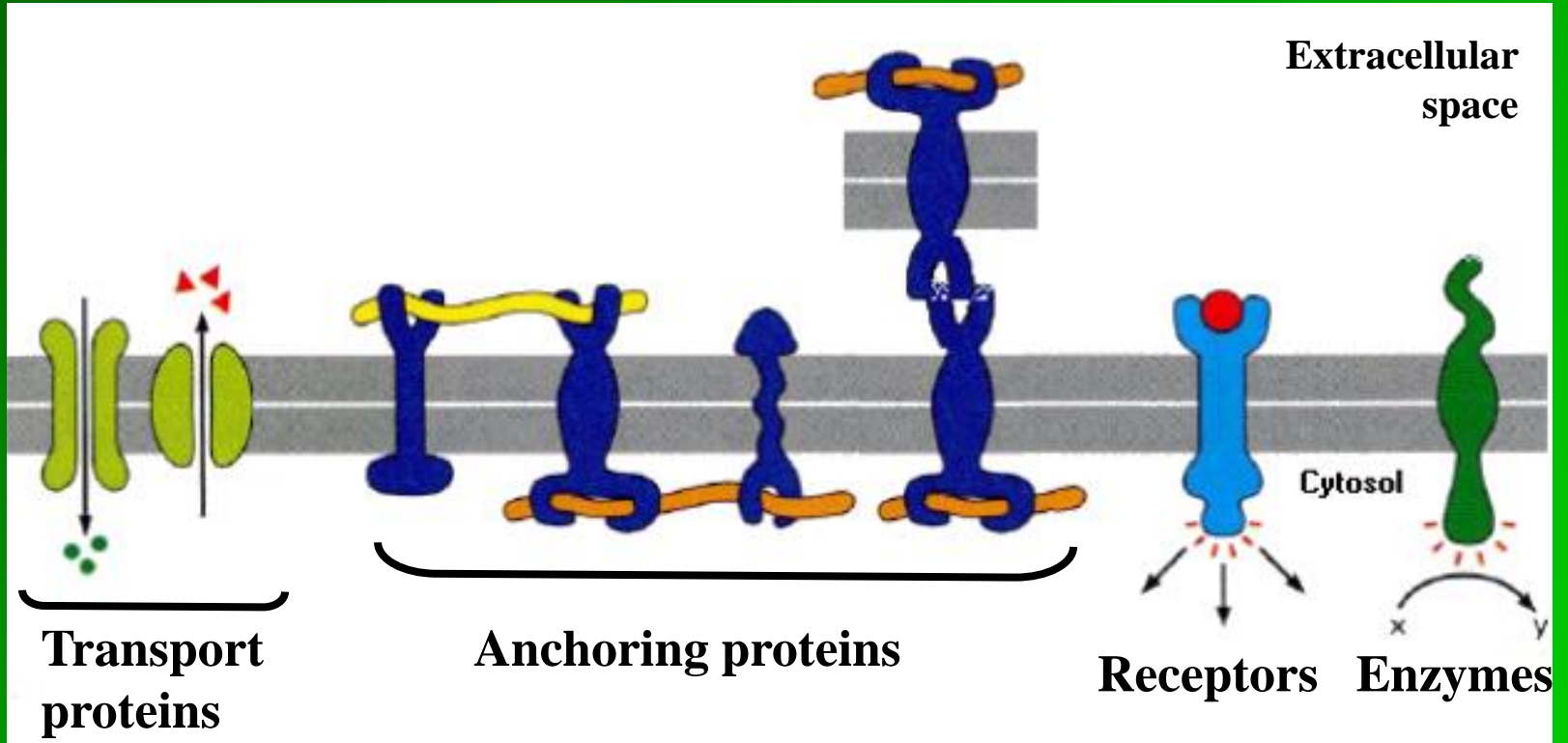
3 layers

- outer, 2,5 nm thick dark – electron-dense – layer (hydrophilic parts of the lipids and membrane-embedded proteins and the directly attached proteins)
- middle, 3 nm thick light – electron-opaque – layer, corresponding to the hydrophobic constituents of the lipids and the hydrophobic regions of the transmembrane proteins.
- inner, 2,5 nm thick dark layer comprising the hydrophilic parts of the lipids and embedded proteins and the directly attached proteins

Biological functions

- Plasma membrane encloses animal cells: maintains essential differences between the cytosol and the extracellular space.
- Due to the semipermeability of the lipid bilayer and the presence of specialized transport proteins, selective, regulated bidirectional traffic of ions and molecules is ensured.
- In nerve and muscle cells: production and transmission of electric signals.
- Membrane proteins serve to anchor cytoskeletal elements, providing stability to the cell.
- Adjacent cells can be bound together by cell adhesion molecules of their plasma membranes
- Plasma membrane receptors allow the cell to alter its behavior in response to environmental cues.

Proteins of the plasma membrane



- **Transport proteins:**

- pores
 - channels
 - carriers

- **Anchoring proteins:**

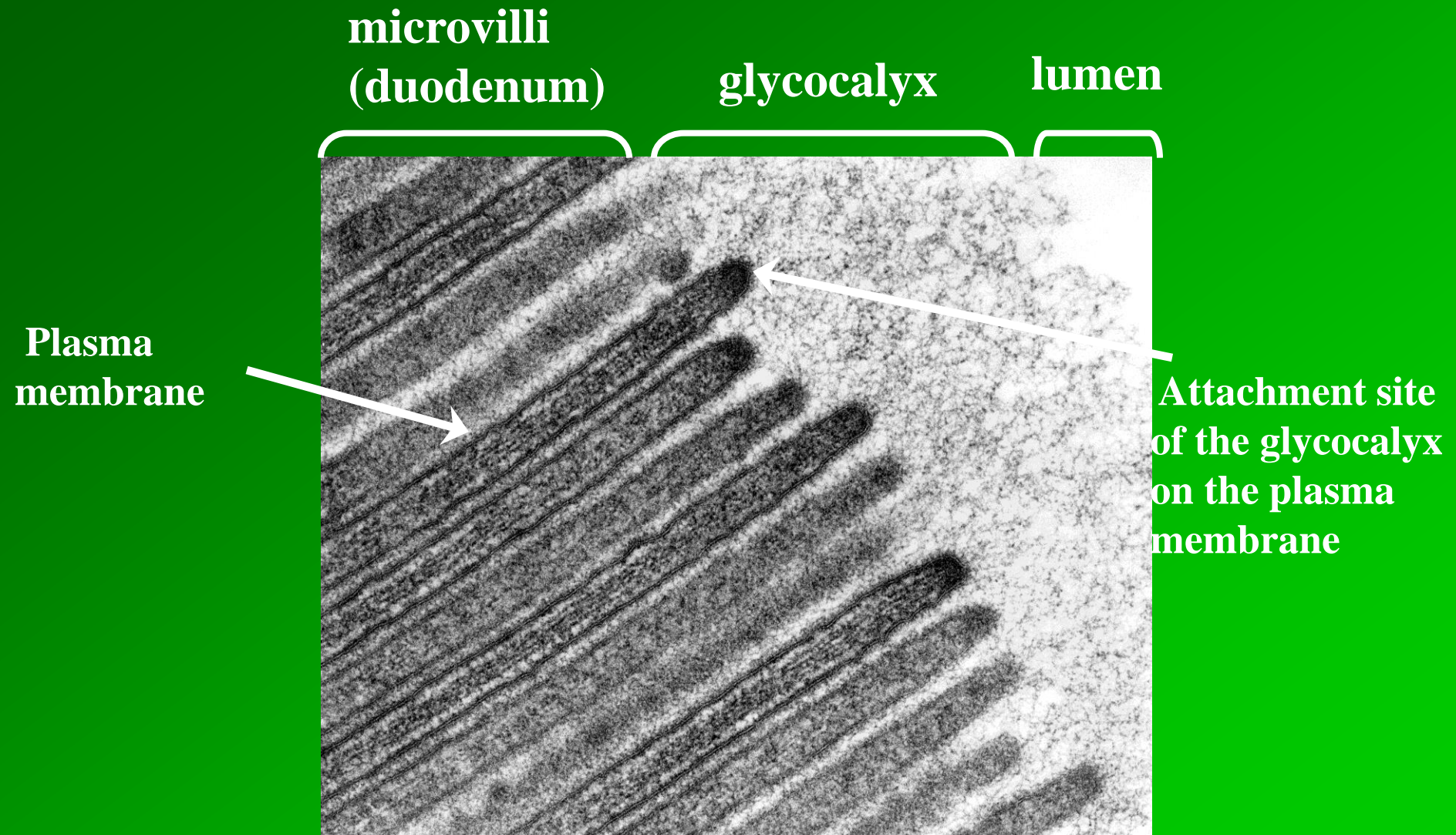
- intracellular binding: to the cytoskeleton

- extracellular binding: to the extracellular matrix (cell-ecm)
to other cells (cell-cell contact)

- **Receptors**

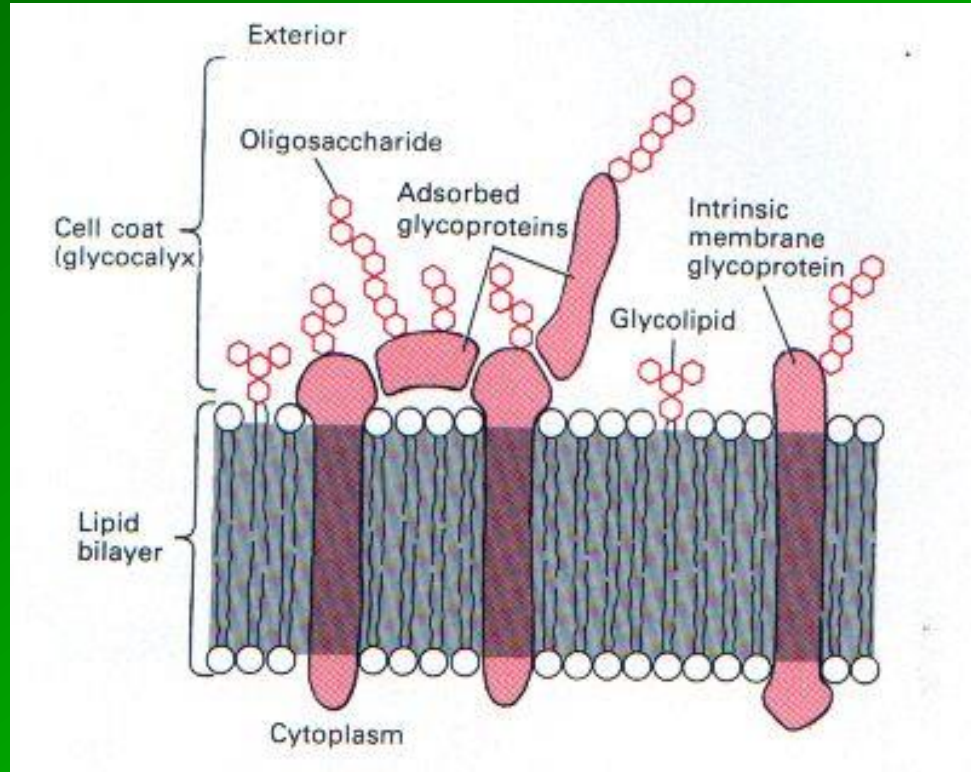
- **Membrane-bound enzymes**

II.1.2.1. The glycocalyx (cell surface coat)



The glycocalyx is exceptionally well developed on the surface of the intestinal epithelium.

The glycocalyx is a carbohydrate-rich zone on the cell surface, made up of proteins and oligosaccharides bound to the outer surface of the plasma membrane.



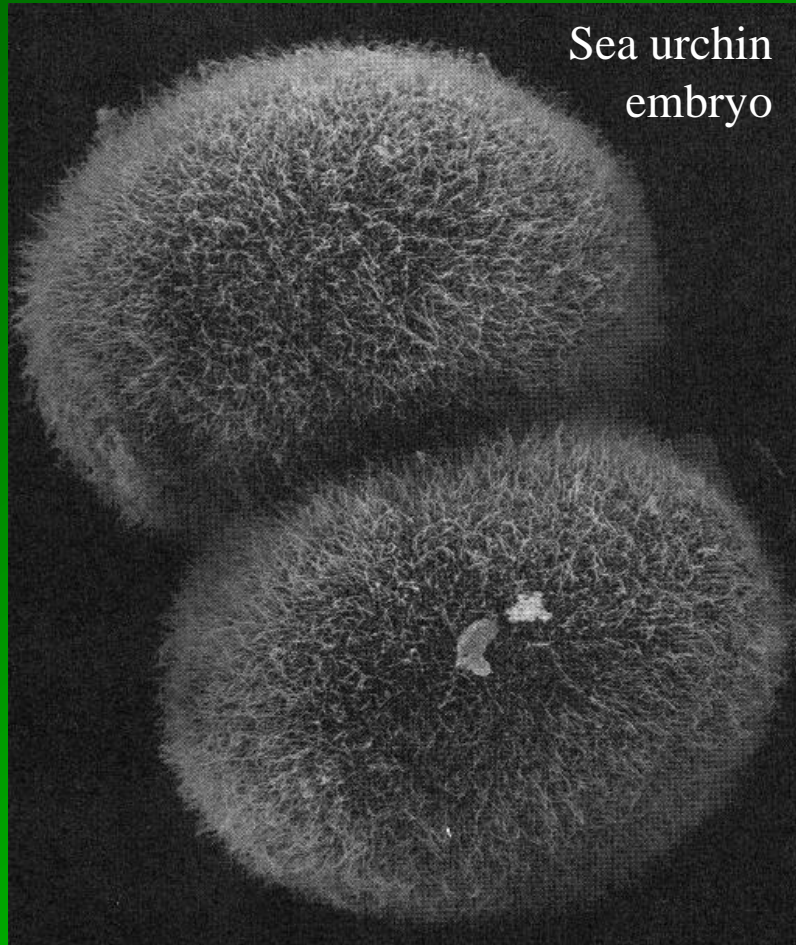
The composition and arrangement of the glycocalyx is cell-specific.

Main biological functions:

- **Protection against chemical and mechanical damage**
- **Cell recognition during embryonic development (tissue formation).**
- **In the adult: immune recognition**
- **Others, e.g. cell-cell adhesion during blood clotting.**

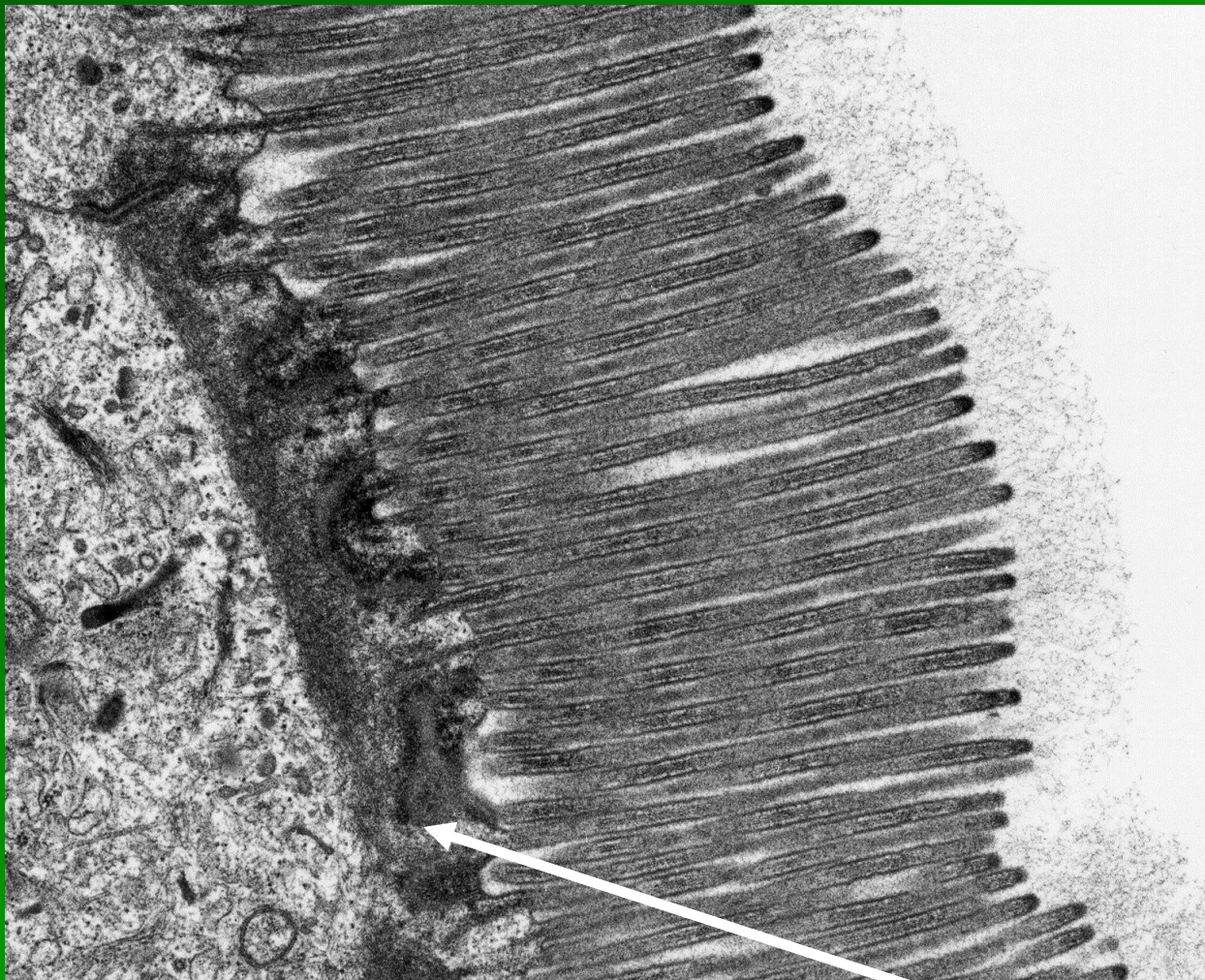
II.1.2.2. Specializations of the cell surface.

- Microvilli



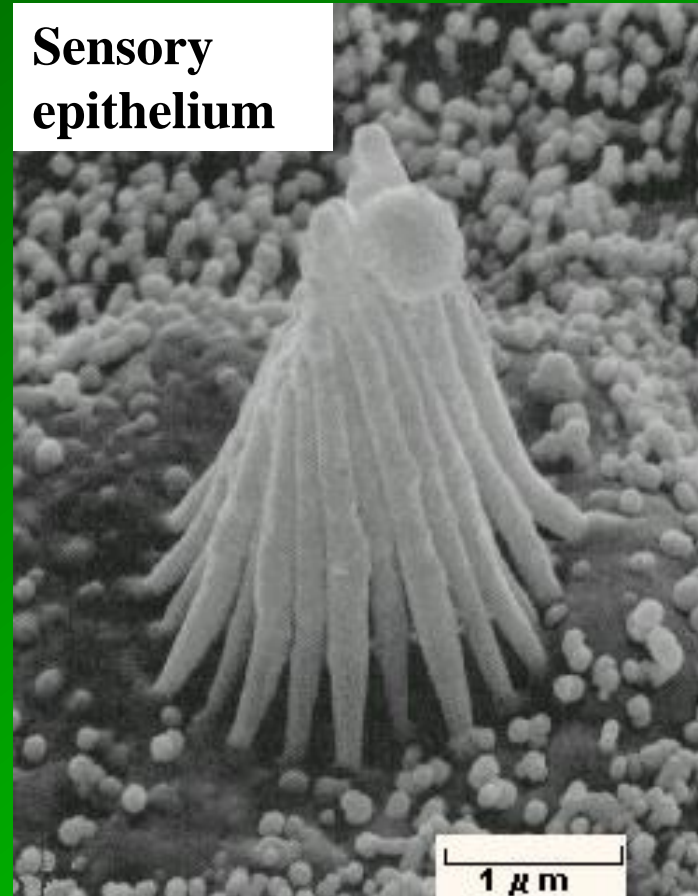
Scanning EM.

Microvilli are fine finger-like projection of the plasma membrane, with limited capability of motion. \varnothing : 50 - 100 nm, length \sim 1 - 2 μ m



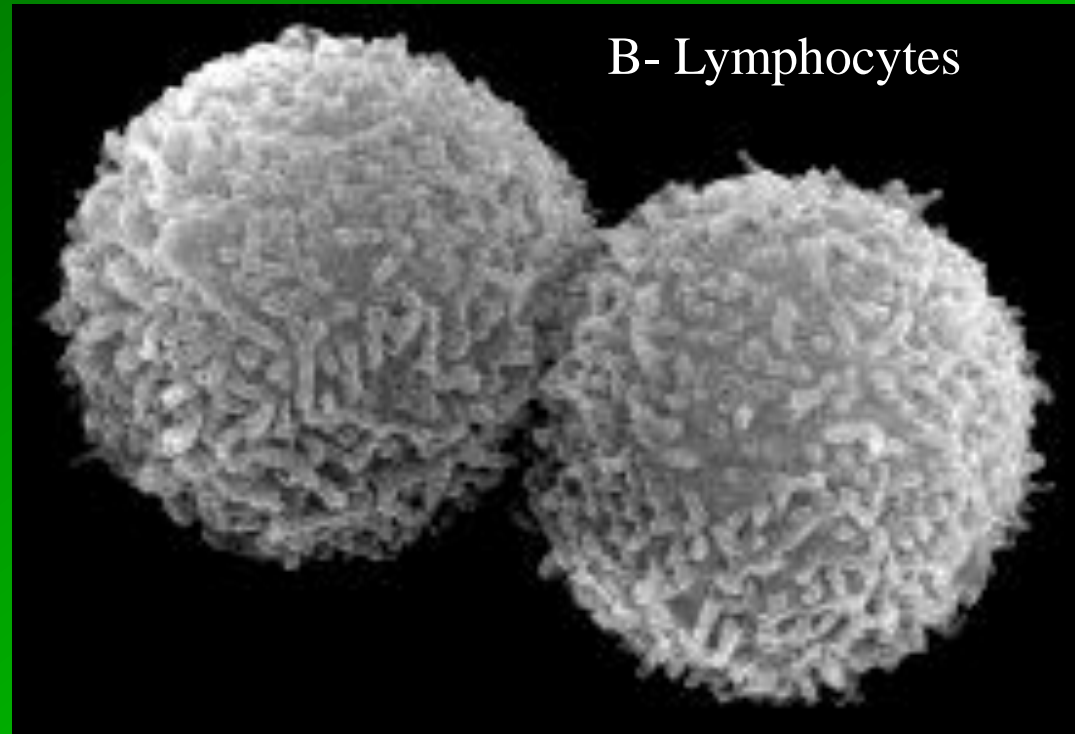
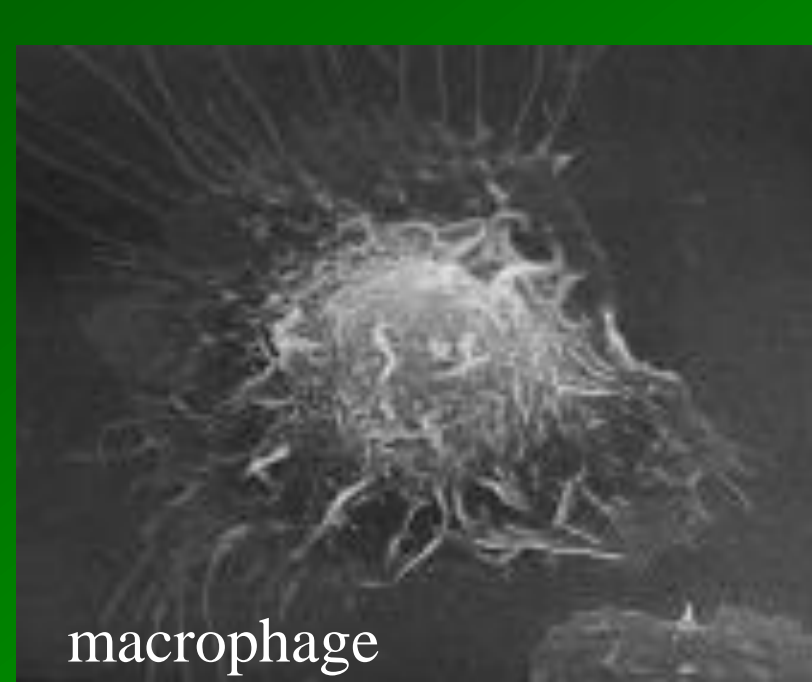
Microvilli are anchored to an electron-dense structure, the terminal web. On the surface of the epithelium of several organs, e.g. in the intestine, they are closely stretching parallel to each other in so called brush border, serving to enlarge the resorptive surface.

- Stereocilia



are long microvilli (3 - 25 μ m), i.e. non-motile long protrusions of the cell membrane. Stereocilia are capable of moving *passively*. They occur e.g. on specialized epithelia (epididymis) and the sensory cells of the internal ear.

- **Pseudopodia**



Pseudopodia are foot-like, long cell projections. Their inner structure resembles that one of the microvilli, but they are less strictly ordered.

- Pseudopodia are formed and withdrawn in several minutes.
- Pseudopodia are capable of active, independent movement.

Their biological role is to ensure the active movement of the cells.

Several cell-types use pseudopodia for locomotion:

Free cells of the connective tissue

plasma cells

granulocytes

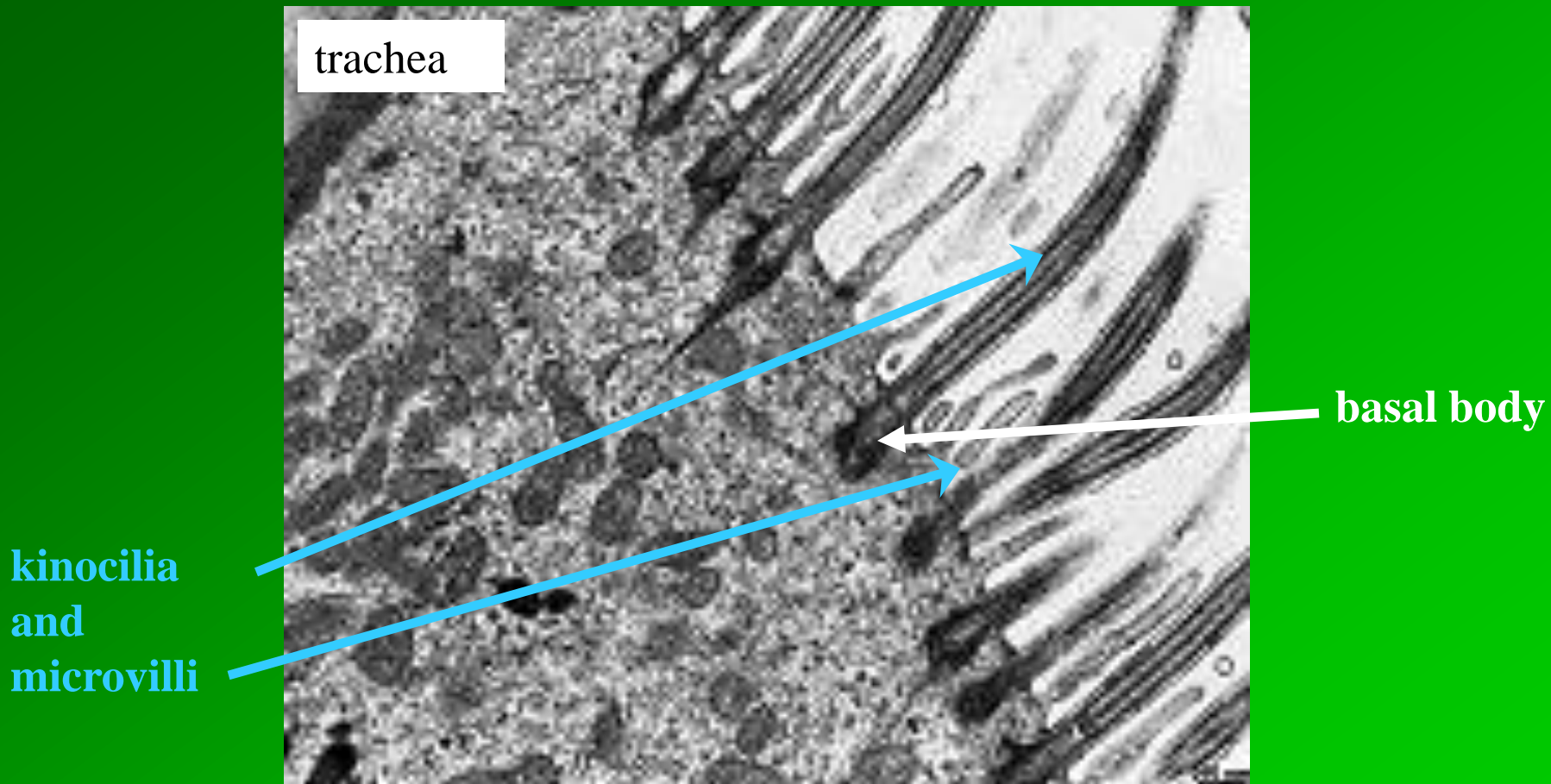
macrophages (phagocytic cells!).

Blood cells

granulocytes

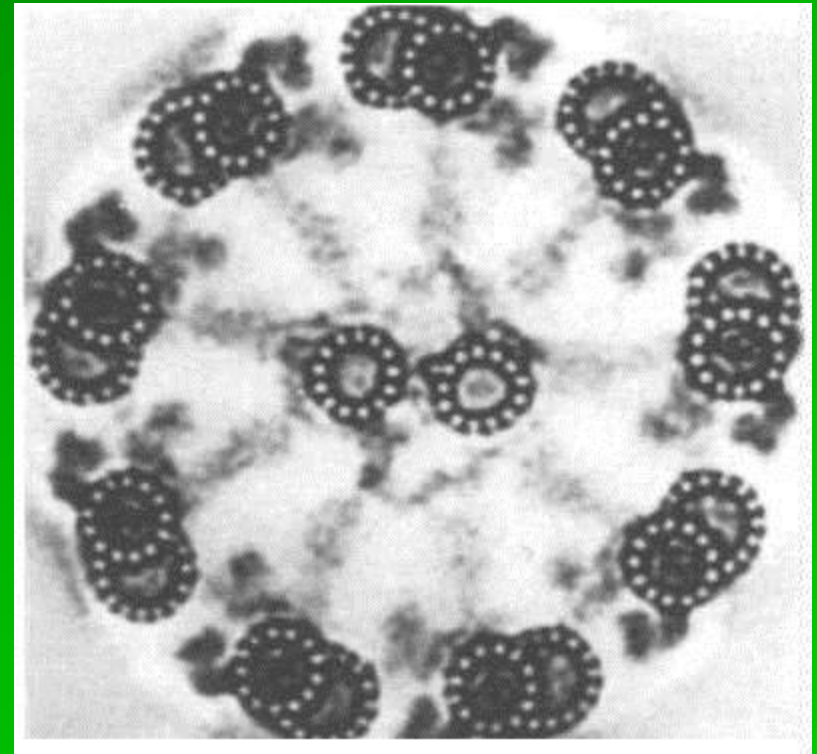
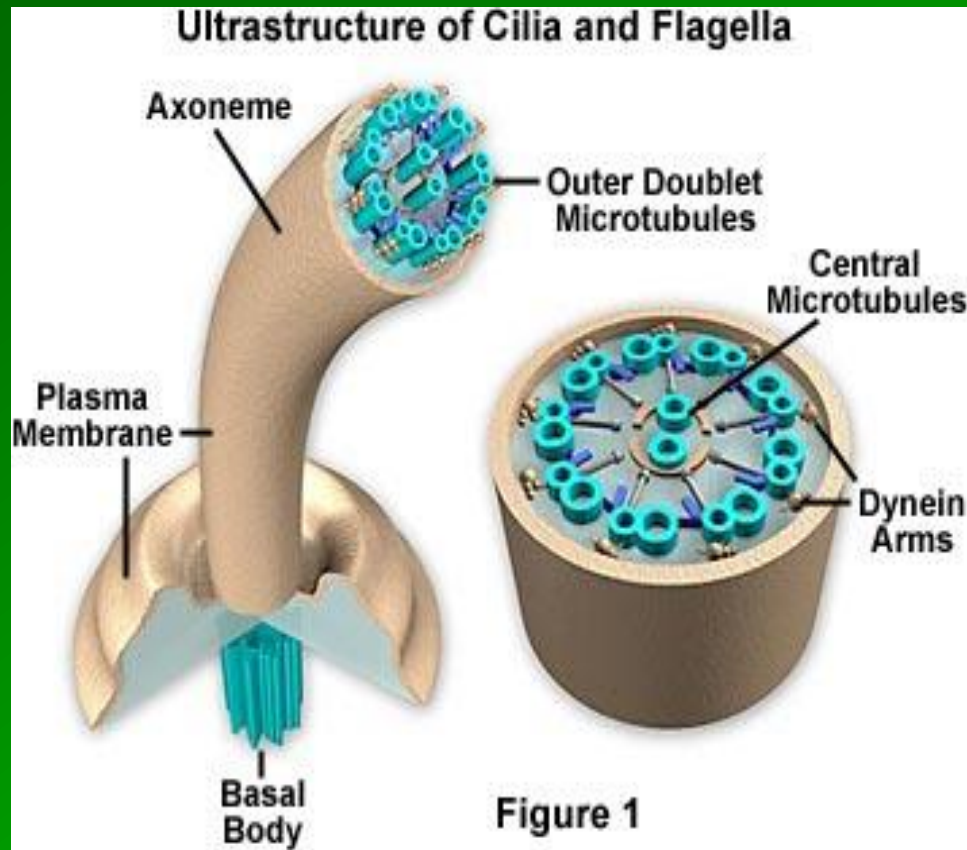
lymphocytes

- **Kinocilia (cilia), flagellae**



Cilia are parallelly oriented, motile, finger-like protrusions of cell membrane (\varnothing 300 nm, length 7 - 10 μ m), connected to an electron dense basal body.

Cilia and flagella are built up by highly ordered microtubular system



- **Kinocilia** are typical for the respiratory epithelium present in the respiratory tract (nose, larynx, trachea and bronchi). Pollen and debris is transported towards the mouth by the coordinated beating of the cilia. Movement is a quick power stroke followed by a slower recovery stroke of the cilia.
- Cilia are present on other epithelia as well, e.g. in the oviduct, where the density is about 10,000,000 cilia per 1 mm² surface.

Flagellae are single, long cilia serving for movement of free cells. They are encountered, e.g. in the tails of sperms which are about 55 µm in length.

II.1.2.3. Intercellular junctions and cell-extracellular matrix (ecm) connections.

1) Occluding junction

- *Zonula occludens*, tight junction

2) Adhering junctions

Interdigitations

Zonula adhaerens, belt desmosome

- *Macula adhaerens*, spot desmosome
- **Focal contact, adhesion plaque**
- **Hemidesmosome**

3) Communicating junctions

- *Macula communicans*, gap junction (nexus)
- *Synapse*

Cell-cell

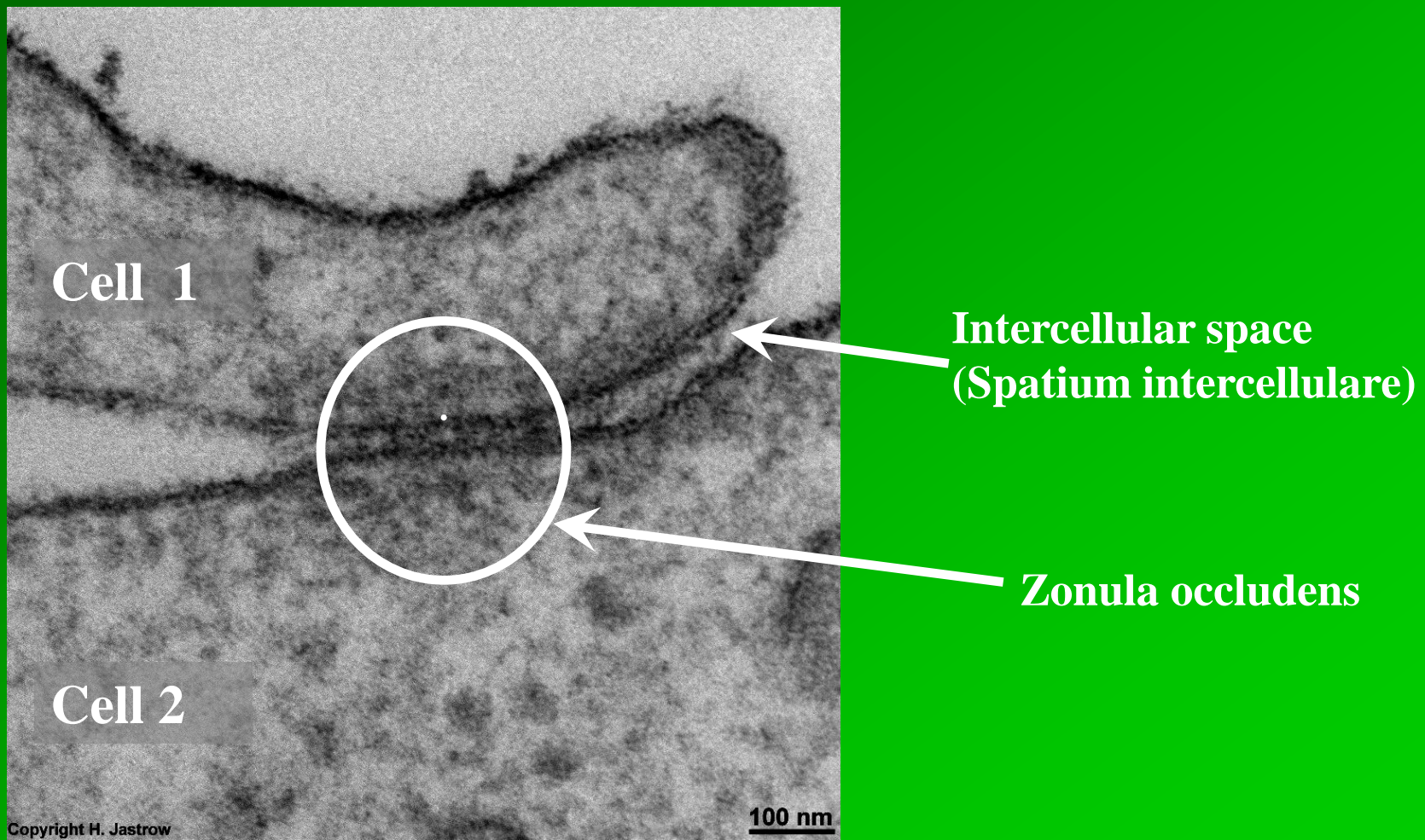
Cell-ecm

II.1.2.3a. Intercellular junctions

- *Zonula occludens*. Tight junction

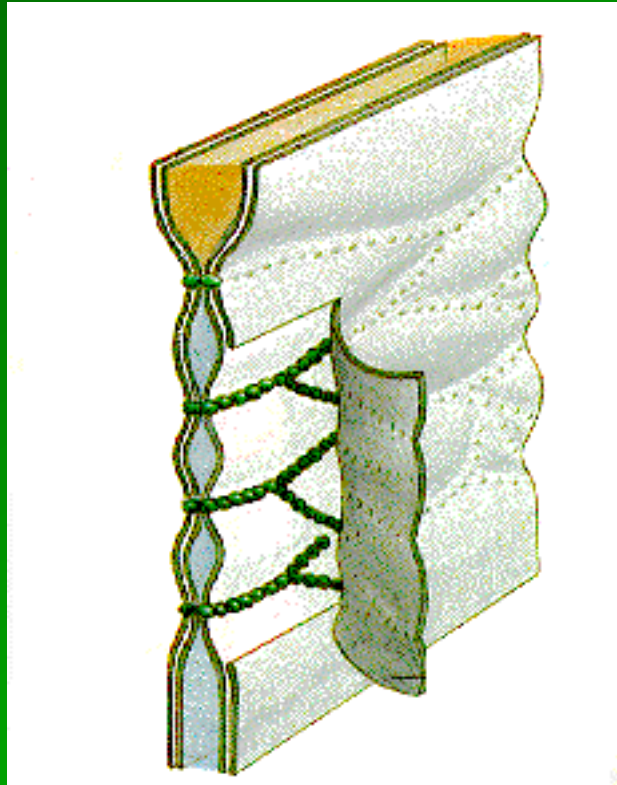
Consists of ridges that seal adjacent cells together. (In EM, the membranes seem to be „melted” together).

Unique to epithelial cells.



Fine structure

Integral membrane proteins of neighbouring cell membranes fuse with each other.

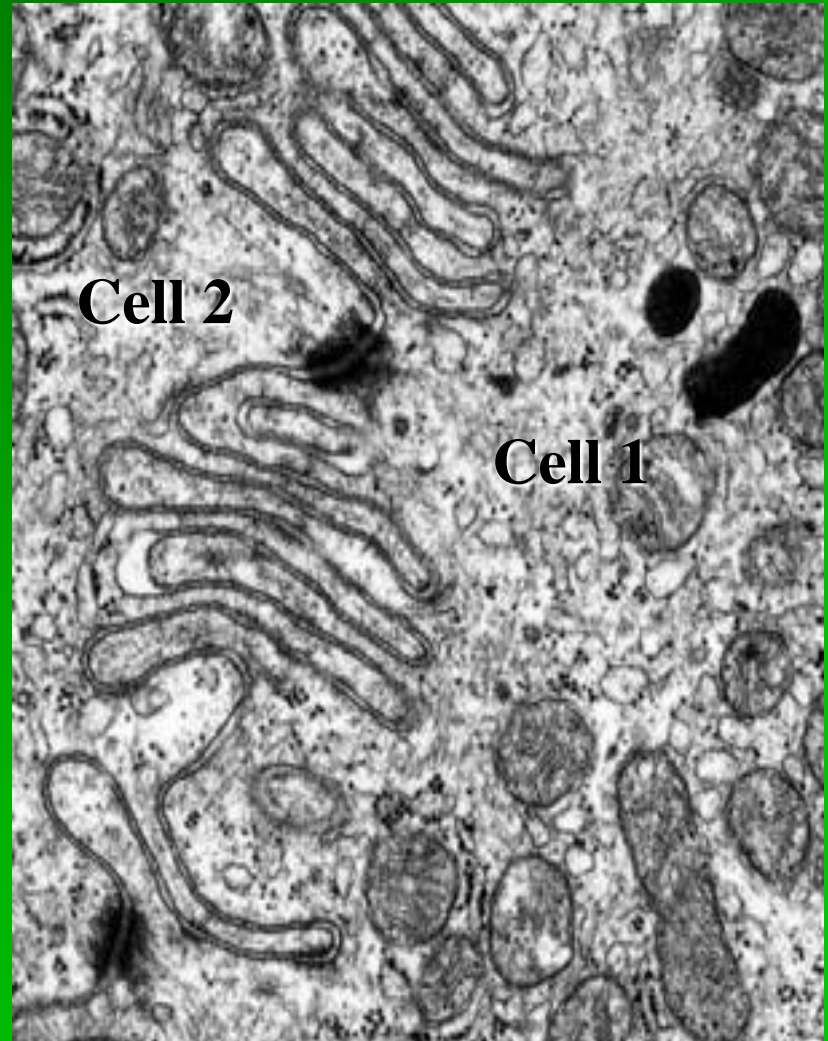
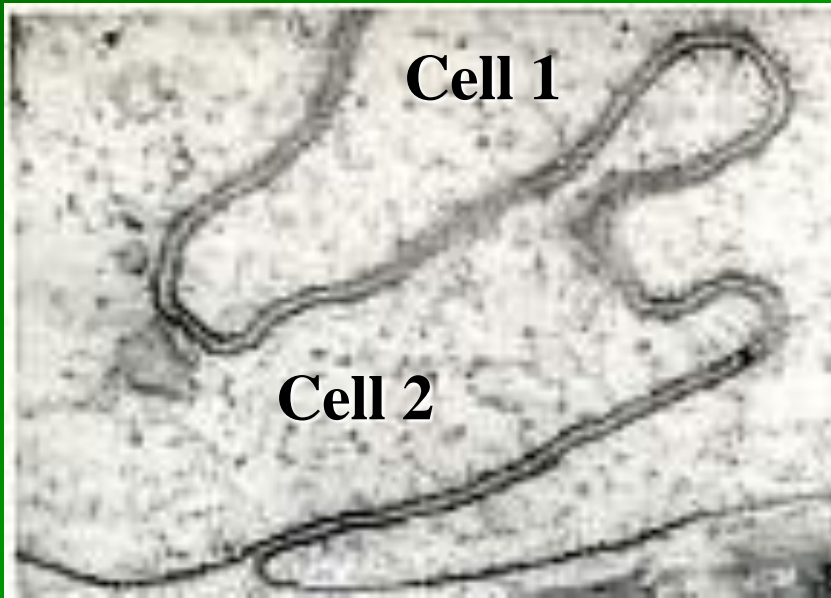


Biological function:

Constituting a barrier to prevent the passage of substances from the organ lumen to the extracellular space and vica versa

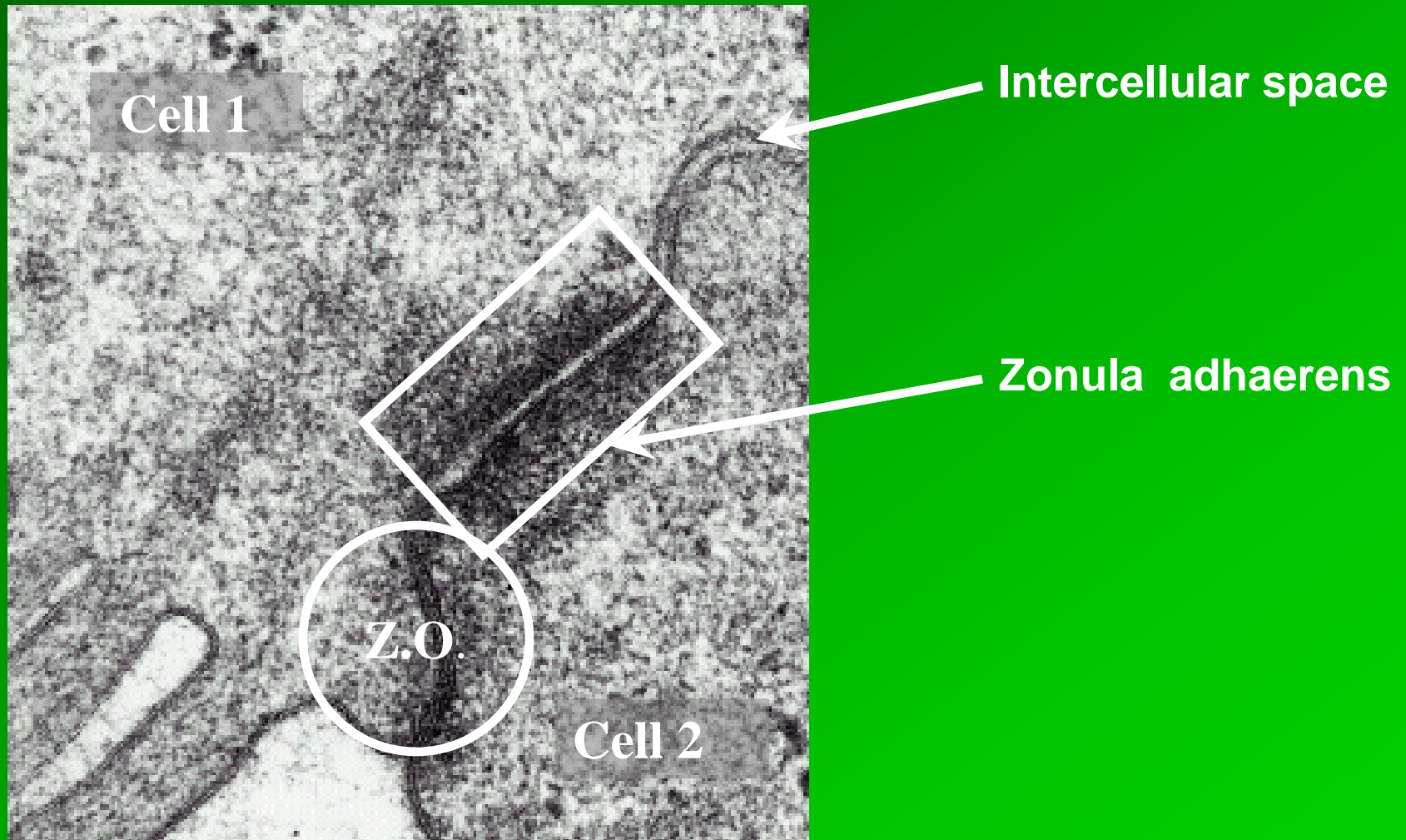
Maintaining cell polarity (apical-basal) by limiting movement of membrane components.

Lateral cell membrane junction:
interdigitation



- ***Zonula adhaerens*. Belt desmosome**

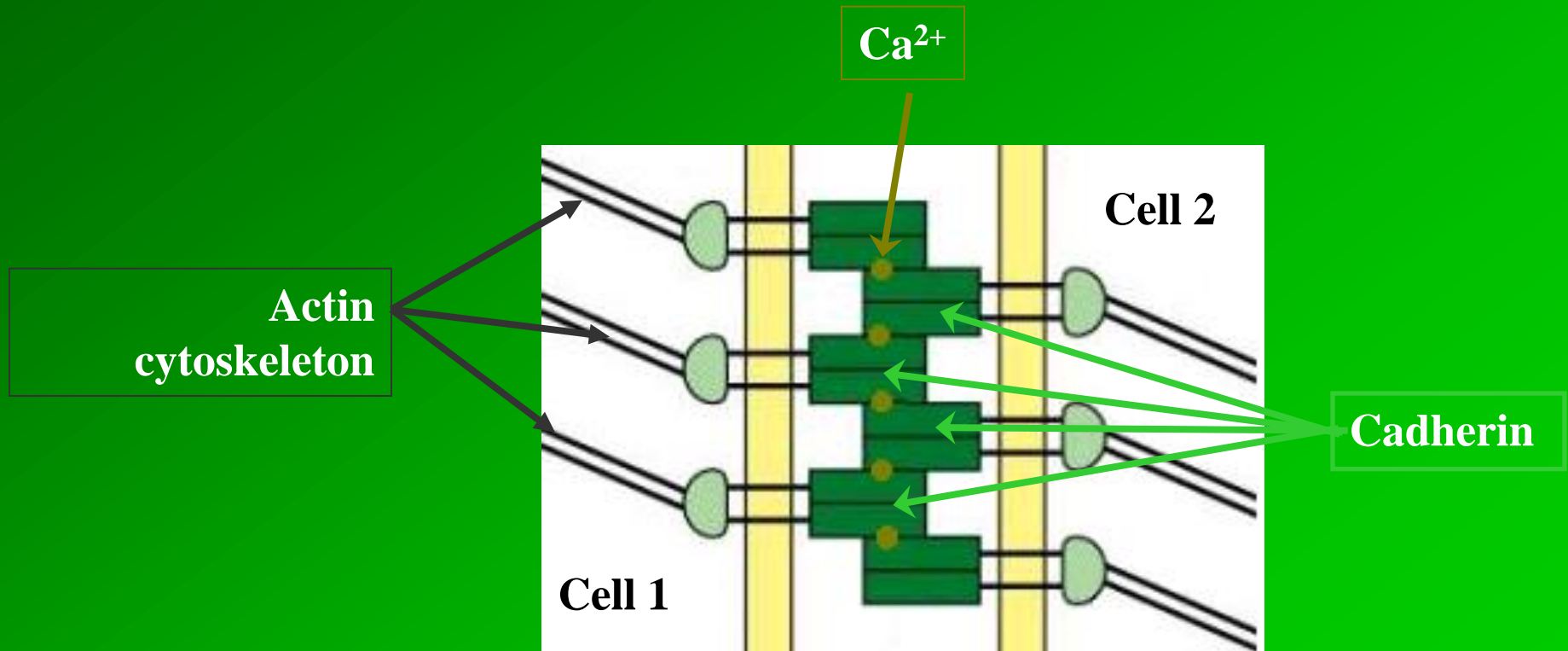
Located below the tight junction in a belt-like fashion. The intercellular space contains filamentous material. Dense plaques appear on the cytosolic surface of the cell membranes.



Fine structure

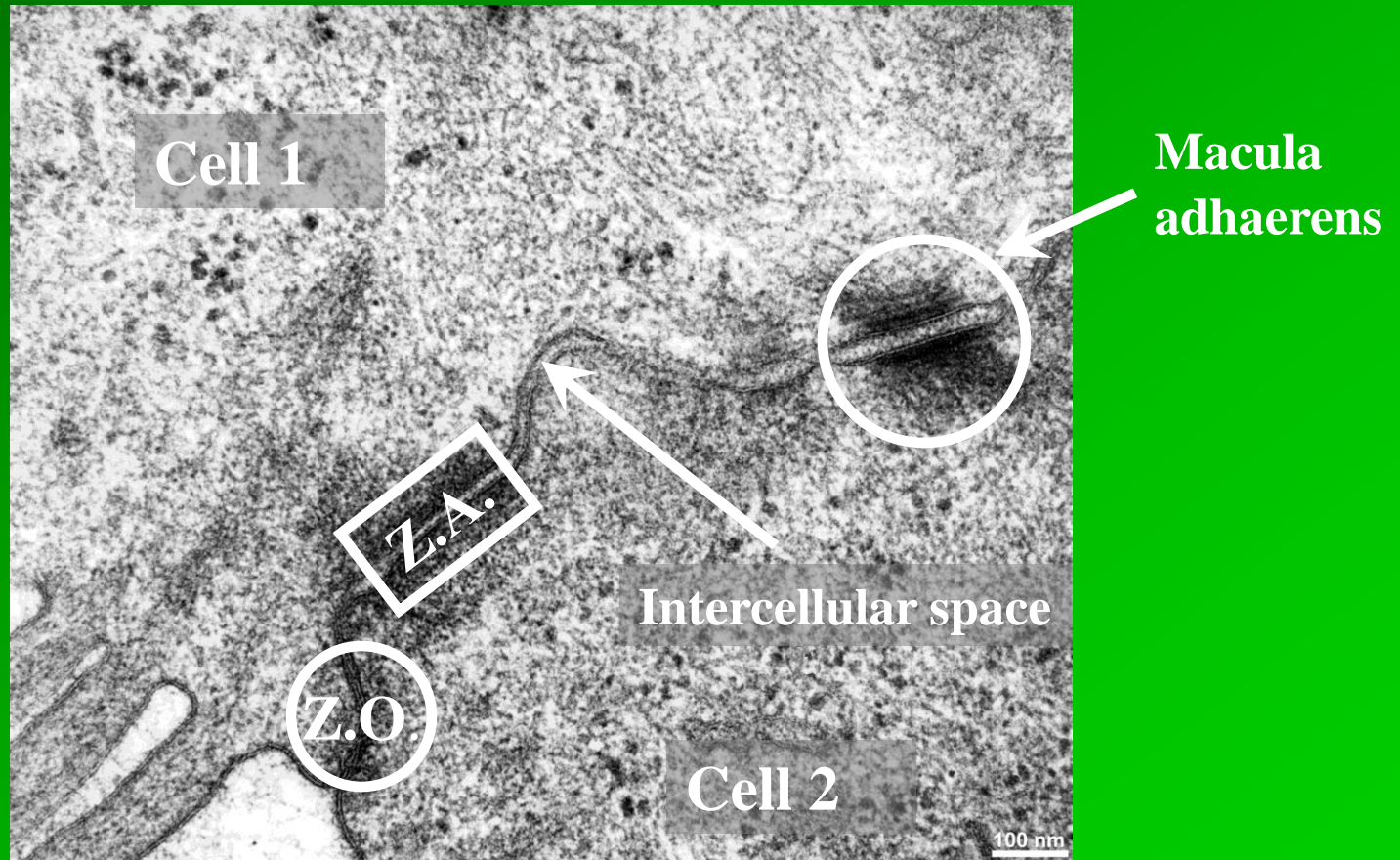
Intercellular anchoring proteins:

Cadherins (Ca²⁺-dependent cell
adhesion proteins)



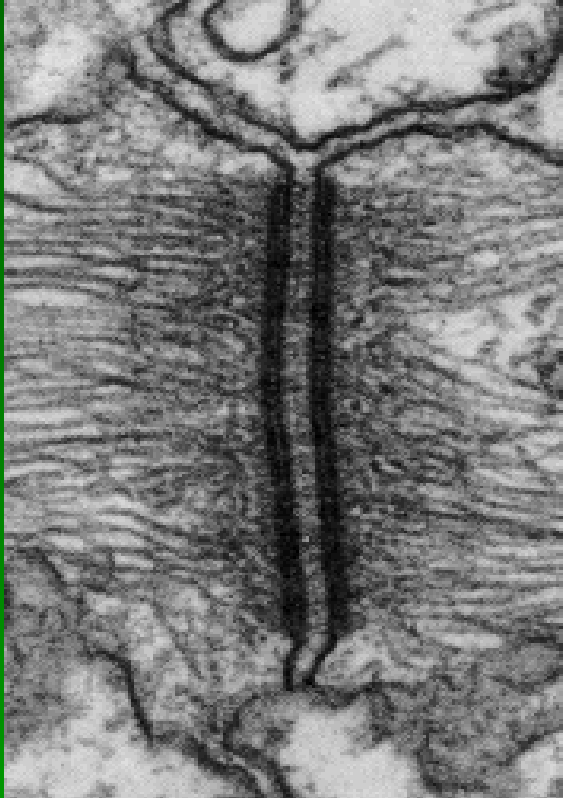
- *Macula adhaerens*. Spot desmosome

Spot desmosome is a disk-like structure. Electron-dense material is present in the extracellular space, that often forms a central line. It can be found between epithelial cells, cardiac muscle cells and several other cell types.



Fine structure

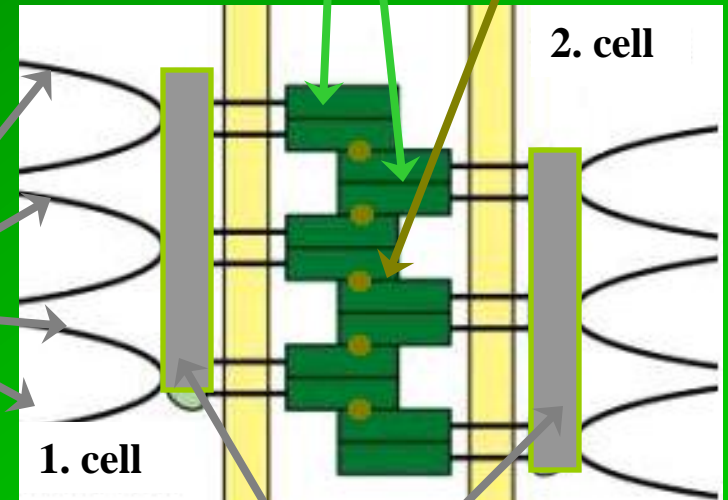
EM



Intermediary
filaments

Cadherin

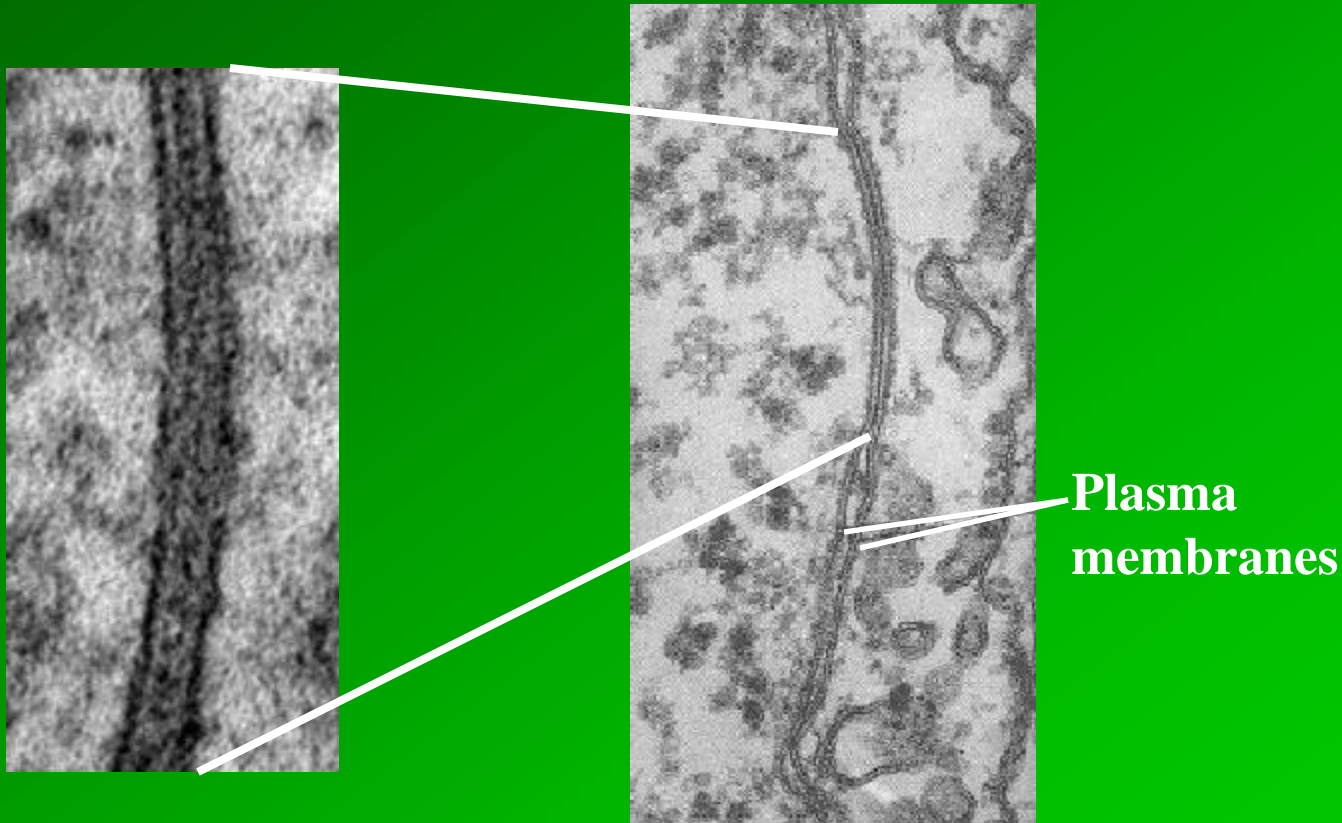
Ca^{2+}



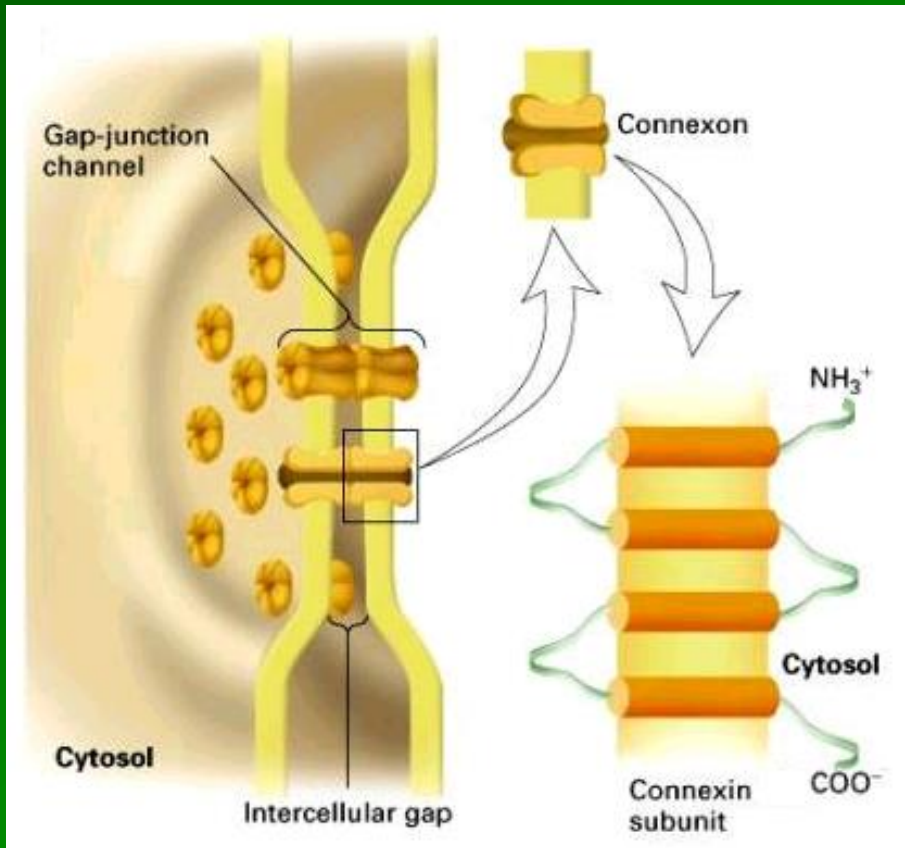
Plaques

- *Macula communicans*. Nexus, gap junction

Disklike zones where the plasma membranes of adjacent cell membranes are in close apposition. Knob-like structures appear on the intracellular sides of two membranes, as well as in the narrow intercellular space between them.



Fine structure



**Small molecules (< 1000 Da):
ions, second messengers,
metabolites etc. can freely pass.**

**The two cells are metabolically
and electrically coupled (in
excitable tissues: electrical
synapse).**

**Enhanced intracellular Ca²⁺
concentration (cell damage!)
results in closing of the nexus.**

**Plasma membrane proteins (connexins) form
a channel (connexon). Connexons of two
adjacent cell membranes join together in the
intercellular space to constitute a continuous
hydrophilic channel, the nexus.**

- *Synapse*

Site of morphologic specialization where a neuron is able to influence the excitability of another cell. Types: interneuronal and neuromuscular synapses.

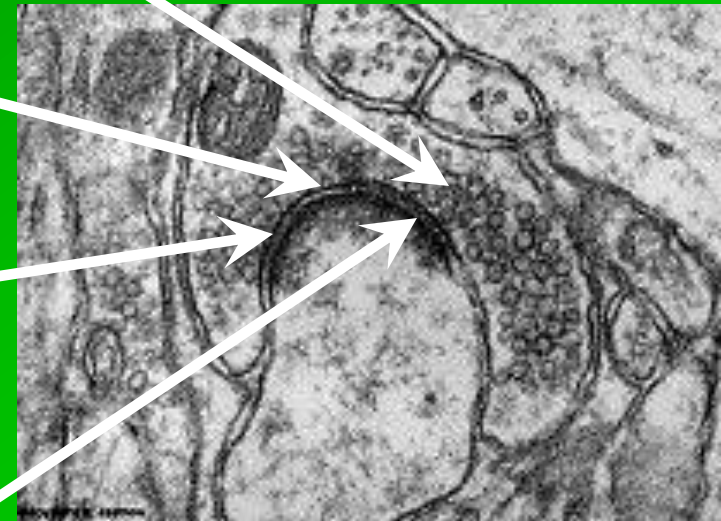
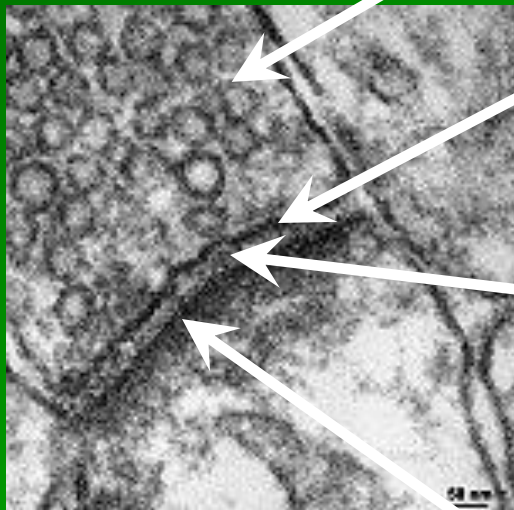
Interneuronal synapse:

Presynaptic site (neuro-transmitter containing) vesicles

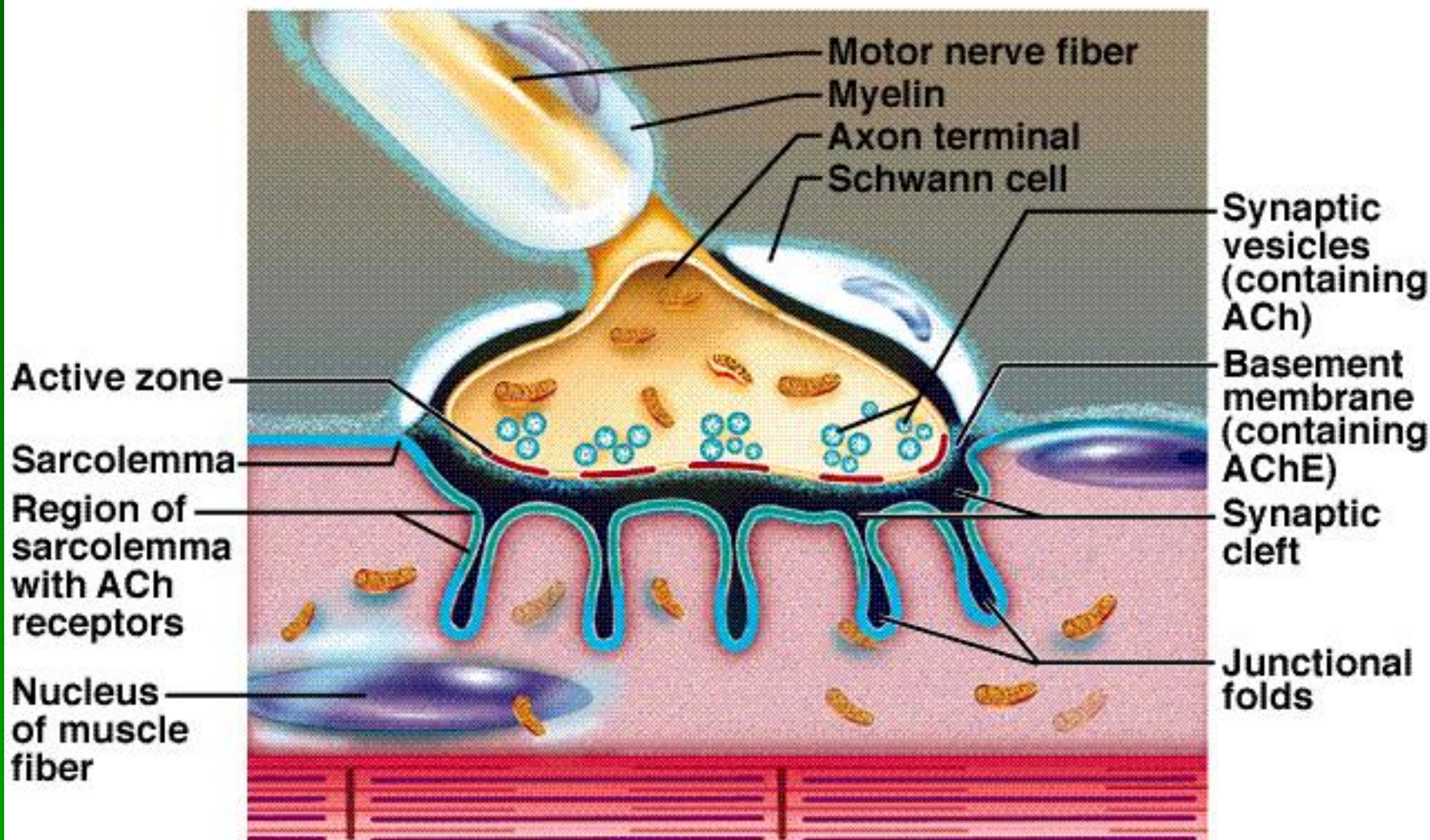
presynaptic membrane

synaptic cleft

postsynaptic membrane thickening



Neuromuscular Junction



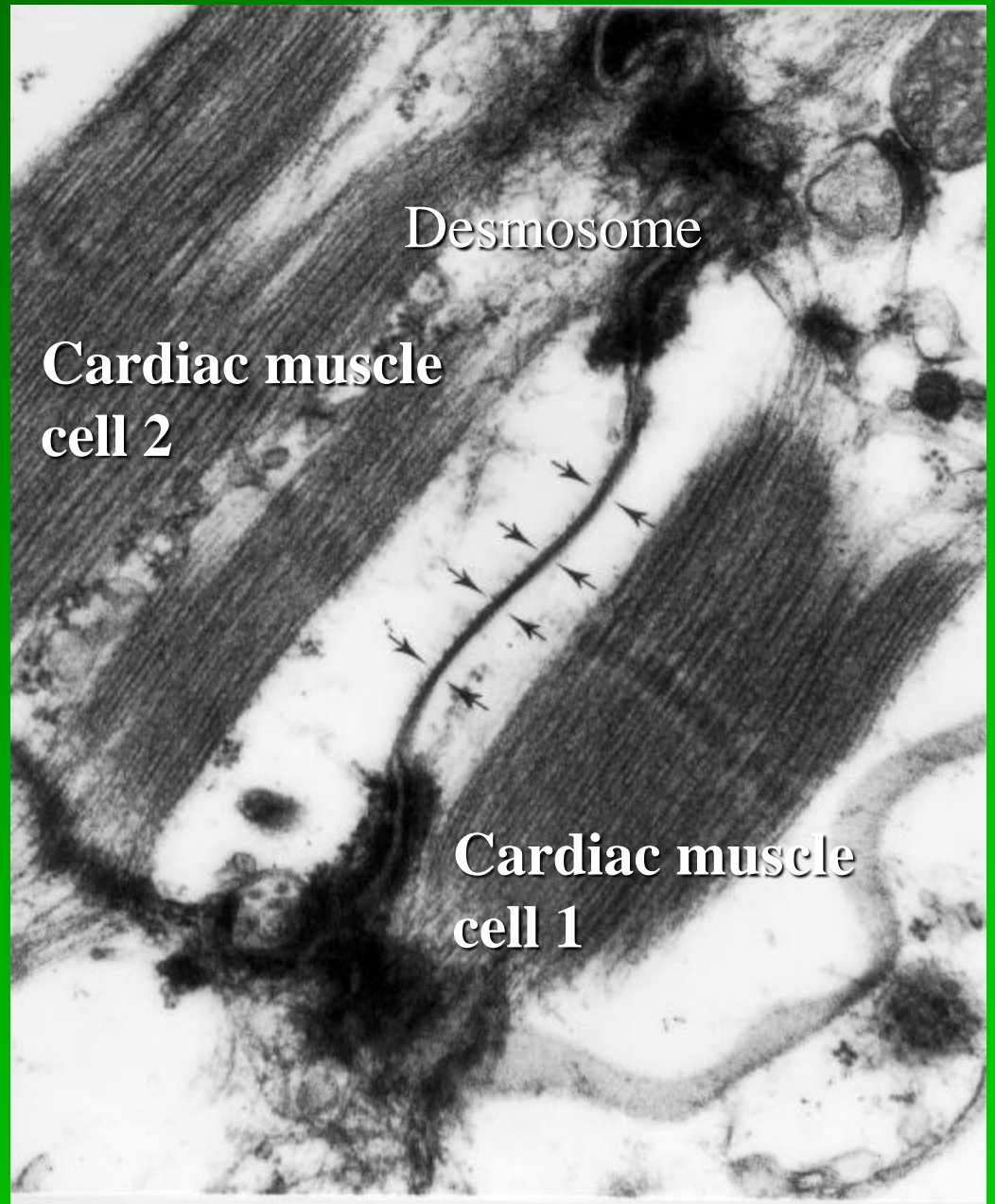
**EM microphoto
of the neuro-
muscular junction:**

- 1- axon terminal with synaptic vesicles
- 2- synaptic cleft with invaginations
- 3- skeletal muscle fibre



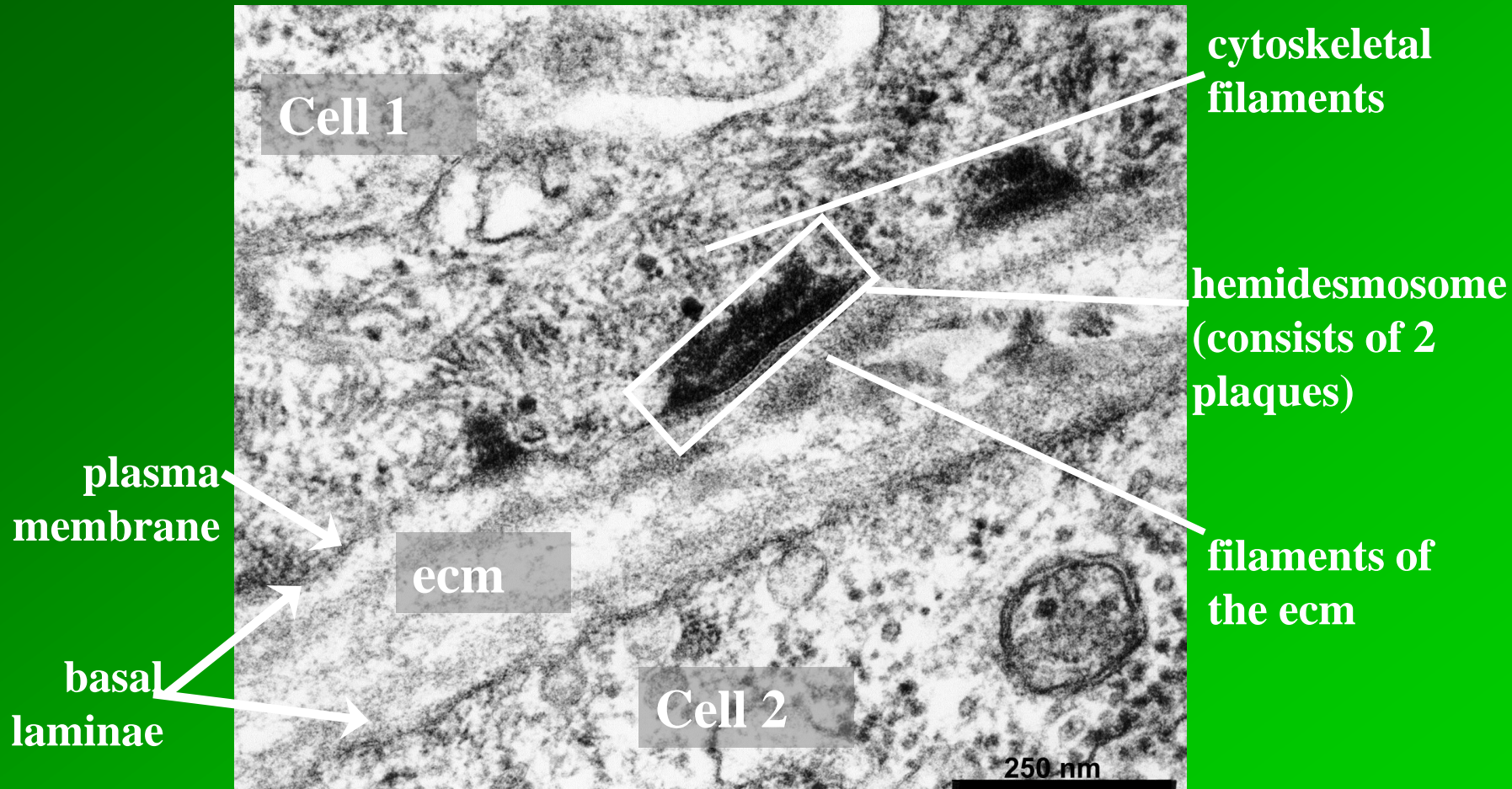
**Complex membrane
junction:
intercalated disk between
cardiac
muscle cells:**

1. Desmosome: mechanical junction
2. Gap junction: (arrows)
communicating junction



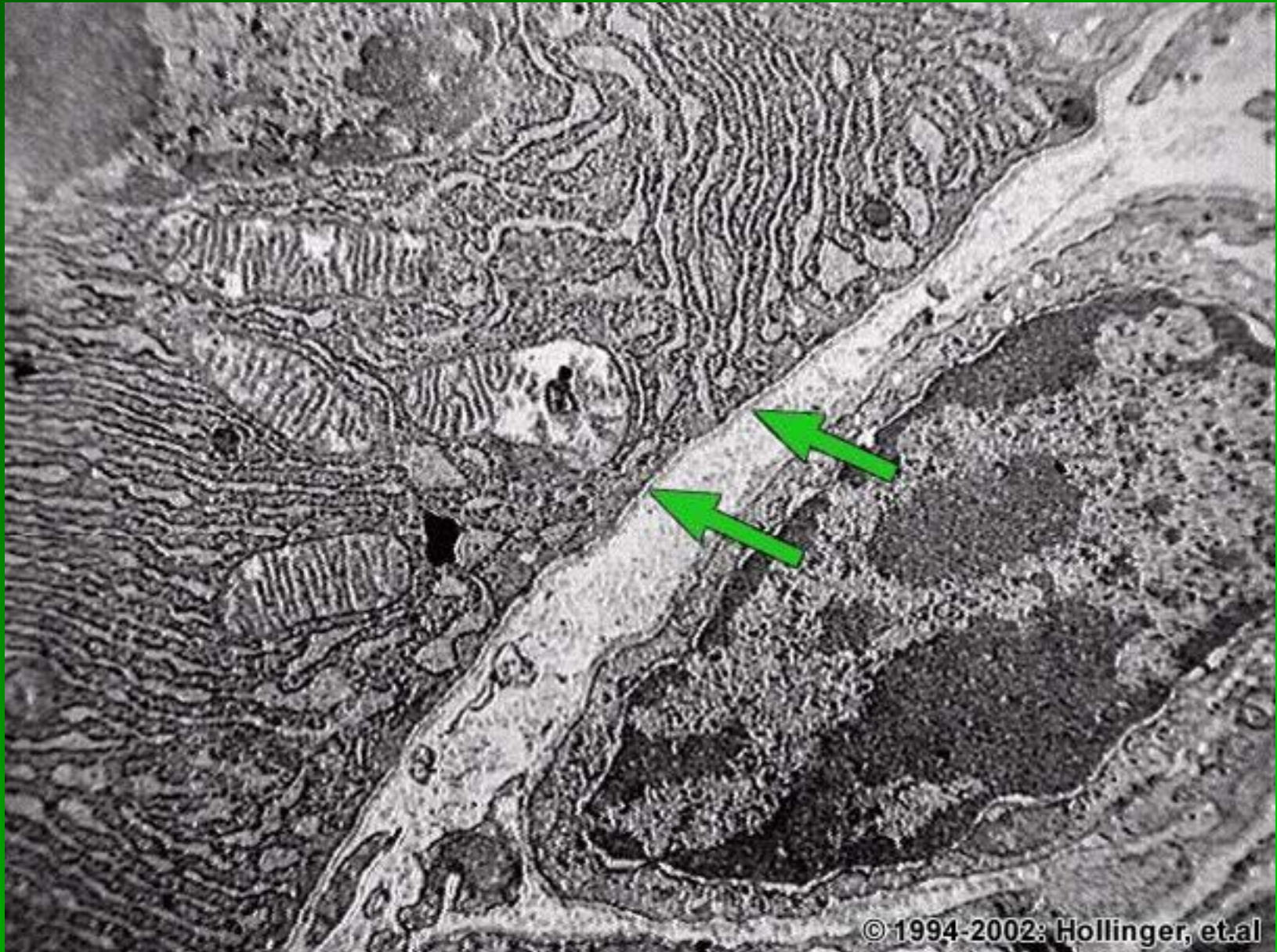
II.1.2.3b. Cell-ecm connections

- *Hemidesmosome*



EM, epithelium of the urinary bladder

Basal striation and basal lamina



BASAL LAMINA:

Very thin structure between epithelial cells and the underlying connective tissue.

It is also present around muscle cells.

It provides attachment for epithelial cells, guide for migrating cells, acts as a selective barrier.

It is composed of type IV collagen, laminin, proteoglycans.

The type IV collagen forms three-dimensional lattice-like structure.

Sublayers:

lamina lucida (40-50 nm thick)

lamina densa (40-50 nm thick)

II.1.2.4. Transport processes, transport proteins

Function of transport processes through the plasma membrane

⇒ regulation of the cell volume

⇒ stabilization and regulation of the pH, ionic and molecular composition of the cell

⇒ to ensure optimal conditions for enzyme reactions

⇒ uptake of energy-rich substances and metabolic intermediates from the extracellular space

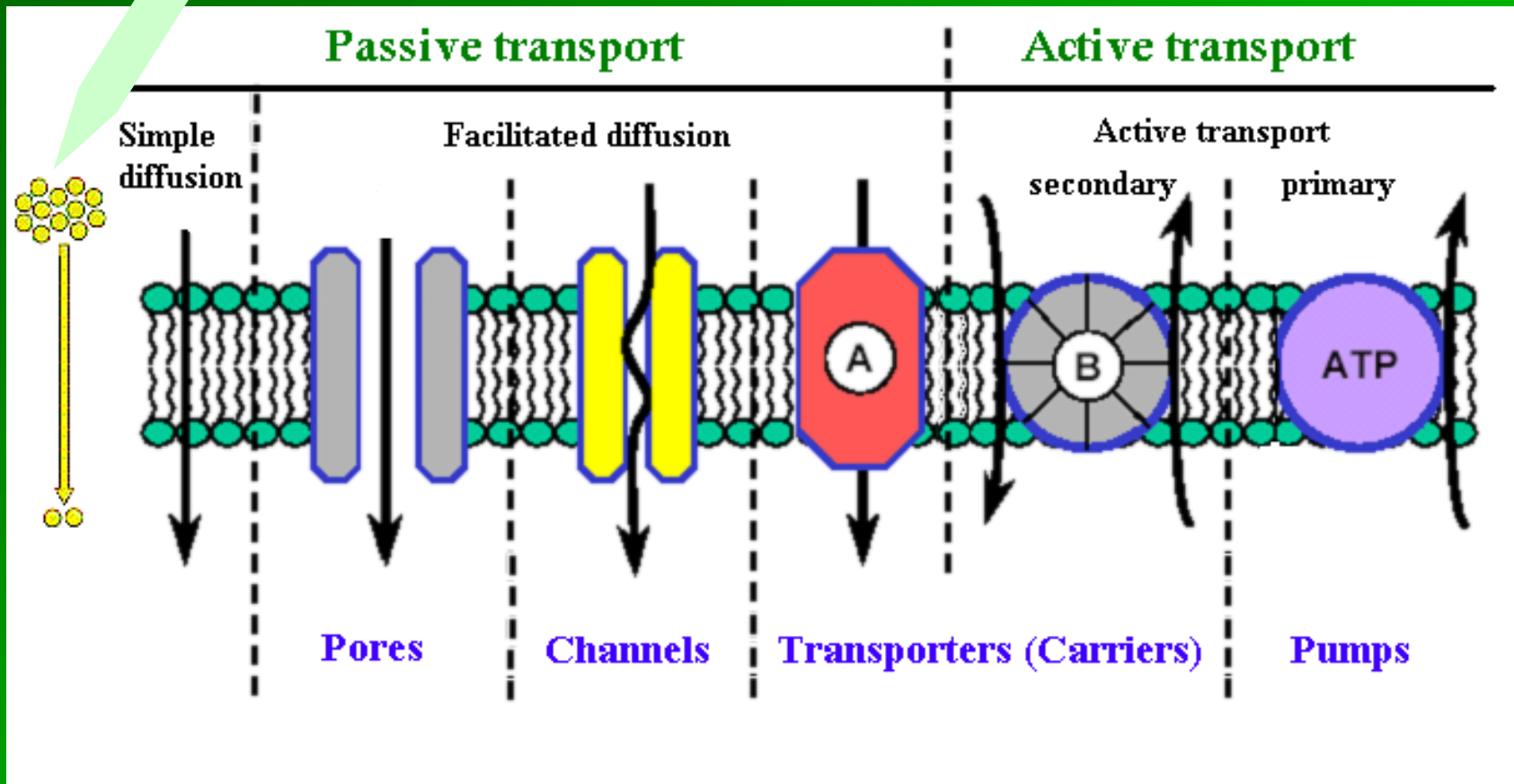
⇒ elimination toxic substances

⇒ formation and maintenance of ion gradients (particularly important for the excitability of nerve and muscle cells)

Transport types

Definition:

(electro)chemical gradient of an ion or a molecule through the membrane

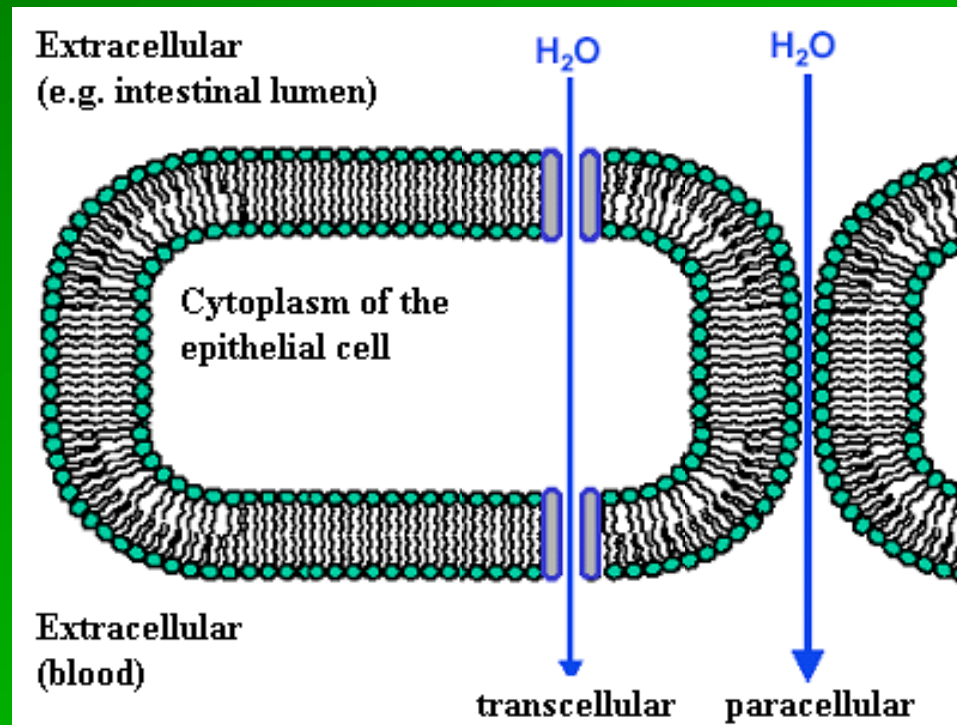


II.1.2.4. 1. Passive transport

- Pores

Example 1.: Nexus (gap junction)

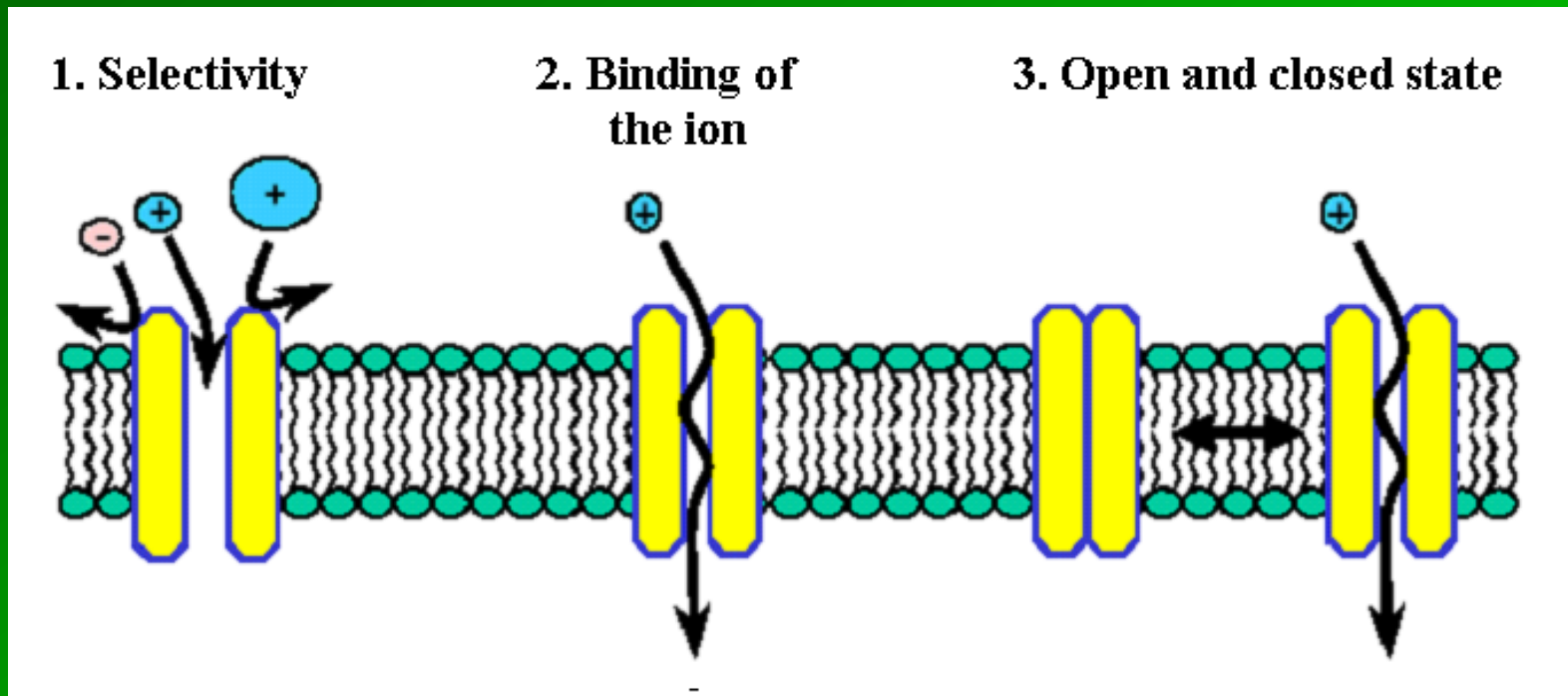
Example 2: Transport of water through epithelial cells (kidney, intestine)



- **Channels**

Ion channels are selective „pores” allowing the passage of ions *in the direction* of their electrochemical gradients.

Properties of ion channels:

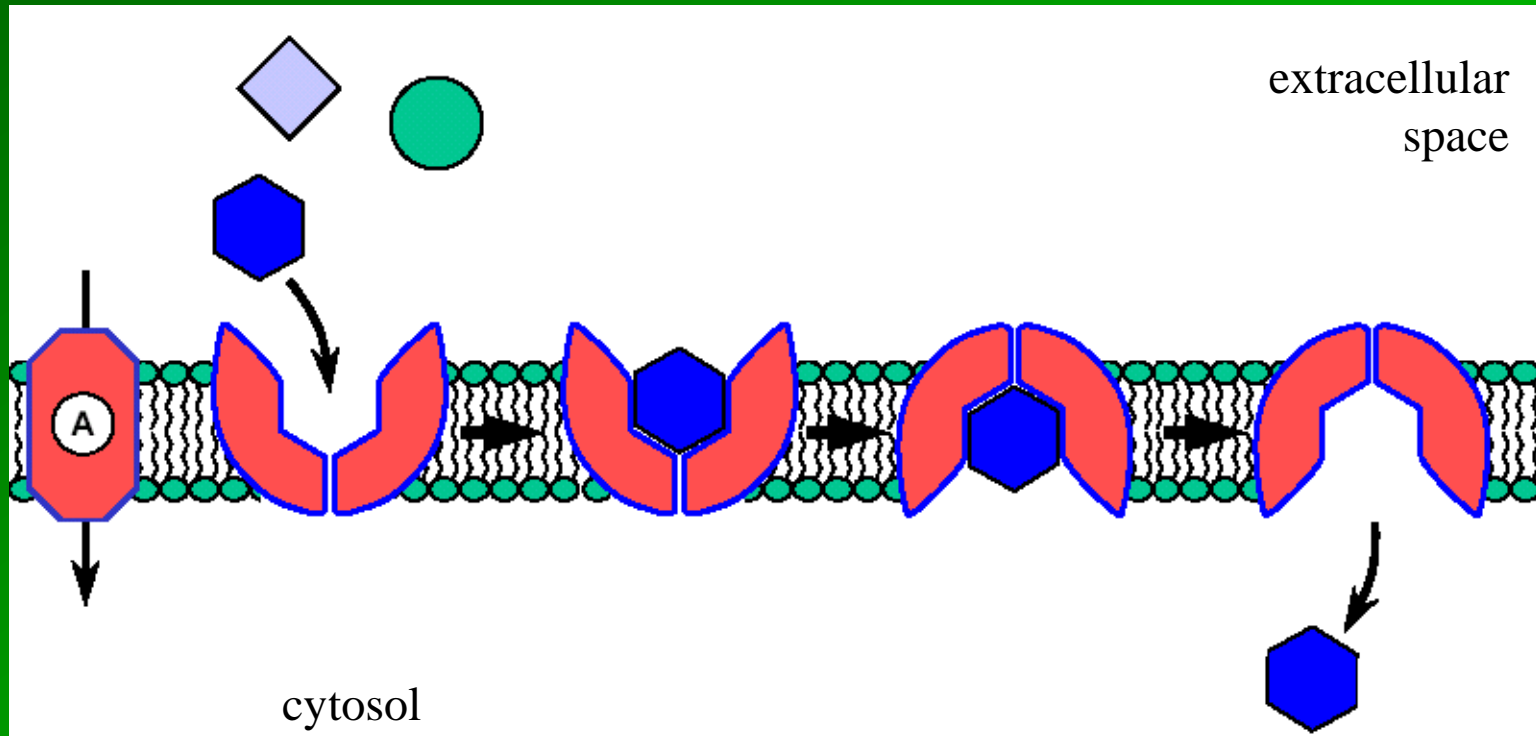


The activity of most of the ion channels is regulated by extracellular signals (e.g. neurotransmitters).

- **Carriers**

Facilitate the transmembrane movement of small organic molecules and ions.

Mechanism of action:



**1. Binding of
the substrate
molecule**

**2. Conformational change of
the protein enables the
substrate to cross the
membrane**

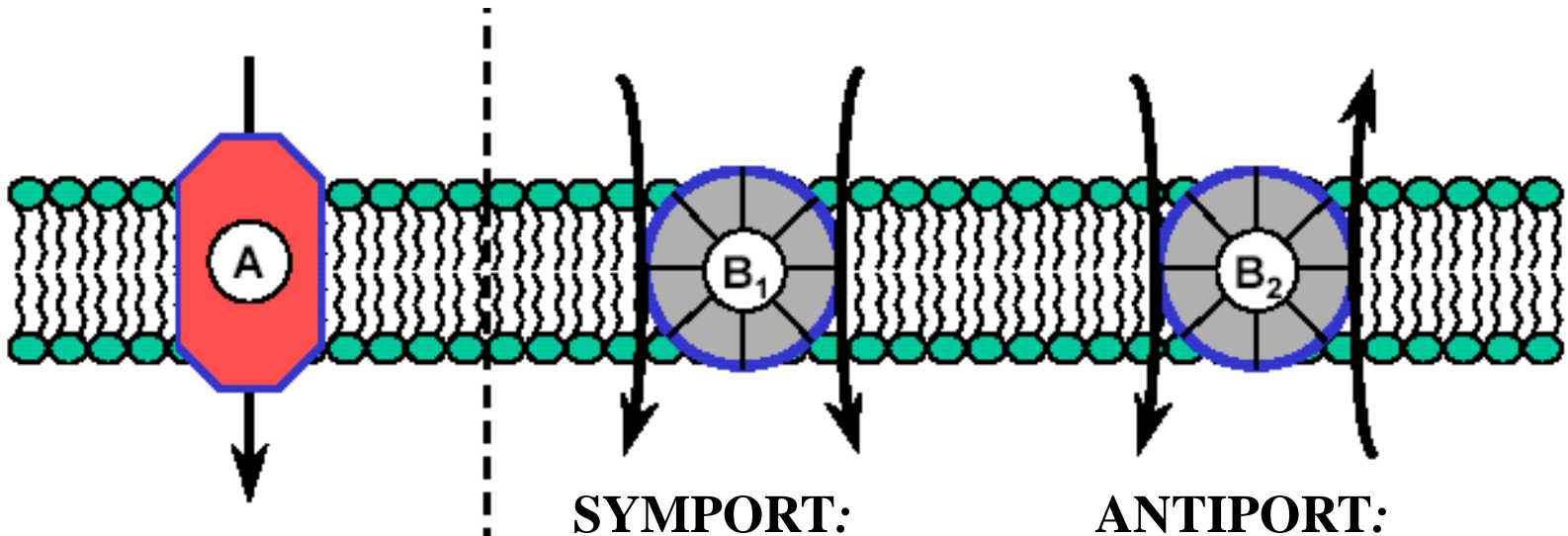
**3. Dissociation
of the substrate
molecule**

Carrier types

A. UNIPORT:

One substrate

(*e.g. glucose*)



One molecule is transported in the direction of its electrochemical gradient.

B. COTRANSPORT:

2 two or more substrates

SYMPORT:

Substrates are transported in one direction (*e.g. Na⁺/glucose, Na⁺/amino acid*)

ANTIORT:

Substrates are transported in opposite directions (*e.g. Na⁺/H⁺, Cl⁻/HCO³⁻*)

II.1.2.4. 2. Active transport

- Primary active transport

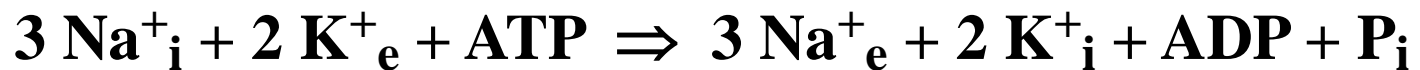
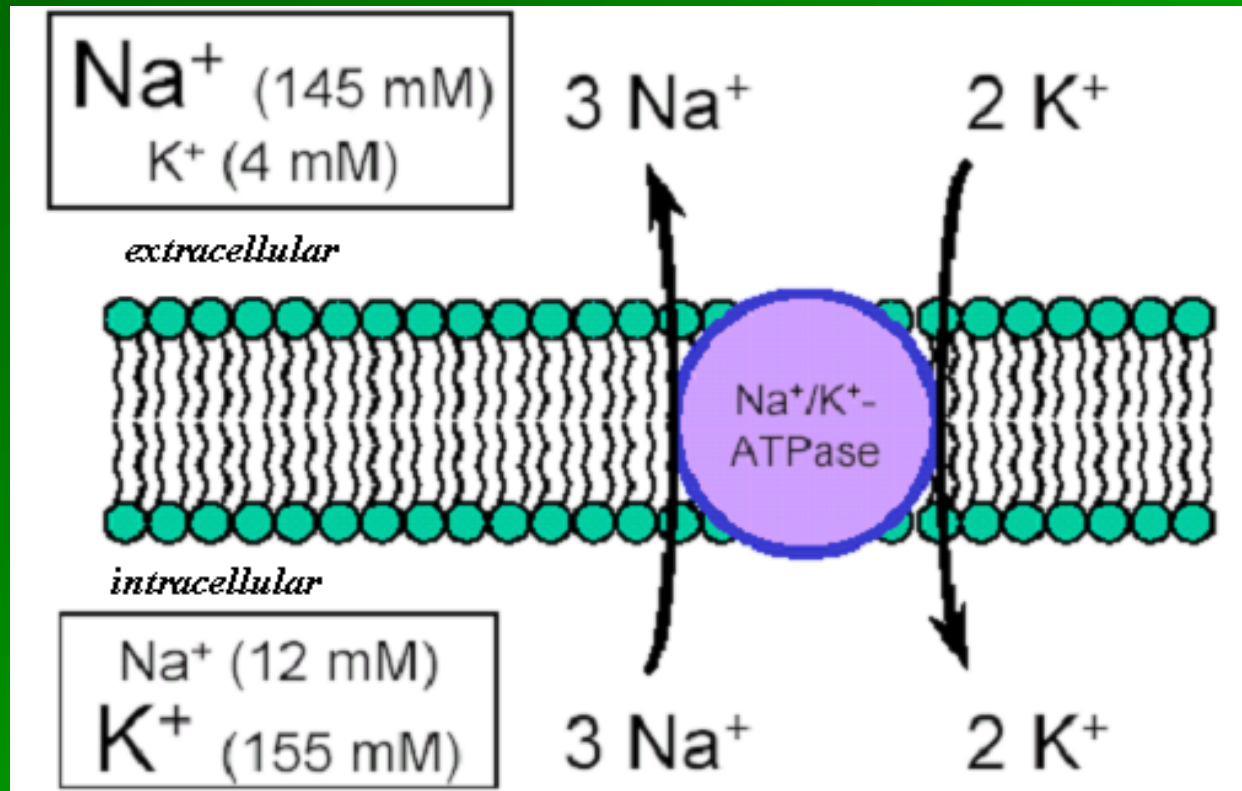
Definition: Transport of ions or molecules *against* their (electro)chemical gradient using the energy stored as ATP.

ATP-driven pumps (pumps, ATPases)

Coupling of a chemical reaction with a transport process.

- ATP is hydrolysed ($\text{ATP} \Rightarrow \text{ADP} + \text{P}_i$): energetically favorable („downhill”) reaction
- at the same time, one or more ion(s) or molecule(s) is transported against its concentration gradient („uphill”)

$\text{Na}^+/\text{K}^+\text{-ATPase}$ ($\text{Na}^+/\text{K}^+\text{-pump}$) (*ubiquitous*)



electrogenic process

- Secondary active transport

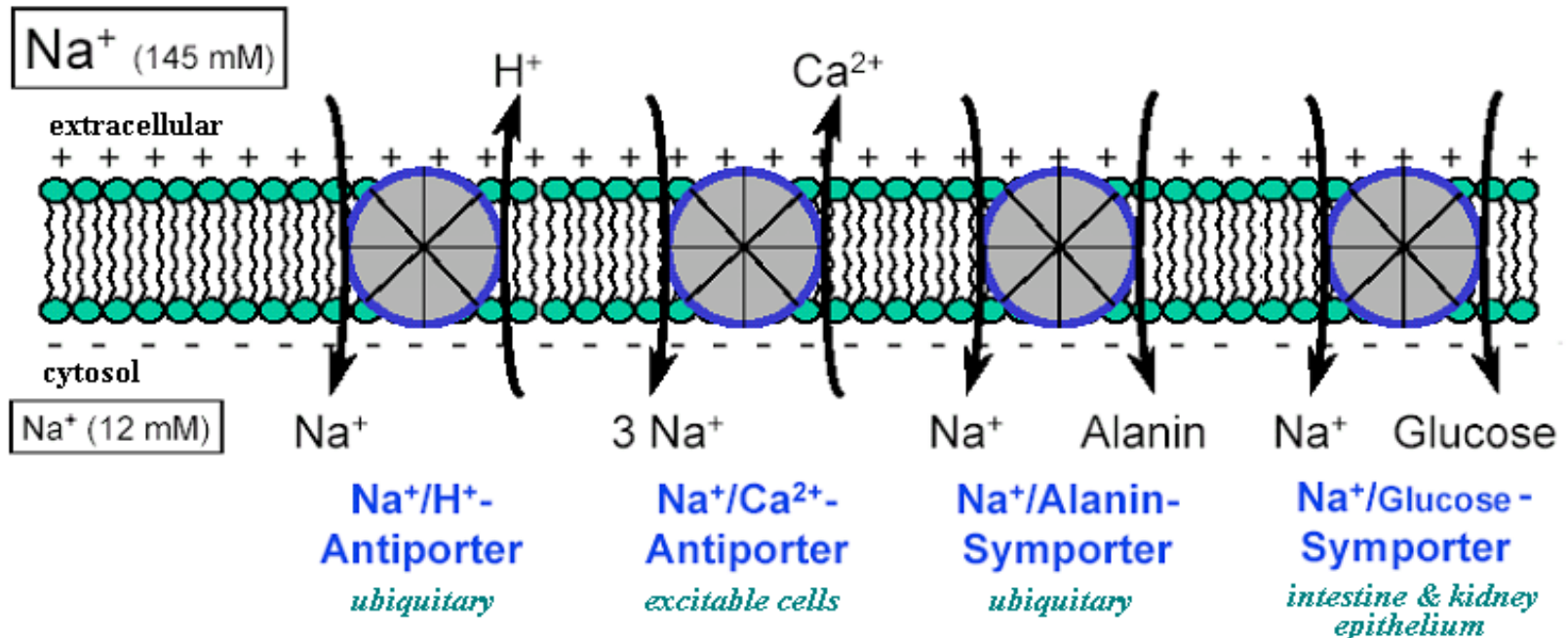
Definition: Transport of ions or molecules *against* their (electro)chemical gradient by using the energy stored as a gradient of another ion.

Carrier proteins, cotransport mechanism (B):

Coupling two transport processes:

- a given ion is transported *along* its concentration gradient – energetically favorable („downhill”) process.
- another ion or molecule is transported at the same time *against* its concentration gradient – energetically unfavorable („uphill”) process.

Na⁺-driven secondary active transport processes



Role of active transport processes in cellular functions

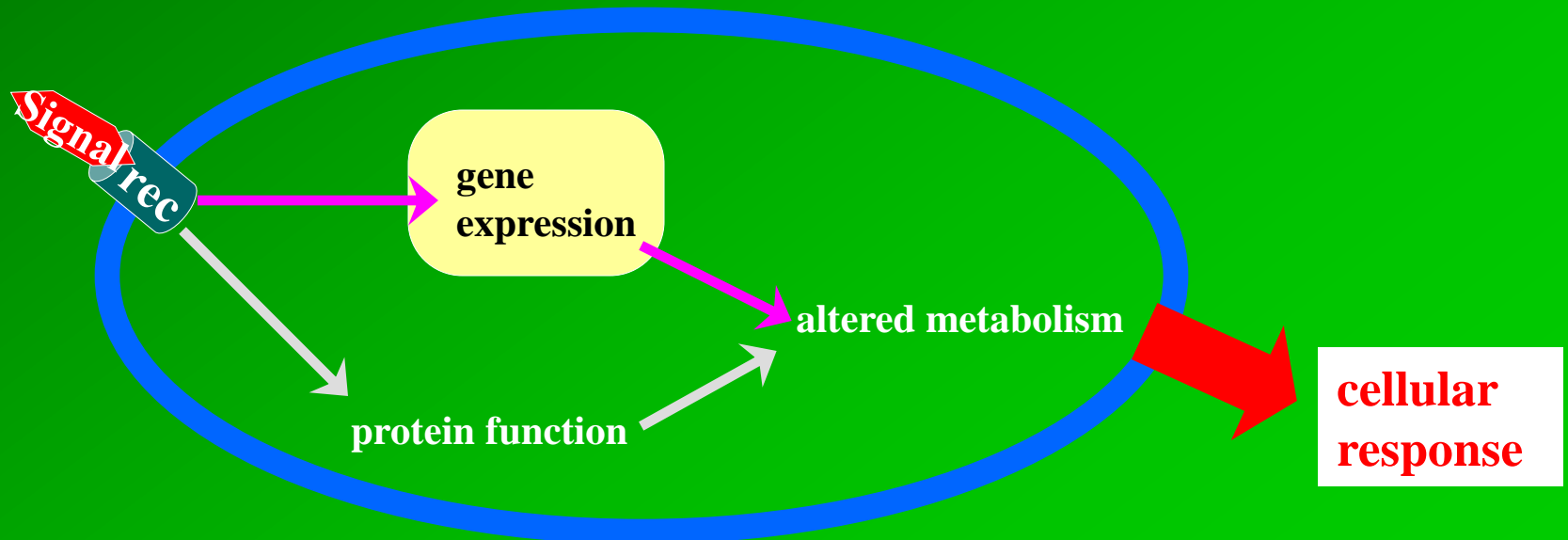
- **Generation of intracellular signals:**
 - regulation of cytosolic Ca^{2+} concentration**
 - in excitable cells: formation and spreading of electric signals.**
- **Uptake of nutrients (e.g. amino acids)**
- **Material transport through epithelia (e.g. intestines, kidney)**
- **Regulation of the pH of the cytosol and organelles.**

II.1.2.5. Receptors, signal transduction

Receptor: (Most of the cases) an integral membrane protein, recognising a specific extracellular signal.

Signal: Stimulus (e.g. light) or a middle molecular weight substance (signal molecule) inducing a cellular response.

Signal transduction: Receiving the signal, its transmission into the cell, and induction of specific cell processes.

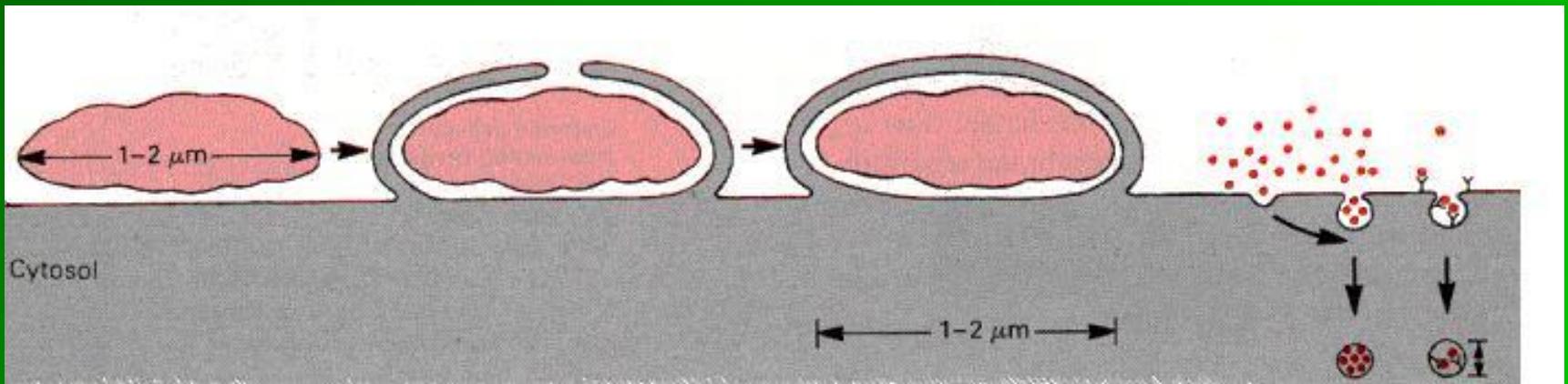


II.1.2.6. Endo- and exocytosis I.

- Phagocytosis, endocytosis

Phagocytosis (cell „eating”)
Uptake of solid particles and cells

Endocytosis
Uptake of relative large portions of substances

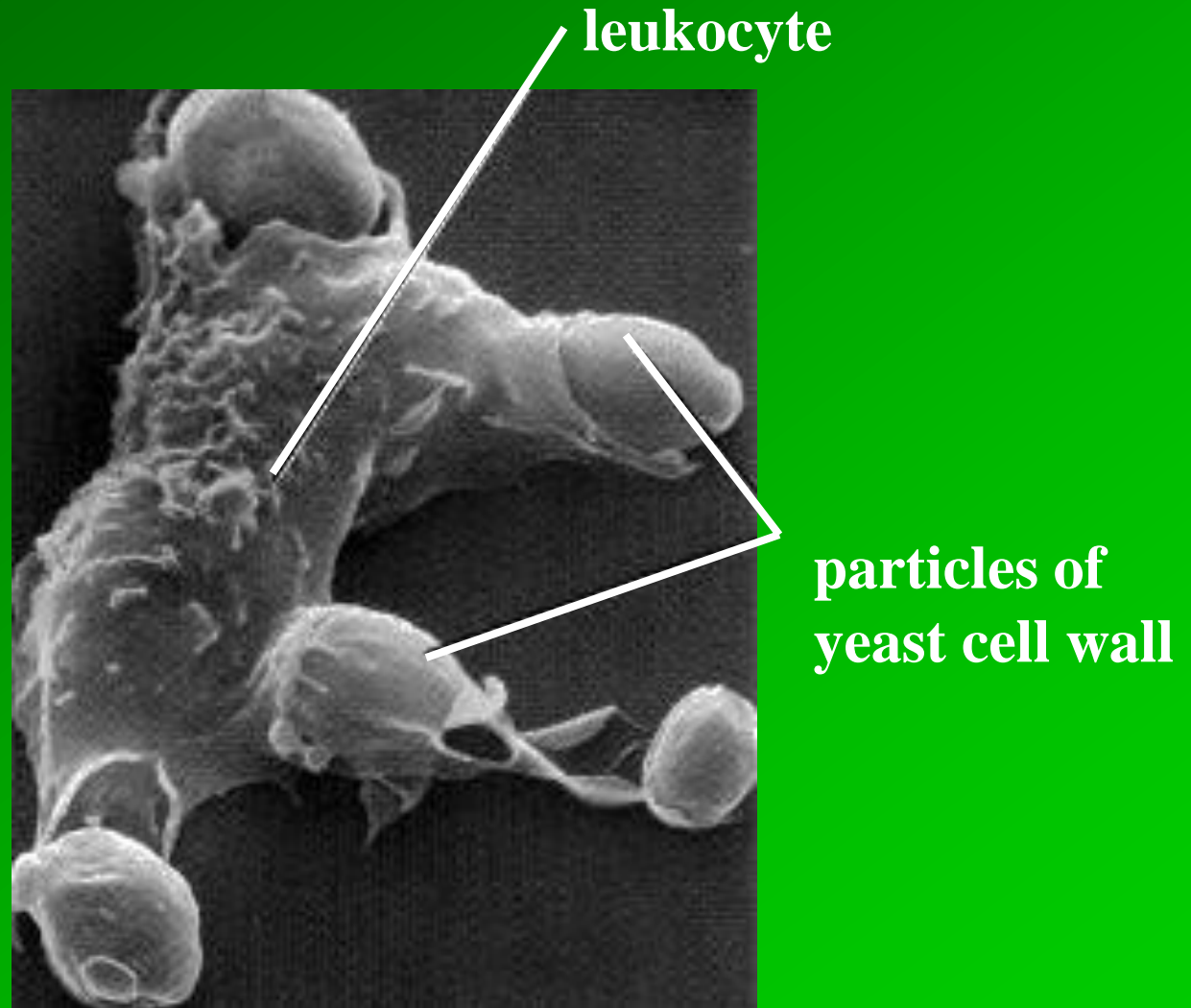


Pinocytosis (cell „drinking”)
Uptake of fluid

**Receptor mediated
endocytosis**

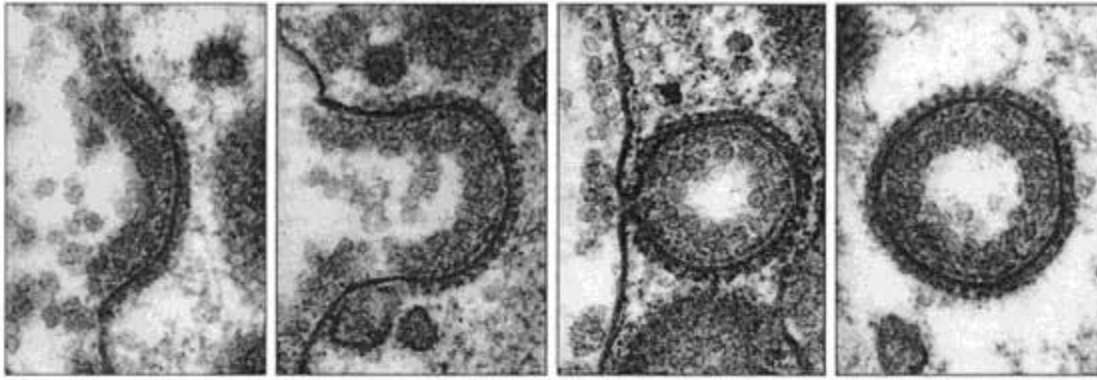
Phagocytosis and endocytosis lead to the formation of membrane coated vesicles within the cell.

Phagocytosis on a scanning EM image



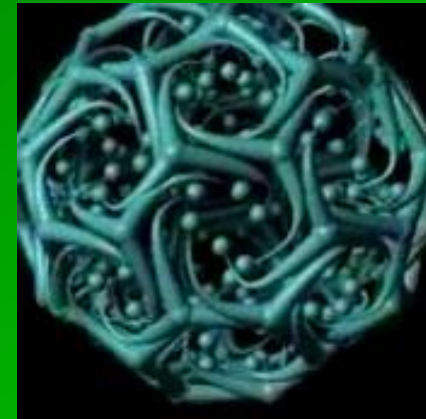
CARRIER-MEDIATED ENDOCYTOSIS:

Formation of Clathrin-Coated Vesicles



(A)

- 2500 every minute
- CCV uncoat within seconds



Model of the clathrin coat

Example: iron is carried by transferrin protein in blood.

The red blood cells have transferrin-binding proteins on their membrane. Endocytosis occurs in clathrin cage, moves inside the cell. Iron released, cage eventually recycles back to cell surface returning transferrin to the extracellular space.

Receptor mediated endocytosis

Uptake of specific substances in higher amount than it is expected from its concentration – highly specific receptor-binding is a prerequisite!

Cell-type-, developmental- and metabolic stage - specific process.

Examples:

cholesterol,

hormons (e.g. insulin)

toxins (e.g. diphtherie-toxin)

viruses (e.g. adenovirus)

antibodies (e.g. IgG) .

- **Exocytosis**

Discharge of substances from the cell (ubiquitous, but particularly important in secretory cells, connective tissue cells and lymphocytes).

The process involves

- ⇒ migration of intracellular vesicles to the cell membrane
- ⇒ fusion of the vesicular membrane with the cell membrane
- ⇒ delivery of the vesicular content into the extracellular space.

Two types: constitutive and
 receptor mediated